HALOGENATED SOLVENTS INDUSTRY ALLIANCE, INC.

FOR HAND DELIVERY

January 18, 2002

Technical Information Staff (8623-D)
National Center for Environmental Assessment
US Environmental Protection Agency
808 17th Street NW, 5th Floor
Washington, DC 20006

TRICHLOROETHYLENE HEALTH RISK ASSESSMENT:
SYNTHESIS AND CHARACTERIZATION
(August 2001 External Review Draft)

COMMENTS OF THE HALOGENATED SOLVENTS INDUSTRY ALLIANCE, Inc

Please find enclosed the comments of the Halogenated Solvents Industry Alliance, Inc that also incorporate contributions from experts in appropriate field as attachments.

HSIA offers to make available bound copies or electronic versions (most probably in the form of CDs) of these comments to EPA for distribution. We will contact NCEA staff to establish EPA's requirements.

HSIA expresses substantial concerns regarding EPA's Synthesis and Characterization and recommends that the document be withdrawn for major revision. Specifically, the Synthesis Document, in its present form, is unsuitable for use as the basis for an update of the IRIS database for trichloroethylene or for any regulatory action. HSIA recognizes the magnitude and complexity of the major revision that is necessary, and offers to assist EPA in any practical way. For example, HSIA would be willing to participate in the establishment of a panel of scientists designated to help EPA to interpret the large and complex set of information.

HSIA appreciates the opportunity to comment on the draft Synthesis Document.

Yours sincerely,

Paul Dugard

Paul H. Dugard, PhD
Director of Scientific Programs

ENVIRONMENTAL PROTECTION AGENCY

TRICHLOROETHYLENE HEALTH RISK ASSESSMENT: SYNTHESIS AND CHARACTERIZATION (August 2001 External Review Draft)

COMMENTS SUBMITTED BY

THE HALOGENATED SOLVENTS INDUSTRY ALLIANCE, INC.

NOTE:

This document is organized into an overview HSIA commentary, plus contributions by individual experts included as attachments. Each attachment is complete in itself and may be read without referring to the overview.

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1. GENERAL COMMENTS, SUMMARY AND RECOMMENDATIONS

1.1 INTRODUCTION

There is an apparent wealth of toxicological and epidemiological information for trichloroethylene (TCE). Unfortunately, this is a mixture of studies that are meaningful, some that have little value, and others that are actually misleading. Although the process used in developing the health risk assessment ("state-of-the-science" papers prepared by scientists with relevant expertise) was presumably intended to assist EPA, the burden of interpretation of a complex database remained EPA's responsibility. HSIA recognizes this burden and the problems encountered, but also has substantial concerns regarding EPA's interpretations and conclusions. HSIA has collected opinions of experts for certain specific areas of scientific concern and these are enclosed as "Attachments". Several of these comments were prepared in relation to a recent proposal by the National Toxicology Program (NTP) to reclassify TCE as a known human carcinogen. These papers are included here because the scientific points made are relevant to EPA's draft Synthesis Document, which is infected with many of the same errors of interpretation that led to the rejection of NTP's proposal by an almost unanimous vote of the NTP Board of Scientific Counselors.

1.2 EPA's INTERPRETATIONS ARE OVERLY CONSERVATIVE

Throughout the draft Synthesis Document EPA has taken the most conservative evidence and applied conservative interpretations. In reviewing the non-cancer endpoints, only studies with positive results are quoted or referenced and the strengths and weaknesses of these studies are not considered. The result is a document that, overall, lacks scientific balance and shows an extreme bias. This is not consistent with scientific principles ("sound science") nor does it meet the requirements of the guidelines that govern EPA's development of health assessments, discussed below.

1.2.1 Epidemiology and Weight of Evidence for Carcinogenicity

Analysis shows that the paper by Wartenberg et al (2000), in an attempt to encompass every epidemiology study that might possibly include TCE, has misled EPA. In fact, the most appropriate epidemiology studies (including almost all of the cohort studies of exposed workers) show no association between any form of cancer and TCE exposure. This is all the more remarkable because, historically, many of the workers studied were exposed daily to levels similar to those used in long-term animal bioassays. TCE itself is not genotoxic, nor are its principal metabolites. The evidence is moving away from supporting any role for genotoxicity in the induction of rat kidney tumors. As more has been learned about TCE's "modes of action" (MOAs) in the induction of tumors in animals, the lower the concern for man has become. Overall the data do not support EPA's classification of "highly likely to cause cancer" and the extensive available epidemiological evidence indicates that a much lower classification is appropriate.

1.2.2 EPA Has Overemphasized the Role of Dichloroacetic Acid (DCA) in TCE Toxicity

Misled by older information, since retracted, EPA has given greater prominence to DCA than the primary metabolite trichloroacetic acid (TCA). This has led to incorrect quantitative treatments, assumptions about MOAs and identification of sensitive sub-populations. This error pervades many aspects of the draft Synthesis Document.

1.2.3 Children and Sensitive Sub-Populations

As indicated by EPA, there are data on the physiological and metabolic processes for children that could have been used for a more detailed examination of the fate of inhaled or ingested TCE. The suggestion that the high end of the range of cancer potency slope factors covers sensitive sub-populations without any supporting evidence is less honest than admitting that the range of sensitivity is unknown and applying a safety factor to an appropriate no-observed-adverse-effect level.

1.3 THE SYNTHESIS DOCUMENT DOES NOT FOLLOW THE APPLICABLE SCIENCE POLICY GUIDANCE

In developing a health assessment, EPA must use the best science and risk assessment techniques available. The principles that guide the TCE risk assessment are set forth in the following documents:

- *EPA Guidelines for Carcinogen Risk Assessment* (SAB Review Draft) (1999), 66 Fed. Reg. 59593 (Nov. 29, 2001)
- *EPA Guidelines for Reproductive Toxicity Risk Assessment* (1996), 61 Fed. Reg. 56273 (1996)
- *EPA Guidelines for Developmental Toxicity Risk Assessment*, 56 Fed. Reg. 63798 (1991)
- *EPA Guidelines for Neurotoxicity Risk Assessment*, 63 Fed. Reg. 26926 (1998)
- Office of Management and Budget (OMB), *EPA Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies*, 67 Fed. Reg. 369 (Jan. 3, 2002)

1.3.1 EPA Guidelines

The only guidelines for carcinogen risk assessment finally promulgated by EPA were issued in 1986. 51 Fed. Reg. 33992. Thereafter, in 1996, EPA published proposed revised guidelines for carcinogen risk assessment. 61 Fed. Reg. 17960. More recently, EPA made available for comment a revised and expanded version of the *Guidelines for Carcinogen Risk Assessment* following three reviews of the 1996 proposal by its Science Advisory Board (SAB). In making the 1999 *Guidelines* available, EPA stated "[u]ntil final Guidelines are issued, the July 1999 draft revised Guidelines will serve as EPA's interim guidance to EPA risk assessors preparing cancer risk assessments." 66 Fed. Reg.

59593 (Nov. 29, 2001). A significant recent decision by a federal appeals court holds that EPA action based on the best science available at the time (in this case an "interim" determination under the Safe Drinking Water Act that chloroform was a threshold carcinogen) is binding on EPA. *Chlorine Chemistry Council v. EPA* 206 F.3rd 1286, 1291 (D.C. Cir. 2000).

Almost every deficiency in EPA's assessment of the potential carcinogenicity of TCE in the Synthesis Document reflects an inexplicable failure to apply the *Guidelines*. Indeed, the Synthesis Document makes surprisingly little reference to the *Guidelines*. In its discussion of weight-of-evidence (§ 1.3) the Synthesis Document states that "under EPA's proposed (1996, 1999) cancer guidelines, TCE can be characterized as "highly likely to produce cancer in humans," and also discusses classification of TCE under the 1986 "current" cancer guidelines. This is a remarkable example of EPA suggesting that it has discretion to apply whichever of the three versions of its guidelines that it would like. Having stated two months ago that the 1999 *Guidelines* are in effect until final guidelines are issued, EPA does not have the discretion to revert to a classification of evidence that does not even appear in the 1999 *Guidelines*. This point, and others relevant to application of the various EPA guidelines, are covered following discussions of substantive scientific issues below to which they apply.

1.3.2 OMB Guidelines

The Office of Management and Budget (OMB) recently issued *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies*. These *OMB Guidelines* require that "with regard to analysis of risks to human health, safety, and the environment maintained or disseminated by the agencies, agencies shall either adopt or adapt the quality principles applied by Congress to risk information used and disseminated pursuant to the Safe Drinking Water Act Amendments of 1996 (42 U.S.C. § 300g-1(b)(3)(A), (B)). 67 Fed. Reg. 369, 375 (Jan. 3, 2002). This standard directs EPA and other agencies, "in [any] document made available to the public in support of a regulation [to] specify, to the extent practicable -- (1) each population addressed by any estimate [of applicable risk effects]; (2) the expected risk or central estimate of risk for the specific populations [affected]; (3) each appropriate upper-bound or lower-bound estimate of risk; (4) each significant uncertainty identified in the process of the assessment of [risk] effects and the studies that would assist in resolving the uncertainty; and (5) peer-reviewed studies known to the [agency] that support, are directly relevant to, or fail to support any estimate of [risk] effects and the methodology used to reconcile inconsistencies in the scientific data." *Id.*

The *OMB Guidelines* further require that any important scientific or statistical information that an agency relies upon be reproducible and that its methods of analysis be transparent. The *Guidelines* require objectivity as well. Objectivity is defined as including a requirement that the information relied upon be "presented in an accurate, clear, complete, and unbiased manner," as well as involving a focus on ensuring accurate, reliable, and unbiased information. This generally means that the original and supporting data should have been generated, and the analytic results developed, using sound

statistical and research methods. Data and analytic results that have been subjected to formal, independent, external peer review may be presumed to be of acceptable objectivity. *Id.*

As in the case of the *EPA Guidelines*, examples of failure to comply with the *OMB Guidelines* are discussed where appropriate in the text below.

1.4 RECOMMENDATIONS

The draft Synthesis Document displays an unacceptable degree of bias and significant errors of interpretation, does not represent the application of standard scientific principles, and fails to meet standards clearly established in the applicable legal guidelines. As a basis for updating the IRIS database for TCE, it is clearly inadequate. The draft Synthesis Document must, therefore, be withdrawn for major revision. Recognizing the enormity of the task faced by EPA in rewriting the analysis, and the complexity of the scientific issues, it is recommended that a panel of experts be assembled to assist the Agency in a consensus effort to analyze and interpret the available data in an objective and unbiased fashion. It is further suggested that organizations such as Toxicological Excellence in Risk Assessment (TERA) or International Life Sciences Institute (ILSI) could provide the management to organize the proposed panel. HSIA remains available to assist EPA in its efforts, whichever direction is taken.

2. EPIDEMIOLOGY

2.1 CANCER CLASSIFICATION/WEIGHT OF EVIDENCE

There are many studies purporting to address the cancer epidemiology of TCE. The character of these studies is extremely varied: experimental design, number of subjects, extent to which known confounding factors have been addressed, and quality of the TCE exposure assessment are among the factors that differ. These, and many other elements, make analysis of the data base problematic in the absence of a clear and consistent association between a specific cancer type and TCE exposure. EPA has chosen to rely heavily on the "state-of-the-science" paper by Wartenberg et al (2000), an analysis commissioned by EPA in support of the TCE reassessment. Wartenberg et al sought to combine data from many studies. The studies were subdivided according to study type (cohort, case-control, or population) and, for cohort studies, the degree of specificity of exposure to TCE. Despite the efforts made to bring this complex database together, there are substantial concerns regarding Wartenberg et al's treatment and the conclusions EPA draws from it in the Synthesis Document. Important areas of concern are (1) the methods used to develop the risk averages, given the character of the data, (2) the selection of studies for inclusion in the calculations, (3) the failure of the evidence reviewed by Wartenberg et al to support an association between TCE and various cancers, and (4) the nonreproducibility to date of the Brauch et al (1999) result.

2.1.1 Derivation of Risk Averages by Wartenberg et al (2000)

The analytical methods employed by Wartenberg et al are reviewed in Attachments 1 (Kelsh et al) and 2 (Bloemen). In both these reviews, the failure to take into account the heterogeneity (in the statistical sense) of the epidemiology study results is identified as a significant weakness. The comments in Attachment 1 also address concerns that only limited consideration has been given by Wartenberg et al to exposure response information, and the absence of sensitivity and influence analyses. Wartenberg et al state that theirs is not a true meta-analysis, although meta-analytical techniques have been used, and acknowledge that failure to account for heterogeneity is a limitation. Other limitations are also identified and discussed by these authors.

Kelsh et al (Attachment 1) describe a number of significant abstraction errors and inconsistencies in the data used in the Wartenberg et al calculations. Dr. George Bonney (Professor, Microbiology Director, Statistical Genetics and Bioinformatics Unit, National Human Genome Center, Howard University), in his role as one of the two primary reviewers for TCE at the NTP Board of Scientific Counselors' Report on Carcinogens Subcommittee meeting of December 13, 2000, described Wartenberg et al's numerical treatment as "the kind of average that gives statistics a bad name" (transcript of BSC Subcommittee meeting). It is evident that the average risk values published by Wartenberg et al are based on incorrect data, are inaccurate, and have been derived by procedures that are inappropriate for the complex TCE database. The risk averages and their confidence intervals should not be used by EPA as evidence for associations between specific cancers and TCE exposure. Wartenberg et al do not rely entirely on the numerical outcome of their analyses to support their conclusions, since they also steer the reader towards the "consistency" of results. The validity of this "consistency" is discussed below.

Bloemen (Attachment 2) has also provided a comment stimulated by a recent addition to the Wartenberg et al review (Wartenberg and Scott, 2002).

2.1.2 Selection of Studies by Wartenberg et al (2000)

It is true that Wartenberg et al have identified virtually all epidemiology studies that could conceivably involve exposure to TCE. However, when casting the net this wide, it is necessary to assess critically the extent to which it is, in fact, possible to link observations with exposure to TCE. Thus the Tier III (also labeled Tier IV) cohort studies are of drycleaners or drycleaners and laundry-workers, and, although some of the members of these cohorts may have been exposed to TCE, the great majority will not have experienced significant TCE exposure at all. Even the Tier II cohort studies do not provide sufficient reassurance that TCE exposure occurred. For example, in one study (Sinks et al, 1992), the judgment that there had been exposure to TCE was based on the presence of a Material Safety Data Sheet (MSDS) for a product containing TCE.

Wartenberg et al made no attempt to grade the case-control studies on any basis, despite the fact that they are extremely heterogeneous with some studies more likely than others

to involve TCE exposures. In addition, it is almost impossible to ascribe findings in the population studies to TCE. Without a much more rigorous review of the characteristics of the case-control and population studies, they cannot be employed to support observations in the much more robust occupational cohort studies. One example of a case-control study that EPA uses in support of an association between TCE and kidney cancer is that of Dosemici et al (1999), which shows an apparent elevation in female workers (Odds Ratio 2.0, 95% CI 1.0-4.0). Dr S. Zahm (Deputy Director, Division of Cancer Epidemiology and Genetics, NCI), a primary reviewer for TCE during the NTP Board of Scientific Counselors Report on Carcinogens Subcommittee meeting of December 13, 2000, observed that ". . .confidence in it [the job exposure matrix employed by Dosemici et al (1999)] really starts to go down when you talk about individual solvents" (transcript of BSC meeting). Dr Zahm has extensive personal experience in epidemiology studies involving job exposure matrices.

For the reasons discussed above, the primary sources of epidemiological information for TCE are, of necessity, industrial cohort studies where reasonably reliable characterizations of exposures are possible. However, there are particular concerns regarding the use of one such study in Wartenberg et al's Tier I.

Comments on the Value of Henschler et al (1995a): Perhaps because the criterion of "the specificity of exposure" to TCE was used to assign cohort studies to tiers, Wartenberg et al included the study by Henschler et al (1995a) in Tier I. Discussions of kidney toxicity and carcinogenicity are important for TCE, and thus the Henschler et al (1995a) paper is significant, both as part of Wartenberg et al's analysis, and in its own right in EPA's Synthesis Document. The conclusions of the Henschler et al (1995a) study have been criticized by a number of scientists (Bloemen & Tomensen, 1995; Swaen, 1995; Attachments 2 and 3) and its inclusion in Wartenberg et al's Tier I has also been judged to be inappropriate (Borak et al 2000; Attachments 1 and 2). The fundamental concern is that the TCE-exposed kidney cancer cases in Henschler et al's cohort were drawn from a cluster of cases that had been already recognized; this has been acknowledged subsequently by the authors (Henschler et al 1995b; Vamvakas et al 1998). It is a general tenet in epidemiology that such a study can only be judged to be "hypothesis setting" and thus adds little or no weight in the assessment of the carcinogenicity of TCE. This was the reason that this study was not given any weight in the deliberations of the IARC Working Group (IARC 1995). It also means that the results of this study should not be used in meta-analyses or numerical combinations such as those of Wartenberg et al.

Concerns regarding the Henschler et al (1995a) study extend beyond the over-interpretation of a cluster-based investigation. The authors characterize this study of workers at a cardboard factory in Germany as a "retrospective cohort study" in which the incidence of kidney cancer in TCE-exposed workers was compared with that in unexposed workers and with cancer registry information from other countries (Denmark and the German Democratic Republic -- the plant was in the Federal German Republic). The study was of a small group of 169 workers in job areas regarded as involving exposure to TCE: locksmiths, electricians, and board machine areas. The in-house

control group consisted of 190 workers not exposed to TCE. There were no cases of kidney cancer in the control group (0.648 expected based on the Danish registry) and Henschler et al (1995a) reported 5 cases in the TCE-exposed group (0.628 expected). The resulting SIR of almost 8 appears to strongly support an association. However, there are certain modifiers to apply: One of the 5 cases was urothelial cell cancer of the renal pelvis, and this is histopathologically distinct (more akin to bladder cancer) from renal cell carcinoma hypothesized to be related to TCE. Of the remaining subjects, one had worked for only three years as an electrician, an area assumed to involve relatively low exposure, before diagnosis. Although there is a possibility that this case was linked to TCE, it seems unlikely. Thus there are probably only three cases that should be considered, and in the work group as a whole one case would have been expected. Another factor beyond country that may affect the number of cases detected is that abdominal sonography was employed in the factory as a screen for kidney tumors and this clearly is not the basis for incidence in cancer registries.

One reason given for the distinct difference between the findings from Henschler et al (1995a) and the other, larger, industrial cohort studies is that workers in the cardboard factory were exposed to very much higher levels of TCE than other workers. In particular, the board machine area was associated with pre-narcotic dose levels of TCE. Apart from the fact that only one of four renal cell cancer cases worked in the board machine area, it should be noted that the exposures in this area were periodic, not daily, and that high-level exposures occurred for 8 to 10 hours per month. This can be compared with exposures assessed by Stewart et al (1991) for a substantial number of subjects in the Spirtas et al (1991)/Blair et al (1998) cohort. These were probably approaching the high levels in the cardboard factory but were a daily event. The same high dose levels almost certainly occurred routinely for a significant number of workers in the Morgan et al (1998) cohort. The exposure differences have been analyzed by Cherrie et al (2001) and Bloemen (Attachment 2).

Why is the Henschler et al (1995a) study so significant? In the calculations of Wartenberg et al for the Tier I studies, the Henschler et al (1995a) study population contributes only 1.32% of the TCE-exposed population (1.97% of the overall study population) but increases the average relative risk by 74% (Borak et al 2000). For kidney cancer incidence, the average risk is 1.7 (95% CI, 1.1-2.7) with Henschler et al data, and 0.98 (CI, 0.58-1.66) without. Similarly, mortality data for kidney cancer in the Tier I cohort studies does not support an association with TCE exposure after omitting data from Henschler et al.

Comments on Vamvakas et al (1998). This case-control study is frequently paired with the Henschler et al (1995a) "cohort" study as providing evidence for an association between renal cell carcinoma and high levels of TCE exposure. However, substantial concerns have been expressed regarding the reliability of the findings. Among the concerns expressed have been the following: Test and control groups were drawn from different populations; there was a significant differential in age between test and control subjects (controls were younger); interviews were conducted by a physician fully aware of the test/control status of the interviewee; the classification of the magnitude of

exposure is suspect. These and other concerns have been explored in detail in Lash & Green (1999), Mandel (2001), Attachments 2 and 3. As for the Henschler et al (1995a) study, the high odds ratio for renal cell cancer has been attributed to unusually large exposures to TCE. In contrast, analyses by Cherrie et al (2001) and Bloemen (Attachment 2) show that the exposures would have been comparable to those in the large occupational cohort studies which do not support an association.

2.1.3 Robustness of "Associations" Identified by Wartenberg et al (2000) and EPA

According to EPA, an astounding range of tumor types are associated with exposure to TCE. These presumed associations are, without exception, improbable or simply not supported by the evidence.

Kidney Cancer. As discussed above, the epidemiological evidence, when critically assessed, does not support an association between kidney cancer and TCE exposures.

Non-Hodgkins Lymphoma (and Lympho-hematopoietic Cancers). As discussed by Mandel et al (Attachment 3) and Lash et al (Attachment 4), the pattern of incidence across Tier I cohort studies is typical of that where no association exists. An analysis by Bloemen (Attachment 2) of the Non-Hodgkins Lymphoma incidence in the most reliable studies shows that there is no association with TCE exposure in the aggregate.

Cervical Cancer. As acknowledged by EPA, this cancer was "sparsely reported" in Tier I cohort studies. Given the known etiology of the disease and the significance of socioeconomic factors in its incidence, it is unwarranted to link the observations to TCE exposure. Because significant TCE exposures are unlikely, the Tier III cohort studies of dry-cleaners and dry-cleaners and laundry workers cannot be used to support an association between cervical cancer and TCE.

Prostate Cancer. Although marginal elevations are seen in the Tier I studies. These are not sufficient to indicate an association with TCE exposure. The slight elevations cannot be attributed to TCE given the number of differences between workers and the general population with which they have been compared.

Liver and Liver/Biliary Cancers. Although marginal elevations were seen in several of the Tier I cohort studies, none was statistically significant. Overall, there were a small numbers of cancers in these categories and potential confounding factors were not considered. EPA claims that an association is supported by Anttila et al's(1995) observation that the relative risk is higher for those with the longest time since first exposure. However, there were only three cases in this category.

Similarly, the suggested increased risk with increasing potential TCE exposure and increasing time since first exposure reported by Ritz (1999) are based on a single case and cannot be used to support these relationships. Mandel et al (Attachment 3) and Lash et al (Attachment 4) show that the pattern of incidence of liver and liver/biliary cancers is compatible with there being no association between TCE exposure and increased risk.

2.1.4 Von Hippel-Lindau (VHL) Gene and Renal Cell Carcinoma

Background information relating to the VHL gene and tumor suppression in humans and experimental animals is provided in Attachment 5, written by Dr James Gnarra, a respected scientist in the VHL field. The human cases studied by Bruning et al (1997) were almost all included in the more extensive study reported by Brauch et al (1999). These are not mutually supportive studies (as suggested by EPA in § 3.4.3.2 of the Synthesis Document) and only Brauch et al (1999) is addressed here.

Multiple Mutations of the VHL Gene. Brauch et al (1999) reported finding a higher incidence of multiple mutations of the VHL gene in renal cell tumors from patients judged to have been exposed to TCE. As explained by Gnarra (Attachment 5), it is not surprising that mutations of the VHL gene were found to be present. However, Gnarra considers that multiple mutations are unlikely to confer a clonal advantage on cells. Thus it seems unlikely that multiple mutations would be an early event in the development of a tumor cell line. A more recent publication by Brauch herself (Brauch et al 2000) showed that the multiplicity of VHL mutations increases as the stages of the renal cell cancers advance. Brauch et al (1999) did not discuss the stages of the disease in the patients studied.

Mutational Hotspot. EPA has emphasized the Brauch et al (1999) finding of a specific mutation at nucleotide 454 in the VHL gene. This hotspot mutation was found in 13 of the 44 subjects considered to have been exposed to TCE. No similar hotspot was found in the tumors from a sub-sample of non-exposed patients. This is potentially a very important observation. However, Gnarra (Attachment 5) advises that there is a need to confirm the hotspot finding. Any new study must pay particular attention to the assessment of TCE exposure, comparability between exposed and unexposed subjects is necessary, and the physical state of samples and analytical procedures have to be matched -- all of these are of concern in the Brauch et al (1999) investigation. There have been "false alarms" regarding hotspot mutations in the past, and the first small-scale test (Schraml et al 1999) failed to confirm the Brauch et al (1999) observations.

2.1.5 Application of EPA Guidelines

EPA states (§§ 1.3, also 3.4, 3.6.1) that "the weight of the epidemiologic evidence [of TCE's potential carcinogenicity] has become stronger with the state-of-the-science analysis by Wartenberg et al (2000). It is surprising that EPA would make such a statement about a literature review, as opposed to new epidemiologic data. The Synthesis Document relies upon "association of TCE exposure with increased risk of human kidney cancer, liver cancer, lymphohematopoetic cancer, cervical cancer, and prostate cancer," along with animal data discussed below, to support a description of TCE as "carcinogenic to humans."

As discussed above, Wartenberg et al used a methodology that combined studies of greatly varying design, exposure, and other elements. As a result of the methodology used, and the inappropriate inclusion of the single cluster study by Henschler,

Wartenberg et al converted a set of cohort studies of TCE-exposed workers that does not show a statistically significant association with kidney cancer into new "evidence" of human carcinogenicity. Table 1 of Mandel (Attachment 3) shows the 95% confidence intervals for 8 studies of the association between TCE and kidney cancer in occupational cohorts. Seven of the studies do not show an association using the standard test for statistical significance -- "if the confidence interval is so great that it includes the number 1.0, then the study will be said to show no statistically significant association between the factor and the disease." *Brock v. Merrill-Dow Pharmaceuticals*, 874 F.2nd 307, 312 (5th Cir. 1989). In other words, the strongest epidemiological evidence relied upon by EPA shows a statistically significant association between TCE and cancer only when a cluster study that has been rejected by IARC and other reviewers is included. Such an analysis is inconsistent with the requirement in the 1999 *Guidelines for Carcinogen Risk Assessment* (pp. 2-8, 2-9) that "[a]ll studies that are properly conducted, whether yielding positive or null results, or even suggesting protective carcinogenic effects, should be considered and assessed in the totality of the human evidence."

In December 2000, the Board of Scientific Counselors of the National Toxicology Program reached a near-unanimous conclusion that TCE should not be considered a "known human carcinogen." (How EPA could interpret this (§ 1.3) as "making a case for a stronger classification in the future," as opposed to a clear rejection of the position in the Synthesis Document, is unexplained.) The Agency for Toxic Substances and Disease Registry (ATSDR), in its most recent Toxicological Profile Update, concludes (p. 151) that "[w]orkers who have been exposed to trichloroethylene show no higher incidence of cancer than controls in numerous epidemiology studies." Even IARC determined that the human evidence could be characterized as no more than "limited" (IARC 1995). The inconsistency between EPA's proposed characterization of the epidemiological evidence for TCE and conclusions reached by other regulatory and scientific authorities is due to EPA's failure to consider negative results and limitations in the data it characterizes as positive, in clear violation of the *Guidelines for Carcinogen Risk Assessment*.

2.1.6 Application of OMB Guidelines

The *OMB Guidelines* require that information relied upon by EPA (or other agencies) in a risk assessment be "presented in an accurate, clear, complete, and unbiased manner." Without repeating the discussion above, it is clear that EPA has not provided the reader an accurate description of the epidemiologic data base for the carcinogenicity of TCE. Any objective discussion of the data base would at the least have emphasized the findings of the authors of these studies. For example, Blair et al (1998) conclude that the relative risks observed do not implicate trichloroethylene as a cause of kidney cancer in their study. Yet, Blair et al and all the other studies that do not report a statistically significant increase in kidney cancer incidence are "combined" with Henschler et al to support the conclusion that "[c]onsistency is strongest for kidney cancer." This is not an objective and unbiased presentation of data. Most importantly, "significant uncertainties" that affect the conclusions reached by EPA are neither identified nor provided for public review.

As discussed above and in the attachments, the epidemiological evidence for the other endpoints is even more marginal, and EPA's conclusions based on these data even less supportable.

2.2 CALCULATION OF CANCER SLOPE FACTORS

It is astounding that EPA should consider any of the epidemiological information satisfactory for calculating cancer potency terms and of even greater concern that the values calculated are used to justify the range of slope factors identified for use by risk managers. Three sources of data have been employed:

2.2.1 Finnish Cohort Study (Anttila et al 1995 Plus Additional Information)

EPA's response to a request for information to permit reproduction and assessment of EPA's calculations based on this cohort did not, unfortunately, contain the necessary data to allow duplication of the results. Concerns regarding the inability to reproduce the calculations are discussed by a leading expert (Clewell) in Attachment 6.

There are other basic concerns regarding the use of Anttila et al's data for this type of calculation:

- The small number of tumors for each of the end-points (kidney and liver cancers, non-Hodgkins lymphoma). This is exacerbated by taking liver cancers 20 years or more after the first determination of urinary trichloroacetic acid (TCA). Any tumors due to confounding factors would have an undue effect on calculations.

- There are no data on duration of exposure available and thus a true measure of total exposure is not possible. In § 4.5.1.1, EPA states that the liver tumor incidence used in calculations was for workers "exposed for more than 20 years." This must be incorrect since exposure duration data was not available.

- The urinary TCA measurements averaged only 2.7 per worker and took place a relatively short time apart. Their timing suggests that they may have been relatively recent (lower TCE exposure levels likely in more recent times). The TCA measurements are "snapshots" and may not be representative of exposures under normal conditions (*e.g.*, workers take extra pains to avoid exposures on days of urine collection).

- It is not clear how kidney cancer incidence was used in the calculation because a deficit of kidney cancer was reported by Anttila et al (1995).

2.2.2 New Jersey Drinking Water Study (Cohn et al 1994)

There are many uncertainties regarding information in this study that make it unsuitable as a basis for calculating cancer slope factors. The true exposures to TCE are unknown and the populations were known to be exposed to other water contaminants. When

comparing human populations, there are likely to be many differences beyond the level of TCE in drinking water -- there are therefore many potential variables. The assumption, necessary for the calculation, that TCE exposure is the only relevant factor is inappropriate.

2.2.3 German Cohort (Henschler et al 1995a)

The shortcomings of this study have been discussed at length above. A study that cannot be used in qualitative assessments certainly is not suitable for the calculation of a cancer slope factor.

2.2.4 Application of Guidelines

Section 3.6 of the 1999 *EPA Guidelines* states that "[t]he exploration of significant uncertainties in data for dose and response and in extrapolation procedures is part of the assessment." Some of the most significant uncertainties in the epidemiological data are ignored in the risk estimates derived (§ 4.5.1) in the Synthesis Document. For the reasons discussed above, Henschler et al (1995) is not a suitable data set for quantitative assessment. The estimates based on Anttila et al (1995) and Cohn et al (1994) are equally unacceptable. The *OMB Guidelines* make clear that data which are significant to an assessment must be capable of being substantially reproduced. This means that "independent analysis of the original or supporting data using identical methods would generate similar analytic results, subject to an acceptable degree of imprecision or error." 67 Fed. Reg. at 378. As evidenced by the statement of Clewell (Attachment 2), it is simply not possible to reproduce the calculations in the Synthesis Document in the absence of access to the underlying data. Similar problems affect the reproducibility of the estimates based on Cohn et al (1994).

3. ANIMAL STUDIES AND CANCER

The Synthesis Document explores the underlying "modes of action" (MOA) for tumor types in order to address the relevance of animal tumors to man, the manner of interspecies dose response conversions, and the method of extrapolating from the experimental dose levels to low doses. Unfortunately, the Synthesis Document ignores or plays down existing evidence and MOAs that are well supported in favor of speculative and poorly supported hypotheses.

3.1 ANIMAL TUMORS AND MODES OF ACTION

3.1.1 Mouse Liver Tumors

It is generally accepted that metabolites are responsible for the induction of tumors in animal studies rather than the parent TCE, as EPA acknowledges. For liver tumors seen in susceptible strains of mice, the focus has been the TCE metabolites trichloroacetic acid (TCA) and dichloroacetic acid (DCA), both of which are known to induce mouse liver

tumors in their own right. EPA has recognized that the mouse liver tumors are unlikely to arise via a genotoxic MOA. Concern arises because of the emphasis that EPA places on the role that DCA plays in the development of mouse liver tumors.

At the October 1999 review of the first draft of the Synthesis Document, several "state-of-the-science" authors expressed the view that the role of DCA in relation to carcinogenicity had been overstated. This opinion is supported by published information and interpretations (*e.g.*, Merdink et al 1999; Barton et al 1999). In the event, EPA chose not to accept the recommendations of the state-of-the-science authors with respect to this issue. The evidence and its significance are reviewed in the analysis by Clewell and Bounds (Attachment 7). The reasons for considering that TCA, in conjunction with a peroxisome proliferation receptor related mechanism, is primarily responsible for induction of mouse liver tumors are founded in a range of experimental evidence and the low level of formation of DCA from TCE. Thus the heavy speculation regarding the role of DCA in section 3.5.1 of the Synthesis Document is inappropriate. Similarly, calculations of unit risk factors in section 4.5.2 that involve DCA are not compatible with current information and its interpretation.

As argued by Clewell and Bounds(Attachment 7), the emphasis in the Synthesis Document should have been placed on TCA and a mechanism involving interaction with PPARα. It is generally accepted that this should be considered a "non-linear" MOA for risk assessment purposes. However, there is a strong body of scientific opinion supporting the view that humans are unresponsive to peroxisome proliferators, at least as far as the factors that are probable contributors to the generation of rodent liver tumors. Chevalier and Roberts (1998) capture the general basis for this opinion and Walgren et al (2000) provide direct evidence for TCA and DCA. If this view is accepted (as it is by US FDA and European Union and Canadian authorities), the mouse liver tumors induced by TCE are of low concern for predicting human responses and should not be employed in calculations of risk. At the very least, EPA should recognize the non-linear character of the dose response relationship and the much lower responsiveness of humans than mice for this MOA.

3.1.2 Rat Kidney Tumors

It is generally accepted that small numbers of renal tumors are induced by TCE in some strains of rats. Non-neoplastic renal toxicity has been observed in all situations where increased renal tumor incidence has been recognized. A plausible hypothesis was developed that involves the conversion of the TCE metabolite dichlorovinylcysteine (DCVC) to reactive products by the enzyme β-lyase in renal tubular cells. This MOA was presumed to contribute to the non-neoplastic kidney toxicity and, directly or via prolonged damage, to tumorigenesis. In the Synthesis Document, EPA indicates strong support for this hypothesized MOA. However, it should now be recognized that, given the known properties of DCVC and its level of production from TCE, the proposed MOA does not fit the spread of experimental evidence. Green explores the evidence in Attachment 8 and concludes that TCE metabolism to DCVC cannot explain either the non-neoplastic kidney damage or the induction of tumors. In Attachment 9, Green tests

the DCVC-related MOA against EPA's own criteria for acceptance of an MOA for risk assessment purposes, and makes clear that the criteria are not met. EPA dismisses a recently developed hypothesis that involves the urinary excretion of large amounts of formic acid when rats are exposed to levels of TCE equivalent to those in the bioassay (Green et al 1998). Nevertheless, the experimental evidence indicates that sufficient formic acid is excreted to explain the observed non-neoplastic kidney damage, and the question as to whether this damage and continuous repair leads to development of the small number of tumors seen is an open question.

Since the DCVC/β-lyase MOA hypothesis does not appear to be supported by evidence in rats, even for bioassay dose levels, the lower production of DCVC (or precursor DCVG) and lower β-lyase throughput in man reduces the likelihood that this MOA is of significance to man (Dekant, 2001). EPA refers to the hypothesis that reactive chlorothioketenes produced from DCVC were responsible for the specific hotspot mutation seen by Brauch et al (1999). The suggestion is that the C to T mutation is the result of DNA adduct formation. However, Volkel and Dekant (1998) have found that chlorothioketenes from DCVC do not form stable adducts with cytosine in an aqueous medium.

3.1.3 Mouse Lung Tumors

Inhalation studies in some strains of mice have shown increased incidence of lung tumors in response to TCE. Other strains of mice and rats did not display this response, and there is no indication of an elevation of lung cancer associated with TCE exposure in epidemiology studies. Green (2000) in a "state-of-the-science" paper addressed the possible MOAs leading to mouse lung tumors. The most likely MOA is identified by Green and this involves the P450-rich Clara cells found in the lungs of mice and their responses versus the situation in rats and man. It is true that the evidence does not "prove" that this MOA underlies the mouse lung tumors, or that this is a "mouse specific response". However, all the information gathered to date is compatible with these conclusions. EPA should not reject this MOA in favor of less likely mechanisms.

3.1.4 Rat Testicular Tumors

The Synthesis Document refers to an increase in benign testicular interstitial (Leydig) cell tumors in a gavage study in rats of the Marshall strain. In fact, this was a positive trend test only, and the dose levels in this study almost certainly exceeded the maximum tolerated dose. Also, this strain displays a very high and variable spontaneous incidence of these tumors. In the same study, Osborne-Mendel rats, which display a low spontaneous incidence of Leydig cell tumors, showed no response. The other study used as a positive indicator was the inhalation study by Maltoni using Sprague-Dawley rats. EPA leaves the impression that this was a typical 104-week study. The Maltoni protocol was one of dosing for 104 weeks but then maintaining animals for their lifetime, for as long as 159 weeks in this case. Leydig cell tumors are regarded as tumors of the aging rat -- the average latency in the Maltoni study was approximately 110 weeks. It has to be questioned whether the increased incidence of Leydig cell tumors seen by Maltoni were a

direct action of TCE or secondary to prolonged general toxicity and, effectively, an acceleration of a process normally taking place with aging. The significance for man of rat Leydig cell tumors has been debated (Cook et al 1999), and the pattern of incidence for TCE certainly suggests low concern. Incidence of this benign tumor should not be used as evidence of the carcinogenicity of TCE in man, or to calculate a "risk-specific dose" for cancer.

3.1.5 Mouse Lymphomas, Lymphosarcomas and Reticulum Cell Sarcomas

According to EPA the sarcomas were just appearing in female B6C3F1 mice after 90 days dosing of TCE by gavage (NCI 1976). However, the results were not statistically significant and the TCE contained mutagenic stabilizers. As numerous reviewers have recognized, these data carry minimal weight. Malignant lymphomas were increased in female B6C3F1 mice in a gavage study involving a single dose but this did not achieve statistical significance. Only in the inhalation study by Henschler et al (1980) was a statistically significant trend demonstrated for lymphoma in female NMRI mice. Indeed, Henschler et al (1980) concluded that the experiments did not show tumorigenic potential for TCE, recognizing that lymphoma is a common spontaneous tumor with a high and variable incidence in female mice.

EPA uses these animal data to support a role for TCE in the induction of human leukemia. This link is not apparent in the robust cohort studies, which casts doubt on the increase seen in a small number of population studies. The Agency also mentions the most recent study of the Woburn, MA, childhood leukemia cluster (Mass DoH, 1997). This is an example of a recognized cluster being included in a study -- a relationship was bound to be established for some parameter and thus can only be regarded as "hypothesis setting". In this case, leukemia in children seems to be elevated for women potentially exposed to contaminated drinking water (TCE was one of the contaminants). This relationship was not statistically significant. Many of the uncertainties regarding exposures at Woburn and their interpretation were explored by EPA (EPA-ORD, 1988). The ORD Workgroup concluded not only that TCE could not be identified as a cause of leukemia but "[i]t cannot be determined if leukemia and drinking water in the community are causally associated."

It is clear that EPA is stretching to establish epidemiological associations and to find supporting data in animal studies. The result is unconvincing.

3.1.6 Application of Guidelines

The 1999 *Guidelines* (§ 2.6.1) require that "each relevant study must be reviewed and evaluated as to its adequacy of design and conduct as well as the statistical significance and biological relevance of its findings." They provide that "[a]dditional information bearing on the qualitative assessment of carcinogenic potential may be gained from comparative pharmacokinetic and metabolism studies," which help elucidate potential modes of action and biological fate and disposition. TCE is a compound for which a wealth of pharmacokinetic and metabolic data are available. The 1999 *Guidelines*

(§ 1.3.2.2) also make clear that "[i]f adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent *per se*, then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent." Finally, the *EPA Guidelines* require corroboration of results in animal studies in two or more appropriate species.

EPA's discussion of the animal studies in the Synthesis Document is inconsistent with the 1999 *Guidelines* in several ways. Alternative mechanisms for the mouse and liver tumors caused by TCE exposure have been postulated for at least 20 years. The Synthesis Document should discuss these alternative mechanisms and should not use a linear default approach. Similarly, several postulated modes of action for the small increased incidence of kidney tumors seen in some studies have been advanced. These are more consistent with the available biological evidence than the genotoxic MOA hypothesized by EPA. As to mouse lung tumors, the Synthesis Document largely ignores the state-of-the-science chapter by Green (2000) which provides a persuasive rationale for why the effects seen in the Clara cells in mice lungs were not seen in rat lungs and would not be seen in human lungs. Finally, the data concerning increases in rat testicular tumors, prostate cancer, and mouse lymphomas would not seem to be of sufficient strength to meet the requirements of the *Guidelines* for strength of evidence.

3.2 CALCULATIONS OF RISK BASED ON ANIMAL DATA

3.2.1 Mouse Liver Tumors

Although it is an entertaining exercise, the exploration of biologically based risk assessments (Chen, 2000) does not provide acceptable methods for calculating slope factors useful to risk managers. This is explored further by Clewell (Attachment 7). For the Synthesis Document, concerns arise both because of the contrived manipulations of the calculation methods as well as the data that are input. As discussed above, DCA is given too much emphasis in relation to TCE toxicity and this is particularly evident in the calculations of slope factors based on mouse liver tumors. It should be noted that the PBPK treatments of Clewell and Fisher include the older overestimates of DCA production from TCE that have been acknowledged by their authors (Merdink et al 1999; Barton et al 1999). The PBPK models should be adjusted to accommodate the new information. Much is made of the difference between the Fisher and Clewell PBPK models and the "improvements" introduced by Bois (2000a and b). Concerns expressed by Clewell and others regarding the Bois treatment have been analyzed and developed by Hays (Attachment). It is recommended that the PBPK models for TCE be updated and that any apparent differences between models be examined at a biological level before statistical treatments are applied, with input from the scientists responsible for the PBPK models.

As discussed above, there are reasons to believe that mouse liver tumors induced by TCE are not relevant to man. If EPA feels compelled to use mouse liver tumors in risk calculations, the approach should be to use a non-linear treatment based on TCA acting via PPARα and linear treatments should not be included. In its current non-linear

treatment, EPA allows for humans to be three-fold less responsive than mouse to a given level of TCA; this factor should be increased. The uncertainty factor for human variability of 50 - 60 is unreasonably large and is driven by differences between PBPK models. It is recommended that the PBPK models be updated and that differences be resolved at the biological level before statistical treatments are applied. If statistics are used, the scientists responsible for the PBPK modeling should participate.

3.2.2 Mouse Lung Tumors

The discussion of the role of chloral hydrate and Clara cells in the induction of lung tumors is rational. This is an area where the lack of response in rats could be factored in and a numerical comparison with the results of epidemiology studies could be informative.

3.2.3 Rat Kidney Tumors

This is clearly an area for further development. It does appear that the evidence is moving away from the involvement of a genotoxic mechanism. Thus treatments founded on cytotoxicity are appropriate. However, the extrapolation of rat data to humans will require a rational assessment of TCE's ability to cause kidney damage in man.

3.2.4 Rat Testicular Tumors

As discussed above, the Leydig cell tumor incidence for TCE is of low concern for man and should not used as a basis for calculation of risk estimates.

4. CARCINOGENICITY CLASSIFICATION

According to the 1999 *Guidelines for Carcinogen Risk Assessment* (§ 2.6), EPA will conduct:

> "[a] weight-of-evidence . . . evaluation of all pertinent information so that the full impact of biological plausibility and coherence is adequately considered. Identification and characterization of human carcinogenicity is based on human experimental data.
>
> . . .
>
> Judgment about the weight-of-evidence involves considerations of the quality and adequacy of data and consistency of responses induced by the agent in question. The weight-of-evidence judgment requires combined input of relevant disciplines."

The *Guidelines* require use of standard descriptors as a way to summarize the biological evidence. These descriptors are:

- Carcinogenic to humans

- Likely to be carcinogenic to humans

  - Suggested evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential

  - Data are inadequate for an assessment of human carcinogenic potential

  - Not likely to be carcinogenic to humans

Amazingly, the draft Synthesis Document provides a description of the weight of evidence for the carcinogenicity of TCE that does not even appear in the 1999 *Guidelines*. The draft Synthesis Document states (§ 3.6.2) that "TCE could be described as 'carcinogenic to humans'" and that "[a]lternatively, TCE could be described as 'likely to be carcinogenic to humans'." Rather than attempt to classify TCE under these guidelines, the draft Synthesis Document opts for "a strong characterization as 'highly likely to be carcinogenic to humans'." As described above in the discussion of the epidemiology, it has been recognized by all reviewers, including the NTP Board of Scientific Counselors and ATSDR, that TCE does not meet the standard for classification as "carcinogenic to humans." In apparent recognition of this fact, EPA creates a new weight-of-evidence category descriptor. This is not authorized under the *Guidelines*.

EPA's semantic efforts to exaggerate the carcinogenic potential of TCE in the draft Synthesis Document are reminiscent of previous efforts by the same office within EPA to subvert previous classification systems. For example, when EPA's classification system used the descriptors "probable" and "possible" human carcinogen, and the data did not support classification in the former category, EPA invented the terminology "close to a probable human carcinogen" specifically to apply to TCE. This effort was rejected on several occasions during Science Advisory Board reviews in the 1980s. It is unfortunate that EPA continues to engage in such transparent efforts to overstate the available data. It is also directly inconsistent with the *OMB Guidelines* discussed above.

The strongest classification that EPA could conceivably support under the 1999 *Guidelines* is "likely to be carcinogenic to humans." Any other classification would violate the *Guidelines* by failing to take into account the available information, discussed in the attachments and by the state-of-the-science chapter authors, that show that TCE causes tumors in certain laboratory animals by mechanisms that are unlikely to be relevant to humans. Most significantly, any stronger classification would fly in the face of the available epidemiology, which (1) IARC has concluded constitutes at most "limited evidence" for the carcinogenicity in humans of TCE, (2) other reviewers, including the NTP Board of Scientific Counselors and ATSDR, agree shows that workers who have been exposed to TCE show no higher incidence of cancer than controls, and (3) has been relied upon by the American Conference of Governmental Industrial Hygienists (ACGIH) for classification of TCE as "not suspected as a human carcinogen" "on the basis of properly conducted epidemiologic studies in humans." ACGIH, 2001 *Threshold Limit Values for Chemical Substances and Physical Agents*, 68. The review of the data in

the Synthesis Document does not even discuss the conclusions reached by such other scientific and governmental reviewers, much less provide a rationale for rejecting it. The "highly likely to be carcinogenic to humans" characterization of TCE should be rejected.

5. NON-CANCER ENDPOINTS

The analysis of non-cancer endpoints in the draft Synthesis Document is at odds in many respects with the state-of-the-science chapter on the subject by Barton and Clewell (2000). EPA should explain the rationale for the rejection of the authors' conclusions, which led to most of the non-EPA authors disassociating themselves from the Synthesis Document. As now written, the Synthesis Document contains an uncritical collection of positive results. The studies yielding these positive results are not evaluated by EPA and studies presenting contrasting evidence are, in most cases, totally ignored. The result is, of course, an unscientific and biased treatment.

5.1 NEUROTOXICITY (§ 3.4.1)

Considering that neurotoxicity is a significant effect attributable to TCE and that it forms the basis for the calculation of EPA's RfC, the review of neurotoxicity information in the Synthesis Document is surprisingly brief. Important studies have been omitted and there is no evaluation of other studies which are referenced. More than one third of the brief assessment of neurotoxicity is taken up with information on the properties of DCA. The small amounts of DCA generated from TCE (rodents and man) cannot be of concern for neurotoxicity. This analysis should be expanded and the more important studies should be critically reviewed.

5.2 LIVER TOXICITY (§ 3.4.2)

Many more studies, human and animal, could have been brought into this section. The increase in liver weight in rodents should have been discussed in the context of peroxisome proliferation and related effects. The minor changes reported at low dose levels in man, if real, appear to be adaptive changes; frank toxicity at higher levels would have been a more important situation for a proper review. It should be noted that the results in the papers by Goh et al 1998 and Chia et al 1997 are based on a single study in which comparability of exposed and unexposed workers is questionable. The control population differed from the exposed workers with respect to ethnicity, diet and lifestyle.

5.3 KIDNEY TOXICITY (§ 3.4.3)

Once again, a more complete review of all the evidence is essential. It is by no means certain that humans experience kidney damage as a result of occupational exposures to TCE. There are a number of reports where no response has been found, and those studies reporting effects are, in some cases, open to question. Much more is known about responses of the rodent kidney to TCE in the short, medium and long term than has been displayed here.

5.4 IMMUNE AND LYMPHO-HEMATOPOIETIC SYSTEMS (§ 3.4.4)

A brief comment on this section has been provided by Dr Holsapple of The Dow Chemical Company (Attachment 11). It indicates that all the human studies cited have weaknesses and should be investigated before being used to support the conclusion that TCE has immunotoxic effects. Discussion of how the autoimmune disease-prone mice relate to the human population is necessary to put results from this animal model into perspective. A number of well-conducted animal studies that did not display positive results should be discussed (*e.g.*, NTP, 1992 a, b, c; White et al 2000).

5.5 DEVELOPMENTAL TOXICITY AND REPRODUCTIVE TOXICITY, ENDOCRINE EFFECTS (§ 3.4.5)

EPA has included a higher proportion of available studies than for other end-points but, once again, the review is superficial. As the developmental studies are extremely varied in design, a skimming of the results is insufficient. Also, as for other end-points, EPA has concentrated on studies with apparent positive results while completely ignoring studies reported to have been negative. The study referred to as Graeter et al has now been published (Fisher et al 2001) and made available to EPA. This study employed TCE and metabolites each at a high dose level administered by gavage to CD rats. Fetuses were examined for cardiac anomalies in a highly sophisticated technique. Also submitted to EPA recently was the report of a guideline inhalation developmental toxicity study in CD rats which showed no developmental effects at up to 600 ppm of TCE. This study was sponsored voluntarily by HSIA to fill an ATSDR Priority Data Need and the study design and final report were fully peer-reviewed. These two recent state-of-the-art studies showed no developmental effects of TCE. They should be examined given great weight in a more complete and insightful review of the potential developmental effects of TCE.

The information regarding endocrine and reproductive effects of TCE is not easily interpreted and requires a thorough analysis before conclusions can be reached. It has already been mentioned that the multiple effects reported by Goh et al (1998) and Chia et al (1997) are open to question because of the lack of matching controls. These results have to be confirmed in an independent study with appropriate controls. As for other end-points, negative studies have been completely ignored.

5.6 APPLICATION OF GUIDELINES

Two key deficiencies underlie the treatment of non-cancer endpoints in the draft Synthesis Document. First, there is no reference to the EPA *Guidelines for Reproductive Toxicity Risk Assessment*, *Developmental Toxicity Risk Assessment, or Neurotoxicity Risk Assessment*, although they have long since been adopted by EPA and are intended to guide evaluations by EPA of reproductive and developmental and neurotoxicity data. Second, the discussion illustrates a disturbing tendency of the authors of the draft Synthesis Document to ignore voluminous reported scientific data that bear directly on

the subjects under discussion. This obviously is inconsistent with each of the foregoing guidelines as well as the *OMB Guidelines* discussed above. The deficiency is highlighted in this case by the fact that the state-of-the-science chapter by Barton and Clewell on non-cancer endpoints provides a thorough review of the data. A simple comparison of that chapter and the draft Synthesis Document shows that the authors of the Synthesis Document were apparently motivated by a desire to reach conclusions that exaggerate the potential toxicity of TCE for each of these endpoints, while ignoring data that would be inconsistent with such conclusions.

6. CALCULATIONS OF RfD AND RfC, AND TREATMENT OF UNCERTAINTY

6.1 UNCERTAINTY IN PBPK MODELING

The statistical underpinning to the Bois (2000 a and b) treatment of the PBPK models is generally accepted. However, the specific manner in which this approach was applied to the Fisher and Clewell PBPK models for TCE appears to have created some highly undesirable results (Hays, Attachment 10). Involvement of the scientists responsible for the PBPK models in the development of the statistical treatment would appear to be highly desirable in order to avoid such distortions in the final product.

6.2 CALCULATION OF THE RfD

The oral reference dose has been calculated using the point of departure of 1 mg/kg-day based on liver effects in rodents. This point of departure seems low and may reflect adaptive changes, rather than toxicity. However, major concerns arise because of the application of multiple uncertainty factors which EPA "limits" to 3000-fold (after developing a case for 5000-fold). Using the same point of departure, Barton and Clewell (2000) arrived at an RfD more than two orders of magnitude higher than the draft Synthesis Document. In EPA's treatment there are two uncertainty factors of concern: The 50-fold factor relating to the "average-sensitive human" and the notion that the uncertain exposures to metabolites should lead to a 3-fold factor. The large factor of variation introduced by the PBPK modeling should have been *resolved*, not *magnified* by a statistical treatment. Allowance for highly variable exposure to metabolites is unacceptable because it introduces additional layers of conservatism into what is required to be an objective assessment. That is not to say that these metabolites should not be taken into account, but that the RfD must reflect the properties of TCE itself. It should be remembered that long-term mouse and rat studies were run with dose levels up to 1000 mg/kg-day and survival at doses at or below 500 mg/kg-day was unaffected. Also, workers were exposed each working day to inhalation dose levels at, or close to, levels used in rodent bioassays without any harm becoming evident in the extensive epidemiology studies.

6.3 CALCULATION OF THE RfC

As for the RfD, EPA shows an exaggerated conservatism in the selection of uncertainty factors in the calculation of the reference dose for inhalation. Applying a 1000-fold uncertainty factor to marginal effects in humans is unwarranted scientifically. In this example, Barton and Clewell (2000) derive an RfC that is 100 times higher than that of EPA. Given human experience of high level exposures, a composite factor of 30 would appear to be more than adequately protective.

6.4 HUMAN VARIATION AND SENSITIVITY/SUSCEPTIBILITY (§§ 1.6 and 3.3)

EPA suggests that the 50-fold range that has been recorded for CYP2E1 activity in humans will translate into large inter-individual responses to TCE. EPA also rejects the view that differing levels in enzyme activity would make little difference to TCE metabolism (Kedderis, 1997). In fact, this issue was discussed at the meeting in October 1999 at which the state-of-the-science authors reviewed an initial draft of the Synthesis Document. The authors responsible for the PBPK models (Fisher and Clewell) agreed, and explained to EPA, that the metabolism of TCE is "diffusion limited," meaning that a 50-fold difference in CYP2E1 activity translates into a very much lower factor of difference in the rate of TCE metabolism. This also plays into EPA's speculation that disease states, alcohol intake or exposure to other chemicals might alter enzyme activity, in turn affecting sensitivity to TCE -- the effects clearly could not be as great as suggested in any realistic exposure scenario. See Clewell (Attachment 7) for further review of the significance of disease states.

6.5 DIFFERENTIAL RISKS TO CHILDREN

On behalf of the producers of TCE, HSIA has committed to a long-term program of TCE testing and exposure assessment pursuant to agreements with the Agency for Toxic Substances and Disease Registry (ATSDR) and EPA as part of the Voluntary Children's Chemical Evaluation Program (VCCEP). Significantly, this program will result in peer-reviewed assessments of potential exposure of children to TCE and the potential impacts of any such exposure. The TCE risk assessment should not prejudge these issues by making overly conservative and unrealistic assumptions about children's exposure and using default approaches to complex mechanistic information.

The pharmacokinetic differences between children and adults that appear to be relevant to TCE may not automatically translate into a greater sensitivity. For example, TCE received in drinking water may be less readily metabolized but more readily exhaled in children than adults, resulting in *lower* sensitivity. In this area, there is sufficient information available now for a more detailed analysis.

The section on pharmacodynamic differences between children and adults is pure speculation as far as neurotoxicity or cancer are concerned. As discussed above, the

Mass DoH (1997) should not be interpreted as showing an effect of TCE, particularly in light of conflicting EPA interpretations of the same data.

7. PROCESS COMMENTS

EPA followed a novel approach to the development of the TCE risk assessment. As we understand it, this included a series of meetings of interested parties and experts which led to development of "state-of-the-science" chapters by authors in various scientific disciplines. These chapters were reviewed and published and form the basis for the discussion in the Synthesis Document. The Federal Advisory Committee Act (FACA) (5 U.S.C. App.) imposes a number of requirements on the use by federal agencies of advisory committees. "Advisory committees" include any "committee, . . . panel, task force, or other similar group which is . . . established or utilized by one or more agencies, in the interest of obtaining advice or recommendations for . . . one or more agencies or officers of the Federal government." *Id*. § 3(2). By its terms, this statute would appear to apply to the scientific experts convened by EPA to advise in the development of the TCE risk assessment. EPA should carefully review whether it has complied with the requirements of the Federal Advisory Committee Act in its efforts to date to develop a comprehensive assessment of the health risks of TCE. If EPA has not satisfied the requirements of FACA, it should immediately withdraw the draft Synthesis Document and ensure that the scientific input from outside EPA into the TCE risk assessment fully complies with FACA by providing an opportunity for all members of the public to participate.

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Critique of the Wartenberg et al. Review on the Epidemiologic Evidence of TCE Carcinogenicity

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December 31, 2001

Introduction

EPA's assessment of the epidemiologic evidence of the carcinogenicity of trichloroethylene (TCE) relied heavily on the review paper by Wartenberg et al. (Wartenberg et al., 2000). The Wartenberg et al. paper provided a summary of results for a number of cancers from different types of studies including occupational cohort studies ("Tier I" and "Tier II" studies), cohort studies of dry cleaner and laundry workers (Tier III studies), case-control studies of kidney and liver cancer, and community-based studies (mostly lymphatic and hematopoietic cancers). In this review of the epidemiologic literature on TCE the authors attempted to summarize an extensive body of research by incorporating more studies than previous reviews. They concluded that there was an excess risk for cancers of the kidney, liver and cervix, and for non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma which they attributed to TCE. However, a careful review of the methods they used reveals a number of problems with their analyses that must be addressed before any conclusion can be reached regarding a causal relationship.

In this report, we review the analytical methods used by Wartenberg et al. Although Wartenberg et al. do not claim that they conducted a meta-analysis, results for Tier I and Tier II studies are summarized using meta-analytic techniques. However, their conclusions are based only on a partial meta-analysis of the epidemiologic studies. For example, for TCE and kidney cancer, simple influence analyses presented in this report and tests of heterogeneity show that the results are largely driven by one study (Henschler et al., 1995) and are not consistent across studies. This example demonstrates the limitations in the Wartenberg et al. analysis. Similar limitations apply to other cancers.

What is a Meta-Analysis and How is it Conducted

Meta-analysis is a statistical summary of studies on a specific topic. The unit of analysis is the study, with the source of data usually being the published results. The key elements of a meta-analysis include:

- Specifications of the exposure and disease outcome

- Identification of all relevant studies on the exposure and disease of interest
- Abstraction and compilation of individual study findings and if necessary, a recalculation of individual study findings to conform to a "standard" summary of the literature
- Assessment of homogeneity of findings across the studies to determine the appropriateness of combining the results using fixed-effects models (i.e., simple weighted averages)
- Statistical procedures for calculating meta-analysis relative risks
- Assessment of potential biases: publication bias, lack of adjustment for confounding factors, selection bias, or other biases (e.g. exposure misclassification)

Quantitative summaries of epidemiologic studies such as meta-analyses have advantages over narrative reviews in that they can detect a small, but significant, effect that might otherwise go unnoticed because individual studies lack the statistical power to demonstrate these effects (Greenland, 1998). However, limitations of individual studies (such as bias and confounding) and qualitative differences among studies (such as differences in case definitions) are lost with quantitative summaries alone and, therefore, narrative summaries which discuss these differences and limitations should complement any quantitative analyses. Sensitivity and influence analyses often identify "outlier" studies that merit additional scrutiny. Such is the case for the TCE literature.

Specification of Exposure and Disease Outcome

Wartenberg et al. identified studies with probable TCE exposure, however as they noted, many of the cohorts had other workplace exposures as well. Also noteworthy is the fact that TCE exposures have changed significantly over time, so the comparison of one study to another can be inappropriate if the levels of TCE exposure are vastly different (which is probable). The Swedish study (Axelson et al., 1994) and Finnish study (Anttila et al., 1995) provide data on a biomarker of TCE exposure, urinary trichloracetic acid (U-TCA), a metabolite of TCE, whereas the remaining studies did not have quantitative exposure information. The case-control studies include a variety of different qualitative exposure

surrogates collected using different methodologies, typically job histories from in-person interviews and mailed questionnaires. Therefore, any interpretation of the results from these studies must be made cautiously taking into account the different methods of "exposure" ascertainment and the heterogeneous exposures that have been lumped together.

Wartenberg et al. also did not focus on a few specific cancer outcomes; rather they attempted to summarize nearly all of the cancers that had been reported on in the studies. This has several consequences: 1) the inability to adequately address each of the cancer outcomes and discuss the characteristics of individual studies that could explain the observed heterogeneity, and 2) potential problems of multiple comparisons when so many disease/exposure findings are presented.

Identification of Relevant Studies

It appears that Wartenberg et al. identified all the relevant studies. The methods they used (Medline searches and review of previous studies and published reviews on the topic) are typical of most meta-analysis studies. They did not identify any additional unpublished results, although it was not their stated objective to do so. A recent Danish study (Hansen et al., 2001) published after the their review would likely be classified into the Tier I category. This study assessed exposure through the use of the U-TCA biomarker and records of air measurement from 803 Danish workers, and linked these workers to information from the Danish cancer registry. Hansen et al. reported statistically significant elevations for non-Hodgkin's lymphoma, cancer of the esophagus, and cervical cancer based on 8, 6, and 4 cases respectively. However, because of the lack of a dose-response relationship, the small number of cases involved and potential confounding by other risk factors for these cancers, the authors concluded that these data did not provide sufficient evidence for a causal association. This study found no increased risk for kidney cancer based on 3 observed cases and 3.3 expected cases.

Abstraction of Relevant Results from Identified Studies

In epidemiologic studies, results are often presented in various forms such as "crude" results, results adjusted for confounding factors or results for a subset of the study population, and in some cases, dose-response findings. In abstracting results from each study for the meta-analysis, it is important to be consistent in selecting the findings. Based on a preliminary review in which we compared the abstracted results used in the Wartenberg et al. paper and the results reported in the original studies, we found several inconsistencies and errors. For kidney cancer, the Blair et al. study of Utah aerospace workers (Blair et al., 1998) reported a standardized mortality ratio (SMR) of 1.22 and a rate ratio (RR) of 1.6. Wartenberg et al. selected the RR of 1.6 although they label it as an SMR. For liver cancer, Wartenberg et al. repeated the error that was made for the Blair et al. kidney cancer data (SMR was 1.15, and the RR was 1.7) and reported the RR of 1.7 as an SMR. On the other hand, Wartenberg et al. elected to use the SMR of 1.32 for the TCE exposed cohort from Morgan et al. and not the RR of 1.6 for an internal cohort analysis (cumulative "high" TCE exposed group). Arguments can be made for using either the internal cohort analysis or the SMR analyses, but the selection of results into the meta-analysis should be consistent. Furthermore, a rationale should be provided as to why one result is selected over another when more than one result is published in the source article.

There were also errors in abstracting results from the study of aerospace workers (Boice et al., 1999). Boice et al. reported an SMR for multiple myeloma of 0.9 (Table 8 in Boice et al.). Wartenberg et al. incorrectly used an SMR of 2.8 for this study. Because only two mortality studies contributed to the overall average risk of 1.9 for the Tier I group (which was the only statistically significant finding for multiple myeloma in Tier I and Tier II findings), this error has important implications for the interpretation of the multiple myeloma finding. The average risk of 1.9 for this group would be reduced to approximately 1.1 if the correct data had been abstracted from the Boice et al. paper. Wartenberg et al. did not report any findings for cervical cancer from the Boice et al.

study, however, Boice et al. reported an SMR of 0.7 (based on 17 cases) among the women working in aircraft maintenance or repair. This is the largest number of cases among the Tier I and Tier II studies and would have had an impact on the average risk for the Tier I studies. The average risk of 1.8 as reported by Wartenberg et al. would be less than 1.25 (the unweighted average) with the inclusion of the data from the Boice et al. study.

In reporting non-Hodgkin's lymphoma, Wartenberg et al. used the category "other lymphatic and hematopoietic cancers and lymphosarcomas and reticulosarcomas" from the Morgan et al. study. However, the same data were not abstracted from the Garabrant et al. study, which included data grouped into the same categories of "other lymphatic and hematopoietic cancers and lymphosarcomas and reticulosarcomas" (Table IV, Garabrant et al., 1988). The SMR for this combination of outcomes would be 0.8 with 18 observed cases in this cohort. This would slightly decrease the summary average risk reported by Wartenberg et al. More importantly, it raises concerns about the completeness, consistency and accuracy of the data abstraction procedures applied in the Wartenberg et al. review.

Another important consideration in the interpretation of the average risk across studies is an assessment of dose-response and a determination and acknowledgement if such trends provide support or refutation of the average risk estimates. For kidney cancer Wartenberg et al. reported suggestions of a dose-response from the Morgan et al. and Wong and Morgan studies (which are analyses of the same cohort) and no evidence from the Boice et al., Anttila et al. and Blair et al. studies. These inconsistent results should be considered as important factors when evaluating the weight of evidence, especially considering the problems of heterogeneity and the strong influence of one study (Henschler et al., 1995) on the overall results.

The above examples are not drawn from a comprehensive review of all data used in the Wartenberg et al. review, yet they highlight a number of inconsistencies and errors that merit further investigation. From just a cursory review, we identified several issues that

could affect the overall data interpretation for several of the cancers, which Wartenberg et al. concluded are associated with TCE. The inconsistencies and errors noted from our cursory review, mainly from Tier I studies, indicate that data used in the Wartenberg et al. paper should be carefully checked before accepting and using the results to develop regulatory policy.

Statistical Procedures and Assessment of Heterogeneity

Assessment of Heterogeneity

The inverse variance weighting method used to calculate summary effect estimates is a common meta-analysis procedure that adjusts for the different sizes of studies, weighting those with more subjects more heavily than smaller studies. However, this method does not address the heterogeneity of findings among studies. There is an ongoing debate regarding what should be the appropriate goal of a meta-analysis: estimating an overall average risk or identifying important differences in levels of risk across studies and trying to explain these differences (Greenland, 1994a, Greenland 1994b, Shapiro, 1994). Central to this debate and the process of establishing study objectives for the meta-analysis, is the assessment of heterogeneity. If substantial heterogeneity is detected among the studies selected for analyses, then the assumption about a common effect across all populations is unrealistic and argues against summarizing across all studies. Under a scenario of heterogeneity, one goal should be to identify important subgroups of studies and summarize these separately. In the concluding paragraphs of their report, Wartenberg et al. recommended a meta-analysis "to focus carefully on the possible heterogeneity among studies". Despite the lack of such an analysis, Wartenberg et al. concluded that the studies reviewed had consistent results and that summary risk estimates demonstrated strong evidence of carcinogenicity for several cancer outcomes. However, the inconsistent results across studies argue against such a conclusion.

Random Effects Meta-Analysis

In meta-analysis there are two methods for combining data to estimate a single summary effect. The fixed-effects approach assumes that each study is estimating the same value for the effect size. In this case, the effects estimated were the rate ratio (RR), standardized mortality ratio (SMR), or standardized incidence ratio (SIR). In the fixed-effects approach, the only source of variation in each study's estimate is that attributable to within-study random variation. Standard statistical tests can be used to judge whether study estimates are likely to meet this assumption. For example, a chi-square statistic is often calculated over all k studies, which is a weighted average of the difference of each study's effect from the common (pooled) effect from all k studies. Each study's weight is the reciprocal of its sampling variance.

$$Q = \frac{\sum_{i=1}^{k} w_i \frac{(O-E)^2}{E}}{\sum_{i=1}^{k} w_i}$$

If k is sufficiently large, this statistic is distributed according to the chi-squared distribution with k-1 degrees of freedom. A large p-value (more than 5%) is an indication of homogeneity among the k studies.

An alternative approach, the random effects model, assumes that the effect size estimated by each study is actually a random selection from a population of effects with some mean effect size and variance. That is, the actual effect size being estimated by each study varies according to a pre-specified statistical distribution. Use of random effects models, indicated by significant heterogeneity across studies, generally does not change the summary point estimates substantially, but may increase the width of the associated confidence intervals, reflecting the additional variability due to effect size heterogeneity.

This procedure does not overcome the basic problem of heterogeneity across studies; it simply reflects the additional uncertainty about the existence of a common underlying risk level.

From the perspective of epidemiologic studies of TCE-exposed persons, without further stratification, it is likely that the underlying effect size being estimated by each study is a random selection from a population of effect sizes. Application of a fixed-effects model in this setting can lead to spurious conclusions. In an attempt to demonstrate the effect of different analytic approaches, we recalculated some meta-analytic rate ratios using both fixed-effects and random-effects models. Unless stratification produced homogeneous groups of studies (as evident by the chi-square test), we used a random effects approach to summarize SMRs and SIRs from the TCE literature. Random effect models were implemented using the statistical software SAS.

Sensitivity and Influence Analyses

Sensitivity analyses evaluate the impact on findings resulting from different procedures, assumptions and classification of data. *Influence analyses* examine the effects of individual data points on overall summary estimates. A repeated calculation of meta-analytic estimates implemented by excluding single studies one at a time is called influence analyses. The results of a meta-analysis should be robust and not influenced by the inclusion or exclusion of single studies. If exclusion of a single study drastically changes the conclusion of the meta-analysis, that study may be an outlier, or the underlying assumptions of estimated effects may not fit the model being used to summarize the data. In either case where one study has a significant influence, it highlights the limitations of the research data; usually pointing to limited statistical power, and indicates the need for caution in interpreting the summary meta-analysis findings.

To demonstrate the importance of these techniques and highlight why they should be part of a meta-analysis or review of TCE studies, we conducted sample sensitivity and influence analyses for TCE and kidney cancer studies for Tier I and Tier II studies (Table

1). Several aspects were examined. First, is it appropriate to combine SIR and SMR results for Tier I studies? In many ways the studies are similar except for the fact that one set relied on cancer incidence data and the other on cancer mortality data. Cancer mortality studies often are reasonable at estimating risk for some cancers, particularly those with a high case-fatality rate. Second, what is the influence of single studies on the summary results? Based on examination of the likely outlier results from the Henschler et al. studies, we excluded these two studies (SMR and SIR) from the meta-analytic summaries (Table 1). We also calculated a random effects summary as well as the fixed-effect summary. The results clearly show the strong influence of the Henschler et al. studies on both SIR and SMR results. The summary relative risk estimates without the Henschler studies included are both close to unity (1.0), indicating no association between TCE and either kidney cancer incidence or mortality. We also observed the large change in homogeneity statistics (chi-square), which switched from significant ($p<0.05$), suggesting heterogeneity, to p values ranging from 0.4 to 0.6, suggesting homogeneity. In combining SIR and SMR studies or Tier I or Tier II, we noted that the chi-square statistic is significant, indicating heterogeneity, but when the Henschler et al. studies are excluded, the p values increase dramatically, suggesting combining across these studies could be considered.

The problems with the Henschler et al. study of cardboard factory workers have been discussed in a recent review (Mandel and Kelsh, 2001). Briefly they include: designing a study around a cluster, potential problems with the matching of an unexposed group, possible miscalculation of follow-up time, questionable smoking data, as well as a number of other issues. There were four exposure groups in the Henschler et al. study: 1) board workers (highest exposed), 2) locksmiths, 3) electricians and 4) non-exposed. Groups 1 to 3 were considered to have been exposed to TCE. Only one of the four original kidney cancers was from the board workers group. The limited exposure information, small size of the study, the fact that it originated from a cluster, and other serious study design problems call into question the classification of the study as a Tier I study. According to Wartenberg et al. the criteria for Tier I studies included the presence of individual TCE exposure assessments and “well-designed epidemiologic studies”.

Neither criterion is met by the study of the cardboard factory workers (Henschler et al., 1995).

These influence and sensitivity analyses are presented only as examples that demonstrate the limitations of the Wartenberg et al. analyses. Such analyses should be conducted systematically for all relevant cancer outcomes, with influence analyses that assess the impacts of exclusion of all studies (one at a time). We selected the Henschler et al. studies only for demonstration, but studies with low SIR and SMR estimates should also be examined. Based on the funnel plot (Figure 1), we would expect much smaller impacts from these studies than the Henschler et al. study.

Although characterized as "consistent findings" by Wartenberg et al., there are numerous instances of inconsistencies for the cancer outcomes reviewed. For kidney cancer, there were differences between incidence and mortality studies (however these differences were substantially reduced when the Henschler et al. study results are excluded). For non-Hodgkin's lymphoma, there were no statistically significant results reported from cohort studies (with summary estimates ranging from 0.9 – 1.5); however, two case-control studies (Hardell et al., 1981, 1994) reported odds ratios of 4.6 and 7.2, much higher than all of the other case-control studies and the cohort studies. These studies had serious methodological problems that were not addressed by Wartenberg et al. For cervical cancer, only one study reported a significant result from among all of the Tier I and Tier II studies. This finding was based on eight cases. For the Tier I and Tier II study classifications, Wartenberg et al. reported only one study in each group that included findings for cervical cancer. Because all of the studies had only a handful of cases interpretability of these data was limited. In addition, as noted earlier in this report, Wartenberg et al. omitted the cervical cancer findings from one of the Tier I studies (Boice et al., 1999).

These examples of heterogeneity of findings across studies and study types again highlight the need for a more complete review and, where appropriate, a meta-analysis of

the TCE cancer studies. Conclusions about carcinogenicity that are based on partial and incomplete analyses and limited studies are not appropriate.

Assessment of Potential Biases

Wartenberg et al. discussed the potential limitations in their analyses including 1) limited exposure assessment, 2) lack of data on exposure-response trends, 3) lack of adjustment for confounding factors, and 4) the rarity of diseases under study (Wartenberg et al., 2000). They concluded that, despite these limitations, the consistency of results suggested that the limitations were not significant enough to have a substantial effect. As we noted in our discussion of heterogeneity above, the results are not consistent for kidney cancer as shown by the statistical tests for heterogeneity, nor are they consistent for cervical cancer and non-Hodgkin's lymphoma, obvious differences of the findings across different studies and different study types. In addition, for liver cancer, for which Wartenberg et al. also concluded that there was strong evidence of an association, there was also significant variation in study results across the different study types. Results where more studies were included in the summary and more cancers observed, tended to have lower summary effects estimates, especially considering the combined category of liver/biliary cancers (rather than only liver). This finding suggests a "regression-to-the-mean" effect, in which a single or pair of studies initially report an association but subsequent studies fail to confirm the association. In this case, it is likely that the initial studies were in the upper part of the expected distribution of study results, and subsequent studies "regressed" back to the true, underlying mean value. Although it would be desirable to be able to only look at liver cancer – a majority of studies classified liver/biliary cancers into one group, which could not be analyzed separately.

Publication Bias

Combining data in meta-analysis makes the assumption that the studies being summarized derive from an unbiased sample of all possible studies. In this setting, studies with small sample sizes should generally yield point estimates with a large degree of scatter due to random variation alone. In contrast, large studies should tend to cluster around a single mean (presumably the true population mean) or a set of means

representing a distribution of true effects due to differing exposures or populations being studied. Constructing funnel plots in a meta-analysis can be a useful exercise in studying publication bias. In general, it is thought that small studies with negative or null effects may be less likely to be published. In this case, smaller published studies should show the tendency towards higher effect sizes. Plotting study weight (reciprocal of variance) on the y-axis vs. study estimate on the x-axis should yield a distribution in the shape of an upside-down funnel (small mouth at the top, large trumpet at the bottom). Gaps or asymmetrical shape of the funnel plot suggest potential publication bias. Whether the plots conform to the expected funnel shape can be judged "by eye" or with a statistic that tests for the asymmetry of the funnel plot (Elvik, 1998). Usually the bias will appear in the region of small (i.e. studies with large variation) negative studies. Funnel plots can also help identify outlier studies. However, these plots should be interpreted cautiously with consideration of how they are constructed (Tang and Liu, 2000). As an example, we generated a funnel plot for the Tier I and Tier II studies (Figure 1). The Henschler et al. studies (particularly the SIR finding) clearly stand out by being far removed from the rest of the studies, which form a cluster around the rate value of 1.0. This leads us to re-evaluate the pooled common effect without the Henschler et al. studies (Table 1). The findings for females in the Blair et al. study also standout relative to the rest of the studies. The relative risk of 3.6, which was based on 2 observed cases when 0.6 cases were expected, was not found among males where 2 cases were observed and 5 were expected. Overall, there were 4 cases observed and 5.6 expected in this cohort.

Clusters and Meta-Analysis

Many authors have written about the validity of including a "cluster" in a meta-analysis. Including a cluster would be appropriate if the cluster resulted from a planned study, but not if a study followed the report of a cluster and included the actual cluster. If not, the cluster more likely represents a chance occurrence of an effect size drawn from the "upper end" of any particular distribution. Any study designed around a cluster that included that cluster is likely to find positive results. What would not be included – mostly because such reports are unlikely to be published - would be an "anti-cluster" or the chance occurrence of a deficit of cases in any particular occupational setting. Since

such "anti-clusters" are unlikely to be reported, including the reported clusters artificially biases a meta-analysis. Even for published studies of occupational cohorts, a specific cancer may not be elevated or may not have occurred at all in the cohort and thus may not be presented in the published manuscript. These types of findings are missed in the meta-analysis study procedures unless the investigators contact the authors of the original studies and obtain the unreported results. If the cluster is included, at a minimum, influence analysis should be done, studying the effect of excluding the cluster to determine the extent to which the cluster drives the result.

Summary

Wartenberg et al. conducted a partial meta-analysis and acknowledged some of the many limitations of their approach. Despite the lack of consistency across studies, Wartenberg et al. concluded that the epidemiologic research provides "evidence of excess cancer" for kidney, liver, non-Hodgkin's lymphoma, cervical cancer, Hodgkin' disease and multiple myeloma. This is not the same conclusion reached by others who have reviewed published reviews of the epidemiologic studies (Weiss, 1996, McLaughlin and Blot, 1997, Morgan et al., 1998).

We identified some significant problems in the Wartenberg et al. article including: 1) inconsistencies and errors in the abstraction of data from the original studies, 2) limited consideration of exposure response information, 3) lack of, and inappropriate, assessment of heterogeneity both in terms of exposure data and study findings, and 4) absence of sensitivity and influence analyses. Without a complete and properly conducted meta-analysis along with an objective interpretation of the meta-analysis results, the conclusions reached by Wartenberg et al. are not justified.

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Table 1. Example Sensitivity and Influence Analyses for TCE and Kidney Cancer Derived from Wartenberg et al Review[2]

Computation of Average[1] Rate for Kidney Cancer

Group of Studies	Tier(s)	Fixed Effects Rate (95% CI)	Random Effects Rate (95% CI)	Heterogeneity p-value[3]
SIR(Including Henschler)	I	1.73 (1.07, 2.65)	1.66 (1.03, 2.54)	0.001
SIR (Excluding Henschler)	I	1.04 (0.60, 1.70)	-	0.395
SMR(Incl. Henschler)	I	1.15 (0.81, 1.59)	-	0.303
SMR (Excl. Henschler)	I	1.04 (0.73, 1.45)	-	0.577
SIR & SMR(Incl. Henschler)	I	1.38 (1.05, 1.78)	1.50 (1.14, 1.94)	0.002
SIR & SMR (Excl. SIR Henschler)	I	1.11 (0.83, 1.45)	-	0.441
SIR & SMR (Excl. SIR & SMR with Henschler)	I	1.04 (0.78, 1.37)	-	0.666
SIR	II	N/A[4]	-	-
SMR	II	1.29 (0.93, 1.76)	-	0.641
SIR & SMR (Incl. Henschler)	I & II	1.33 (1.08, 1.61)	1.39 (1.13, 1.69)	0.017
SIR & SMR (Excl. SIR Henschler)	I & II	1.20 (0.97, 1.46)	-	0.917
SIR & SMR (Excl. SIR & SMR with Henschler)	I & II	1.16 (0.94, 1.42)	-	0.808

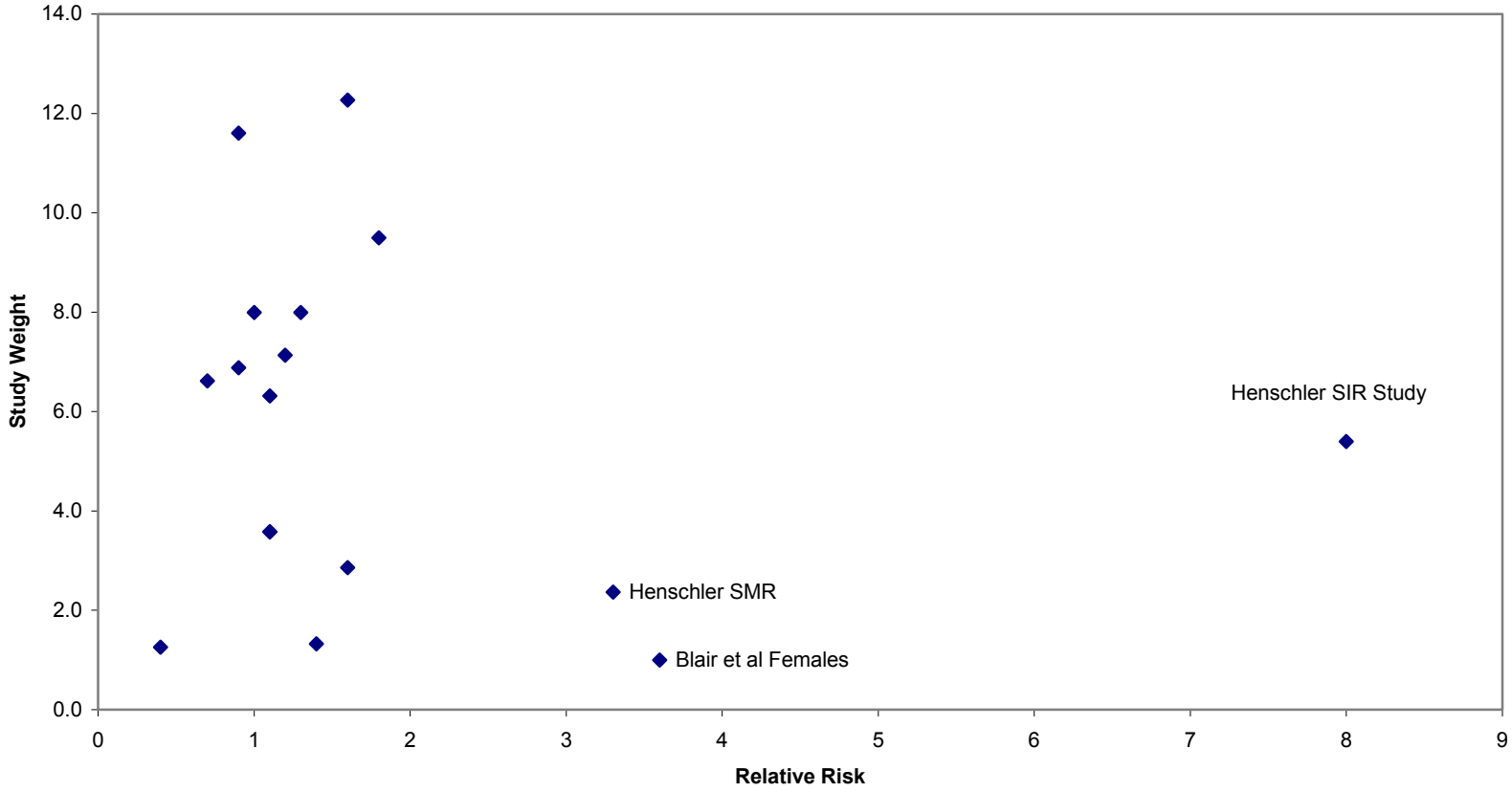
[1] Based on Meta-Analysis Techniques for Fixed and Random Effects Models

[2] Trichloroethylene and Cancer: Epidemiologic Evidence; D. Wartenberg, D. Reyner, CS Scott, 2000;

[3] Based on a Chi-Square test for homogeneity

[4] Only one study reported (Sinks, et al.)

Figure 1. Funnel Graph For Kidney Cancer
SIR & SMR Tier I & II Cohort Studies



Main points of concern on the National Toxicology Program Draft Background Document for Trichloroethylene.

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The criteria for listing Agents, Substances or Mixtures in the Report on Carcinogens stipulate that consideration should be given to all relevant information. The background document completely and systematically ignores the descriptions of historical worker exposure, given extensively for instance in the IARC Monograph No. 63 or the publication by Stewart et al. (1991). In doing so, the document does not meet the criteria for an NTP Background Document. Scientific judgment based on this document can easily be flawed, as is demonstrated below.

The plausibility of the risk of 10 for renal cell cancer seen in the studies by Henschler et al (1995) and Vamvakas et al (1998) depends on the argument of exceptional exposure levels experienced by the study populations. There is ample information the literature which makes clear that use of trichloroethylene described in these studies was consistent with contemporary procedures in general. Hundreds of thousands of workers have been using trichloroethylene this way for many years (IARC 1995). A risk of this size cannot be missed and certainly would have shown up, if not in registry based studies. In reality, no increased risk for renal cell cancer has been seen after eighty years of intensive use of trichloroethylene.

The background document extensively quotes the meta-analysis publication by Wartenburg et al. (2000). This quotation however is biased by only mentioning the results, and ignoring the careful comments from the authors. The comments and suggestions show clearly that the results of the analysis were not definitive, even in the authors' eyes.

The approach used to aggregate the results of the different epidemiological studies used by Wartenburg et al. (2000) is inappropriate for this situation. Many of the required considerations for meta-analysis that were defined in an earlier paper (Blair et al 1995) that includes Wartenberg as a co-author, are ignored here. Wartenberg et al. do not address heterogeneity or differences in exposure levels between studies, but only weigh studies according to precision, which is related to cohort size. Cohort size is only one of the determinants of study relevance. Wartenburg's analysis ignores the negative results revealed by the internal analyses in these studies: risk related to duration of exposure or dose. These omissions make the meta-analysis incorrect and misleading.

The large cohort studies compare disease or mortality patterns in the work force with patterns seen for the general population at country or at best, at state level. The small differences seen in these comparisons are much more credibly explained by bias and confounding, because of comparing unmatched groups, than by exposure to trichloroethylene. The lack of consistent patterns when looking for association with duration of exposure or level of exposure, makes this point very clear.

Completeness of information.

The Criteria for listing Agents, Substances or Mixtures in the Report on Carcinogens by the U.S. Department of Health and Human Services national Toxicology Program, as given in the Draft Background Document for Trichloroethylene, clearly state:

"Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information."

The Background Document explains the use of trichloethylene in seven lines (2.1), indicates an annual production capacity in the US of 160,000 tons in a certain year (2.2) and gives a table which indicates that 400,000 workers were potentially exposed frm 1980 to 1983, 90,000 in the electric and electronic equipment industry and 30,000 while manufacturing fabricated products. This information underlines the point that trichloroethylene is a very important solvent and has been used extensively.

In contrast, no description of use of trichlorothylene and no measurements of workers' exposure are given. Not that this information does not exist. The IARC Monograph on trichlorothylene mentions the results of occupational air and biomonitoring results in reported in a large number of papers.

The systematic ignoring of information on exposure goes so far that the separate publication on the exposure situation in the most important cohort where highest exposures have occurred (Blair et al. 1998) has not even been mentioned and does not appear in the literature list (Stewart et al. 1991). This indicates a clear lack of understanding by the authors of the Background Document of the role of exposure in the interpretation of epidemiology studies.

Clear understanding of the historical use of a chemical and the exposures related to this allows the findings of the occupational studies to be put into context. The lack of this insight prevents readers of the Background Document from getting the proper perspective and to arrive at sound scientific judgement on the carcinogenicity of trichloroethylene.

Historical use of trichloroethylene and levels of exposure

In a discussion on the health effects of exposure to trichloroethylene (TRI), it is important to consider historical use of TRI, and what exposure levels this resulted in.

The extensive information contained in Patty's Industrial Hygiene and Toxicology and the 1995 IARC Monograph 63 on Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals serve as the main source for the following description.

Commercial production of TRI started in 1920's in Germany and the US. It has been produced at a large scale in many industrialised countries. Manufacturing quantities range from 277 thousand tonnes in 1977 in the US and 210 thousand tonnes in Western Europe in 1980, to 60 thousand tonnes in Canada in 1976.

The major use of trichloroethylene is in metal degreasing. It is an excellent solvent for organic matter, it is non-flammable and relatively inexpensive (Grandjean 1955). Degreasing is important in all metalworking and maintenance operations to remove oils, greases, waxes, tars and moisture before final surface treatments such as galvanizing, electroplating, painting, anodizing and application of conversion coatings. Trichloroethylene is used in degreasing operations in industrial groups such as fabricated metal products, electric and electronic equipment and transport equipment.

Metal cleaning operations are of two types: cold cleaning and vapour cleaning. In cold cleaning, trichloroethylene is applied at room temperature; in vapour degreasing, the solvent vapours are condensed on the part to be cleaned. In cold cleaning, metal parts are either dipped in to the solvent solution or the solution is sprayed and wiped onto the object. The cold process is frequently used in maintenace operations and on small parts. The manufacture of metal parts using a lathe, includes frequent dipping of the part into trichloroethylene to remove the cutting oil before measuring its dimensions. Trichloroethylene subsequently evaporates from the parts. Vapour degreasing requires a tank with heating coils on the bottom and a condensing zone near the top. The solvent is heated to boiling (87 ^{0}C), and the hot vapour fills the condensing zone near the top of the tank. Soiled objects are lowered into this zome, where the vapour condenses into a pure liquid solvent on the piece and dissolves and carries off dirt as it drains into the tank. The part dries immediately in the air. The tanks started to get equipped with cooling rings at the top reduce loss of the solvent and reduce exposure at the end of the 70's. Lids were also introduced to obtain closed machines, which are the rule nowadays. The effect of these measures can be seen on the annual use of the solvent in the US for metal cleaning: in 1971 trichloroethylene use for metal cleaning was 200,000 tonnes, this reduced to 84,000 tonnes in 1980.

Exposure levels up to the 80's had average levels of 60 ppm for eight hours and peaks often up to 400 ppm but occasionally up to 1000 ppm (IARC 1995). The TLV applied in the US in the 50's was 200 ppm, and in the UK it was 400 pm (Grandjean et al. 1955).

The smell of trichloroethylene has been described as not unpleasant, etherlike and deliberate inhalation of vapors by workers has been reported repeatedly. At room temperature, air saturation with trichloroethylene occurs at 70.000 ppm. Laboratory experiments have indicated that no effects are seen in man at exposures up to 100 ppm, marginal effects at 200 ppm and slight effects above 300-400 ppm. Eye and nasal irritation are the main adverse effects at or above these levels while CNS effects occur at levels of 1000 ppm or higher.

All this indicates that historical use of trichloroethylene has resulted in considerable levels of exposure for the workers to trichloroethylene. The inclusion of 106 cases of trichloroethylene poisoning in the Finnish cohort of 2198 workers studied by Tola et al (1980) and later by Anttila et al.(1995), and 32 cases of poisoning by trichloroethylene reported during the study period by Malek et al. (1979) underlines this point. Fatalities from industrial exposure to trichloroethylene might be expected to have occurred in this time period and, indeed, a number have been reported (for overviews see Von Oettingen 1955, NIOSH 1973).

This is the background against which the cohort studies must be seen. In In the studies by Axelson (1995) and Anttila (1995), the cohorts were defined based on lists of participants in biomonitoring in during respectively 1955-1975 and 1965 –1982. This ensures inclusion of only people with the potential exposure to trichloroethylene. The investigators then used the results of the biomonitoring program to estimate exposure for the working period of the members of the cohort and conclude exposure levels have been rather low, less than 20 ppm for most of the Axelson cohort. This clearly is incorrect, only a few samples have been collected for each cohort member, no sampling procedure is given while the working period before the start of the study is ignored. In particular against the background of the way trichloroethylene was used, it is more resonable to expect that exposure of the two cohorts was much higher than assumed by the investigators. The inclusion of 106 cases of poisoning by trichloroethylene in the Anttila paper supports this notion.

Morgan et al. (1998) rate exposures low medium and high where work on degreasing machines was classified as high with exposures stated to be above 50 ppm. Boice at

el. (1999) only describe use of trichloroethylene for vapour degreasing. For the initial study of the cohort reported on by Blair (Spirtas et al. 1991), extensive historical exposure assessment was performed which resulted in a separate publication (Stewart et al.1991). In the report, regular cleaning jobs were reported with exposure levels up to 600 ppm, in accordance with what is described above on usage of trichloroethylene.

The only other studies on trichloroethylene which give detailed information on exposure are the studies by Henschler et al (1995) and Vamvakas et al (199 . After identifying a cluster of kidney cancer cases in a cardboard factory descriptions of working practices 20 years ago involving bi-weekly cleaning of machinery using trichloroethylene must have impressed the investigators. They failed to note that cardboard factories are notoriously unpleasant to work in because of the formation of hydrogen sulphide along with other organic sulphur compounds, all of which have an unpleasant, strong smell. The most common of these are methylmercaptan, ethylmercaptan, dimethylsulphide and hydrogen sulphide (IARC 1981). These circumstances are a better explanation for the compliants of the workers. The use of trichloroethylene for the bi-weekly cleaning job, done according to practices normal for that time period, will undoubtedly have contributed to the feelings of distress of the workers involved, but there is no reason to expect the working situation in the cardboard factory to be very different from that in other industries in that period. This is confirmed by historical reassessment of exposures in the publications on cohorts exposued to trichloroethylene by Cherrie et al (accepted for publication, J Clin Oncol and Cancer Research).

Vamvakas et al. (1998) expand the assumption of extremely and uncharacteristically high exposure levels from the cardboard factory to the area of the source population for their study, the area around a country hospital in North Rhine Westphalia, without any justification. They explain that exposures took place predominantly in small premises decades ago. There will undoubtedly have been high exposures amongst the study subjects but there are no reasons to expect the exposure situation to be very different from that of other users.

The argument that very high exposures explain the very aberrant findings in these two studies is not based on any data. Problems with study design and reporting made Adami and Trichopoulos (submitted to NTP) decide that these studies should not considered for regulatory evaluations.

Meta-analysis

Meta-analysis, other than for clinical trials, still is a subject of often heated discusison amongst epidemiologists. Calculating overall risk estimates based on combined raw data, pooling of data, or averaging risk estimates for individual studies is not contentious for experimental clinical trials because of good documentation, very similar study design and dosing strategies for these studies while minumal risk exists for bias and confounding because of the randomized study design.

No concensus exists on methods to obtain a numerical expression of risk seen in observational studies. Many epidemiologists consider that attempting to obtain a numerical results often requires multiple assumptions and therefore the outcome is misleading. The meta-analysis by Wartenburg et al. (2000) has generated an overall risk estimate with confidence intervals for several end-points, based on 7 cohort studies. These studies report on 20,000 workers exposed to TRI since the 50's. Others have reviewed these studies and concluded they concur with a null effect (Mandel et al. submitted for this meeting), while others have made very clear that the studies by Henschler et al. (1995) ad Vamvakas et al. (1999) are of such a low standard that they should not be considered for risk evaluation (Adami & Trichopoulos submitted for this meeting).

The discrepancy between the overall findings by Mandel et al and Wartenburg et al. (2000) can easily be explained by the inappraprate method used by Wartenburg et al. Wartenburg is co-author of a recent paper on meta-analysis in environmental epidemiology (Blair et al. 1995) where a group of experienced epidemiologists give advice on how to conduct these studies. Wartenburg et al. mention some of the considerations from this paper, but in practice many important considerations are completeley ignored.

The main concern when merging study results is that of adding apples and oranges, adding studies that are different. Blair et al. state: “consideration of heterogeneity is central to the decision of whether summary statistics should be produced in a meta-analysis and if so, how they should be produced (e.g. by stratifying the studies by the source of heterogeneity and conducting separate meta-analyses on the different subgroups)”.

The paper by Wartenburg et al. shows no attempt to analyze heterogeneity, so heavily emphasized in his own earlier paper. To calculate the summary statistic, they use the inverse-study-variance method which is based on the the assumption of homogeneity.

Differences in exposure levels experienced by the different cohorts, would be a very good reason not to add studies. Indications for important differences are that Ritz et al. where trichloroethylene is used as a solvent, report only low to moderate exposures while the paper, focussed on exposure assessment of the aircraft maintenance cohort suggests jobs with regular exposure levels up to 600 ppm.

By ignoring these differences, Wartenburg et al. develop an incorrect and misleading summary statistic.

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December 12, 2000

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EPA TRI HEALTH RISK ASSESSMENT/WARTENBERG REVIEW

The first statement by the EPA in the section on lymphatic cancer and leukaemia (section 4.5.3) is the following:

> *Overall, there is an increased, but not statistically significant, risk of NHL (RR=1.5, 95% CI=0.9-2.3, N=22) (Wartenberg SOS).*

This one statement and the basis of its derivation, illustrates all that is wrong with both documents. I have made the following observations on the way that Wartenberg erroneously arrived at that figure.

1. The figure is based only on cancer incidence from Wartenberg's Tier I studies.
2. The inclusion of results by Wartenberg is inconsistent.

Anttila (8 NHL cases)	All subjects exposed to TRI are included (men and women combined).
Axelson (5 NHL cases)	A result is only given for male subjects. Females constitute 14.4% of cohort and Axelson notes that there were no cases of lymphoma in women.
Blair (7 male, 2 female NHL cases)	Results only given for subjects in the highest cumulative exposure group (>25 units – y). A further 12 males in the other TRI exposure categories developed NHL (RR < 0.9[*]) and 1 female NHL case (RR < 0.6[*]). Results are given separately for men and women.

3. The weighting system used by Wartenberg to combine results from the studies unfairly penalises Blair, because the Rate Ratio in this study is calculated by comparing incidence among workers with exposure to TRI to those with no exposure to chemicals. Technically, this is a better comparison than that employed by Anttila or Axelson,

[*] Results not given for combined group.

where the comparison groups are the Finnish or Swedish populations. However, because the populations of Finland and Sweden are considerably larger than the group of unexposed chemical workers in the Blair study, the standard error of the Rate Ratios calculated by Blair are much larger than the standard errors of SIRs in the Scandinavian studies. The standard error in the Blair rate ratio (7 NHL cases) is 5.84 times that of the SIR of Anttila (8 NHL cases) and 3.52 times that of the SIR of Axelson (5 NHL cases). This means that it gets relatively little weight in the combined estimate of relative risk.

4. The weighting system only takes into account the standard error of the relative risk estimate. Consequently a small study of very highly exposed workers gets less weight than a large study of workers with low exposures.

5. A crude combination of the observed and expected from the 3 studies gives the following:

	Obs	Exp
Anttila	8	4.44
Axelson		
Males	5	3.13
Females	0	? (0.5 approx)
Blair		
Males		
>25 units-y	7	7.00
5-25 units-y	4	5.71
<5 units-y	8	8.89
Females		
>25 units-y	2	2.22
5-25 units-y	0	?
<5 units-y	1	1.67
	35	33.06*

Overall, there is little to suggest that there is an association between TRI exposure and NHL cancer incidence. Other information on dose response etc adds further weight to that view, although NHL mortality was elevated in both males and females in the Blair cohort.

* Does not include expected figures for females in Axelson or females with 5-25 units-y in Blair study.

Additional comments on hazard characterisation in EPA risk assessment of trichloroethylene.

Wartenberg and Siegel-Scott (2002) have recently published an addition to the State-of-the-Science paper on the epidemiology of trichloroethylene (1). In the original publication, studies on trichloroethylene exposed workers were divided in tiers, according to certainty of exposure (2). For each tier a summary risk was calculated for the disease of interest by dividing the sum of the observed cases by the sum of the expected cases. Studies were weighted by the inverse variance. The Tier I studies all present analysis of risks for these working populations in comparison with the general population.

One should realize that the diseases of interest all are relatively rare, and in many cases, one is confronted with scarce data and the related, rather wide, random scatter of results around the null value. Focusing on a few of the many results reported in the studies could cause the reader to forget the overall picture of randomness of the results.

A large number of publications have shown that risks for disease are not evenly distributed over socioeconomic strata. Differences in choice of food and in personal habits, as well as differences in access to medical services, are among the explanations given for these findings. Therefore, it should not be a surprise that some consistency can be seen between all these comparisons between working populations and the general population. Wartenburg et al. again make these differences clear in extensive tables.

To tease out any risk factors related to the working environment, additional analysis is required which can highlight dose-effect and latency aspects in relation to the exposure of interest. Risks related to an exposure will increase with increasing dose and latency. For this purpose, groups are identified which differ in exposure intensity, cumulative dose, or time since first exposure, to name a few possibilities. When evaluating the Tier I studies for this aspect, again a random pattern appears, indicating, the exposure of interest, trichloroethylene, is unlikely to be the explanation for the differences seen in the overall analysis.

It is remarkable that Wartenburg et al consistently ignore this last aspect in their analysis, and in their new publication continue the inappropriate practice of just adding up observed (in the working population) and expected (in the general population) from the different studies. It seems for their purpose, the authors of the different studies could as well have finished their work and their article after having calculated and presented the table with the overall results.

It may be unfortunate that at first glance there is no easy way to integrate the results from the internal analysis (exposed versus unexposed workers in the same company), but this does not make the method used now more valid. This unsophisticated work should not be the basis for EPA's hazard identification in humans, in the Trichloroethylene Risk Assessment.

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A Review of the Epidemiologic Studies of Trichloroethylene and Kidney Cancer and with Reference to Liver Cancer and non-Hodgkins Lymphoma

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Summary and Conclusions

On balance the epidemiology studies of workers exposed to TCE do not support a causal relationship between TCE and kidney cancer. Seven occupational cohort studies involving over 130,000 workers consistently show no significant increase in the risk of kidney cancer. The study by Henschler et al. has so many methodological problems that no valid conclusion is possible. The case-control study by Vamvakas et al. also has so many design flaws that it cannot be given any consideration.

Causal inferences from epidemiologic studies are generally based on several criteria including, 1) Strength of the association; 2) Consistency of the association; 3) Temporality of the association; 4) Coherence of the association; and 5) Specificity of the association. Other evaluation criteria are the quality of the exposure assessment, the absence of confounding and bias, and the statistical uncertainty in estimating the risk ratio for the outcomes of interest.

Based on these criteria, it is clear that the available epidemiologic data do not support a causal relationship between kidney cancer and TCE. With the exception of two poorly designed studies by Henschler et al. and Vamvakas et al., the results are not significant and do not suggest elevated risks among workers exposed to TCE.

Other cancers have also been considered in the evaluation of the carcinogenicity of TCE. A summary of the data on liver cancer and non-Hodgkins lymphoma from the above-mentioned studies is presented in Table 2. As can be seen from this summary none of the studies found a significantly elevated risk for these cancers.

Overall, these epidemiologic studies do not provide sufficient evidence of carcinogenicity in humans to support the NTP's classification of "known to be a human carcinogen."

Introduction

This review of the epidemiology of trichloroethylene (TCE) and kidney cancer focuses on the published occupational studies. The emphasis is on kidney cancer although data are provided on both liver cancer and non-Hodgkins lymphoma.

Occupational studies generally provide the most useful information on associations between chemical exposures and cancer. They are generally designed as retrospective cohort mortality studies where a defined group of workers are identified retrospectively from company records and their mortality experience is compared to that of a comparison group, usually the general population. Other types of studies, such as population-based case-control studies, are generally less persuasive primarily because of inadequate exposure information. In a population-based case-control study, cases and controls (or their next of kin if they are deceased) are interviewed about prior jobs they have held and companies where they have worked. These job/company combinations are then converted to exposures. There is generally a high degree of uncertainty in ascribing the exposures to individuals. Furthermore, these studies often suffer from selection bias, information bias and confounding. In some situations, case-control studies nested within an occupational cohort can provide a valid exposure assessment.

This review is presented in two sections. First is a summary of the seven occupational cohort studies conducted in the U.S., Finland and Sweden. This is followed by a more detailed review of the studies of renal cell cancer in Germany that report much higher risks than the other published studies (Henschler et al. 1995, Vamvakas et al. 1999).

Occupational Cohort Studies

The association between TCE exposure and kidney cancer has been studied in eight occupational cohort studies (Table 1): Garabrant et al. 1988, Axelson et al. 1994, Anttila et al. 1995, Henschler et al. 1995, Blair et al. 1998, Morgan et al. 1998, Ritz, 1999 and Boice et al. 1999. Seven of these eight cohort studies provide no evidence that occupational exposure to

TCE causes kidney cancer. The exception is the study by Henschler et al. (1995) which does report a significantly increased risk for kidney cancer. This study, built around a kidney cancer cluster, has many methodological problems and therefore its validity is questionable. Four of the eight cohort studies provide incidence data and only one (Henschler et al.) has a significantly elevated standardized incidence ratio (SIR). For cancer mortality, only the Henschler et al. study has an elevated standardized mortality ratio (SMR), which was not statistically significant.

Garabrant et al. (1988) conducted a retrospective cohort mortality study of men and women employed in an aircraft manufacturing company where it was estimated that 37 percent of the jobs involved exposure to TCE. The mortality experience of the workers from 1958 to 1982 was compared to the mortality experience of the United States population and the population of San Diego County which was the location of the facility. A total of 14,067 workers contributed 222,100 person-years of follow-up. During the study period, 1,804 workers were identified as deceased and death certificates were obtained for all but 84 of these decedents. The observed number of kidney cancer deaths was less than the expected number (SMR=0.93, 95% CI, 0.48-1.64).

Axelson et al. (1994) published an update of a Swedish retrospective cohort incidence study at a TCE manufacturing facility where workers were offered free surveillance for trichloroacetic (U-TCA), a metabolite of TCE in urine. The study included 1670 workers who contributed almost 25,000 person-years of follow-up. There was no statistically significant increase in kidney cancer incidence. The observed number of cases was approximately equal to the expected number (O=6, E=5.2, SIR=1.16, 95% CI, 0.42-2.52).

Anttila et al. (1995) had access to a Finnish database of employees biologically monitored for occupational exposure to TCE during 1965 to 1982. Exposure was measured by urine concentration of trichloroacetic acid. In addition to the workers with urinary measurements of U-TCA, the database included 109 workers with no urinary measurements but who were listed in the registry of occupational diseases with trichloroethylene poisoning. Cancer cases were ascertained from 1967 to 1992 by linking the database of workers to the Finnish cancer registry, and cancer deaths from 1965 to 1991 were ascertained by linkage to the vital statistics records

from the Central Statistical Office in Finland. The study included 1,698 men and 1,391 women who contributed 31,552 and 28,353 person-years of follow-up, respectively. There were fewer kidney cancer cases observed than expected (SIR=0.87, 95% CI, 0.32-1.89) and there was no association with the number of years since the first measurement

Blair et al. (1998) provided an update of a retrospective cohort mortality study of workers at Hill Air Force Base in Utah. The purpose of the study was to evaluate potential disease risks associated with exposure to organic solvents, particularly TCE. A cohort of 14,457 workers who were employed at least one year between 1952 and 1956 were enrolled and followed through 1990. As of 1982, there were over 45,000 person-years of TCE exposure in this cohort (Spirtas et al., 1991). Exposure to TCE was determined through an extensive assessment of jobs, the workplace, chemical inventories, interviews and monitoring data. There was no statistically significant increase in deaths from kidney cancer (SMR=1.22, 95% CI, 0.85-1.74), no significant increase in the risk ratio comparing exposed workers to nonexposed workers (RR=1.6, 95% CI, 0.5-5.1), no increased risk with increased exposure and no significant increase for the most highly exposed group for both men and women. In addition, no significant increases in risk were found for any of the alternative methods of evaluating exposure including low level intermittent exposure, low level continuous exposure and frequent peaks. Incident cancer cases were identified through a linkage to the Utah cancer registry. No statistically significant increases in kidney cancer cases were found for men or women and there was no dose-response effect.

Morgan et al. (1998) studied 20,508 workers (461,618 person-years of follow-up) having worked at least one year during the period 1950-1985 at the Hughes Aircraft Company in Arizona. At this facility, TCE exposure occurred between 1952 and 1977 in vapor degreasing units and prior to 1981 through ingestion of contaminated well water on the site. A total of 4,052 deaths were identified between 1950 and 1993. No statistically significant excess of kidney cancer was found for the overall cohort (SMR=1.14, 95% CI, 0.78-1.61) or for the TCE-exposed cohort (SMR=1.32, 95% CI, 0.57-2.60) or for the TCE high exposed cohort (SMR=1.78, 95% CI, 0.72-3.66). An internal analysis, using Cox proportional hazard models, also did not show a significant increase in risk (RR=1.89, 95% CI 0.85-4.23).

Using data available from the Comprehensive Epidemiology Data Resource (CEDR), Ritz (1999) examined kidney cancer risk in association with TCE, cutting fluids and kerosene among 3,814 at a uranium production facility in Ohio. In this cohort there was 120,237 person-years of follow-up. Plant industrial hygienists classified job titles into TCE exposure groups (none, light, moderate, and heavy), with most workers classified into the "light" exposure category. Approximately 80% of the cohort had at least some exposure to TCE. There were fewer deaths from kidney cancer than expected (SMR=0.65, 95% CI, 0.21-1.51).

Boice et al. (1999) conducted a retrospective cohort mortality study of 77,965 workers, contributing 1,889,795 million person-years of follow-up, at the Lockheed Martin aircraft manufacturing facilities in California. There were fewer kidney cancer deaths than expected for the overall cohort (SMR=0.92, 95% CI, 0.76-1.09), significantly fewer than expected for those workers with the longest duration of employment (SMR=0.52, 95% CI, 0.26-0.93), fewer than expected for those exposed to TCE (SMR=0.99, 95% CI, 0.40-2.04) and a deficit of kidney cancer cases among those with the longest duration of exposure to TCE (RR=0.69, 95% CI, 0.22-2.12).

In summary, these seven occupational cohort studies of workers exposed to TCE, which were based on well-defined cohorts and exposure assessments involving either urine biomonitoring or some type of job exposure matrix, did not find significantly increased risks of kidney cancer.

The articles by Henschler et al. (1995) and Vamvakas et al. (1998) warrant more attention because their results have been quite different from the other epidemiologic studies of TCE exposure and they have been prominent in the more recent considerations of TCE carcinogenicity. The authors of these studies suggest that exposures to TCE significantly and substantially increase the risk of kidney cancer. They attribute their findings, which are contrary to the findings from the cohort studies, to the higher exposures in their study populations relative to the other cohort study populations. This is alleged despite the absence of specific data to substantiate their claim regarding exposures. The fact that these studies have received so much attention may be due to the reported results that show rate ratios in the range of 8 to 10. However, the size of the number should not detract from the numerous and serious methodological flaws with these two studies.

Henschler et al. 1995

Henschler et al. (1995) conducted a retrospective cohort study at a cardboard factory in Germany. One study group consisted of workers exposed to TCE for at least one year between 1956 and 1975. Of the183 eligible workers, 169 were included. A comparison (unexposed) group was ascertained of 190 male workers, matched on age and physical work activities, whose work did not involve exposure to TCE. There were 50 deaths among the exposed group and 52 among the unexposed group. The overall SMRs and 95% CI's were 0.68 (0.48-0.93) in the exposed group and 1.03 (0.77-1.35) in the unexposed group. There were two kidney cancer deaths in the exposed group (SMR=3.28, 95%CI, 0.40-11.84) and 0 (0.60 expected) in the unexposed group. There were five incident cases of kidney cancer (4 renal cell cancer and 1 urothelial cancer) among the exposed group and none among the unexposed group. For the exposed group, the SIR was 7.97 (95% CI=2.59-8.59) when compared to the Danish Cancer Registry and 9.66 (3.14-22.55) when compared to the Cancer Registry of the Former German Democratic Republic. The authors concluded that these results support a causal relationship between TCE and renal cell tumors. A careful review of the paper raises a number of serious issues that cast doubt on their conclusion.

This study appears to be an expanded investigation of a cluster of kidney cancer cases. If true, then causation cannot be inferred. Designing a study around a cluster and including the cluster cases in the study almost assuredly leads to a positive finding. Numerous issues in the design and conduct of the study and in the data presented in the published article suggest many other problems with the study.

The unexposed group was matched on age to the exposed group yet there was a considerable difference in the age distribution between the groups. The median, minimum and maximum ages for the two groups were: exposed: 59, 40, 89; unexposed: 62, 28, 79. The study period was from 1956-1992, a maximum of 37 years (minus the one year enrollment criterion), however the median observation periods for the two groups as shown in Table 1 of the article were 34 years for the exposed group and 32 years for the unexposed group. Given that there were 50 deaths in the exposed group and 52 in the unexposed group, it would appear that all the

deaths would have had to occur toward the end of the study period for the median years of observation to be correct. This is a highly unlikely occurrence.

Other data in Table 1 of the paper are questionable. For example, results for smoking are presented for 175 exposed workers yet there were only 169 workers in the exposed group. It is interesting to note that data were available for everyone in the unexposed group indicating that no one refused to participate yet there were a number of refusals in the exposed group. A rather high percentage (22%) of people in the unexposed group used diuretics. Median blood pressures were identical between the two groups (140/80) despite the differences in the range.

Using the Danish Cancer Registry the authors computed that 0.628 kidney cancer cases would be expected in the exposed cohort (Table 2 of Henschler et al). This is essentially the same as the expected number of deaths presented in Table 5 of Henschler et al., a surprising result given the 5-year survival rate for kidney cancer.

The mortality data presented in Table 5 does not show any significantly elevated SMR except for brain cancer in the unexposed group (SMR=9.38, 95% CI, 1.93-27.37). The authors attribute this to a sensitivity bias. A similar bias could have influenced case ascertainment of kidney cancer in the exposed group since all members of this group received abdominal sonography.

There were no data on TCE air concentrations or on TCE metabolites in urine. Exposures were surmised from "walk-through surveys and extensive interviewing of long term employees". Of the five kidney cancer cases, three had jobs with relatively low exposure to TCE and two were in "highly" exposed jobs. However, one of these highly exposed workers was the urothelial cancer. Thus, it appears that one renal cell cancer case in the cluster worked in a "highly" exposed job.

Because of the many methodological problems and inconsistencies in the data, this study is difficult to interpret. It is likely that the Henschler et al. finding is due to chance based on a cluster investigation presented as a hypothesis testing study, to confounding, or to issues related to the design and conduct of the study.

The Case-Control Study by Vamvakas et al. 1998

Vamvakas et al. (1998) conducted a case-control study. Notwithstanding the earlier comments about case-control studies, this study is reviewed because it has received considerable attention in the evaluation of the carcinogenicity of TCE. The cases were defined as all renal cell cancer patients from the Urology Department of a country hospital in North Rhine, Westphalia who underwent nephrectomy between December 1, 1987 and May 31, 1992. After exclusions due to missing data and refusals, 58 cases and 84 controls were available for analysis. Comparing the "highest" exposed group to the nonexposed group gave an unadjusted odds ratio of 7.9 based on 8 exposed cases and 2 exposed controls. A small degree of misclassification or bias could significantly alter this risk. The authors present the adjusted odds ratio for the highest exposure category as 11.42 (95% CI, 1.96-66.79), the wide confidence interval reflecting the small numbers.

Cases included in an earlier study by Henschler et al. (1995) were excluded even though they might have been eligible by virtue of having undergone surgery at the study hospital. Two justifications for excluding these cases were provided. First, the authors wanted to avoid "double reporting" the cases; second, the authors limited cases to those employed in small, rather than large, factories. However, neither reason is justified, since both could result in selection bias. There is no inherent problem in including cases who might have participated in another study. Omitting selected cases who meet the study criteria could introduce a bias if they are different from cases included in the distribution of risk factors. Using factory size as a basis for exclusion of cases might have been acceptable had the same criterion been applied to controls. Apparently, it was not. A further problem in the case selection procedures is limiting the cases to those who underwent surgery, rather than to all histologically confirmed cases because the included cases may not be similar to the excluded cases in the distribution of risk factors.

An important issue in case-control studies is the selection of controls. Controls should be selected from the same source population or study base as cases (Wacholder et al. 1992). In this study, the authors selected controls from the accident wards of three hospitals, none of

which was the hospital from which cases were ascertained. Controls were selected from patients hospitalized during 1993, rather than from the same period as the cases (1987-1992) and there was no effort to ensure comparability on age between cases and controls. There are at least five reasons why this method of control selection is problematic and would result in selection bias. First, controls were selected from different hospitals than the cases. Without knowing hospital utilization and referral patterns in the area, it is impossible to conclude that controls were from the same study base as cases. Second, controls were selected from a specific diagnostic category. Since Berkson's classic paper in 1946, selection of hospital controls from a single hospital ward or disease category has been discouraged to guard against introducing bias (Berkson, 1946). Third, controls were selected from 1993, whereas cases were selected between 1987 and 1992. Thus, potentially eligible controls admitted to the hospital between 1987 and 1992 were excluded from consideration. This discrepancy between the eligibility dates for cases and controls is striking and highly unusual for case-control studies. Fourth, cases and controls were interviewed at different times, with up to six years between the initial interviews with the cases and controls. Fifth, the age discrepancy between the cases and controls bears directly on exposure potential. In this study, 8.6 percent of the cases were below the age of 50, whereas 44.0 percent of the controls were under 50. Therefore, cases had considerably more opportunity (more person-years of work experience) to experience the exposure of interest. It is especially noteworthy that the cases were first exposed in 1957 whereas the controls were first exposed in 1975 (Table 4 of Vamvakas et al. 1998). Thus, by itself, this design feature almost guaranteed that a positive association would be found. Age is a prominent risk factor for renal cell carcinoma. The age discrepancy between cases and controls would also affect confounding factors such as cigarette smoking, obesity, and diuretic use. It is important to note that adjusting for age would not satisfactorily resolve the concern about the striking age imbalance.

Another important consideration in case-control studies is information bias. This refers to systematic (as opposed to random) error that can occur if information about exposure is not valid. Information on previous jobs and exposures was obtained through a personal interview. The interviewers, who were physicians, were aware of who was a case and who was a control. Apparently, different physicians interviewed cases and controls. For cases who were deceased, information was obtained from former colleagues and relatives. Since none of the controls was

deceased, all of their information on exposures and confounding factors was obtained through a direct interview. Generally, in case-control studies such as this, every effort is made to design the study to minimize the opportunity for obtaining different quality of information from cases and controls. Such strategies would include blinding the interviewers as to case or control status of the participants and utilizing the same interviewers for both cases and controls. Using physicians in the area as interviewers rather than professionally-trained interviewers could result in considerable variability in the manner in which the interview was conducted and hence considerable bias in the responses. Another feature of the study that could have introduced information bias was the follow-back interviews. In this phase of the study, patients who reported any occupational exposure to trichloroethylene or tetrachloroethylene were recontacted to participate in another interview to assess conditions of exposure to these solvents in greater detail. The specific details of this procedure are not stated in the paper so it is not clear what the criteria for inclusion were or if a structured interview was administered.

The assessment of exposure was conducted through interviews with patients or informants. As stated in the paper, air or biological monitoring data were not available for any of the patients. To supplement the self-reported information, the investigators obtained more detailed information on work history from the Employer's Liability Insurance Association. This would suggest that for some, but not all individuals, and presumably those who filed a claim, additional information was obtained. It is likely that this information was more available for cases than controls.

Information on potential confounders was also collected through personal interview. There are a number of important risk factors for renal cell cancer such as smoking and obesity. Bias in the confounder information could also distort the results of the study.

Although it is difficult to know with certainty if this study is biased, there are some clues to suggest it may be. For example, there is a well-established association between renal cell cancer and cigarette smoking. In this study, 48 percent of the cases and 56 percent of the controls had ever smoked suggesting no positive association with renal cell cancer. Another important risk factor, obesity, was also not associated with renal cell cancer in this study. Body mass index was identical between cases and controls. The absence of these well-established

associations reinforces the argument that there was bias in the selection of study subjects and/or in the collection of the data.

Another potential source of bias is nonresponse. Not all selected subjects participated in the study. Overall, 79.5 percent of the cases and 75 percent of the controls agreed to participate. If the participants differed from the nonparticipants in exposure experience or in any of the important confounding factors, bias could have been introduced.

The authors conclude that bias could not account for their results, yet offer no evidence to support their position. Although it is difficult to know precisely the extent to which the many unusual features of this study may have biased the risk estimate, it is likely that the bias is not trivial.

Conclusions

Overall, these epidemiologic studies do not provide sufficient evidence of carcinogenicity in humans to support the NTP's classification of "known to be a human carcinogen."

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Table 1 – Summary of Occupational Cohort Studies of TCE Exposed Workers

Authors, Yr	Study Group	No. of Workers	Kidney Cancer
Anttila et al., 1995	Finnish workers monitored for TCE and other solvents	3,974	SIR= 0.87 (0.32 – 1.89)
Axelson et al., 1994	Swedish workers monitored for TCE	1,670	SIR= 1.16 (0.42 – 2.52)
Blair et al., 1998	Aircraft workers, Utah Air Force base	14,457	SMR= 1.6 (0.5-5.1)
Boice et al., 1999	Aircraft manufacturing workers, Burbank, CA	77,965	SMR= 0.99 (0.40-2.04)
Garabrant et al., 1988	Aircraft manufacturing workers, San Diego CA	14,067	SMR = 0.93 (0.48-1.64)
Henschler et al., 1995	Cardboard factory workers, Germany	169	SIR= 7.97 (2.59-8.59)
Morgan et al., 1998	Aircraft manufacturing workers, Tucson , AZ	20, 508	SMR= 1.32 (0.57 -2.60)
Ritz, 1999	Uranium processing plant workers	3,814	SMR= 0.65 (0.21-1.51)

SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio. 95% confidence intervals in parenthesis

Table 2 – Summary of Occupational Cohort Studies of TCE Exposed Workers

Authors	Study Group	Liver Cancer	NHL
Anttila et al., 1995	Finnish workers monitored for TCE and other solvents	SIR= 2.27 (0.74-5.29)	SIR= 1.81 (0.78-3.56)
Axelson et al., 1994	Swedish workers monitored for TCE	SIR= 1.41 (0.38-3.60)	SIR= 1.56 (0.51-3.64)
Blair et al., 1998	Aircraft workers, Utah Air Force base	SMR= 1.7 (0.2-16.2)	SMR= 2.0(0.9-4.6)
Boice et al., 1999	Aircraft manufacturing workers, Burbank, CA	SMR= 0.54 (0.15-1.38)	SMR= 1.19(0.65-1.99)
Garabrant et al., 1988	Aircraft manufacturing workers, San Diego, CA	SMR= 0.94(0.40-1.86)	SMR= 0.65(0.21-1.52)
Henschler et al., 1995	Cardboard factory workers, Germany	NA	SMR=1.10(.03-6.12)[1]
Morgan et al., 1998	Aircraft manufacturing workers, Tucson , AZ	SMR= 0.98 (0.36-2.13)	SMR=1.01(0.51-1.81)[2]
Ritz, 1999	Uranium processing plant workers	SMR= 1.66(0.71-3.26)	SMR= 1.28(0.90-1.77)[3]

SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio. 95% confidence intervals listed in parenthesis, NA= not available

1. Results are for lymphatic and hematopoietic tissue
2. Results are for cancer of all other lymphopoietic tissue.
3. Results are for lymphopoietic cancer

Comments on the National Toxicology Program's *Draft Report on Carcinogens Background Document for Trichloroethylene* (2000)

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December 1, 2000

CONTENTS

1. Introduction

In this commentary, we analyze the conclusions of the *Draft Report on Carcinogens Background Document for Trichloroethylene*.[1] We focus on the conclusions based on epidemiologic evidence. In so doing, we find that the *Draft Report* is flawed. Neither the methods employed nor the results presented in this *Draft Report* constitute a reliable analysis or a basis for causal inference. Upon reviewing the primary evidence, it becomes clear that the proposal to reclassify ("upgrade") trichloroethylene (TCE) as a chemical "known to be a human carcinogen" should be rejected. This is because the epidemiologic results on TCE, with respect to its possible carcinogenicity, are most consistent with the null hypothesis – that is, with the hypothesis that TCE is not a cause of human cancer. For each type of cancer evaluated, suggestive results are weak, extremely unstable and inconsistent with the weight of the epidemiologic evidence, or better explained by alternative hypotheses.

In the early and mid-1990's, the accumulated epidemiologic data on TCE were judged as insufficient (ACGIH, 1993)[a] or "limited" (IARC, 1995).[2, b] Additional epidemiologic data on TCE have been generated since, so that a re-analysis is timely. As shown below, no coherent analysis of these data would suggest "sufficient" evidence for TCE of human carcinogenicity. Although the *Draft Report* does conclude that the evidence is sufficient, it does so through a selective, incomplete, insufficiently detailed, and insufficiently critical analysis. Such an analysis cannot be relied upon for scientific decision making. Moreover, recent thorough reviews of the epidemiologic evidence on TCE and cancer come to quite different conclusions from those given in the *Draft Report.*[3–5]

2. Method of our analysis

We have organized our comments to follow the summary conclusion of the "Human Cancer Section" of the *Draft Report* (its Chapter 3). That conclusion is:

> The number and sophistication of studies assessing the possible carcinogenicity of TCE is impressive. Although the studies are not

[a] The American Conference of Governmental Industrial Hygienists classified TCE in its "Group A5, Not Suspected as a Human Carcinogen," finding that TCE "has been demonstrated by well controlled epidemiological studies not to be associated with any increased risk of cancer in exposed humans."

[b] The International Agency for Research on Cancer found "limited evidence in humans for the carcinogenicity of trichloroethylene," writing, "Overall, the most important observations are the elevated risk for cancer of the liver and biliary tract and the modestly elevated risk for non-Hodgkin lymphoma in all three of the most informative cohort studies." As discussed here, follow-up studies and new epidemiologic results available since February 1995 (when the IARC Working Group met) alter these observations. Further, even at the time (February 1995), about half of the members of the Working Group felt that the epidemiologic evidence on TCE was "inadequate," not even "limited" (Parker, U.S. EPA, 1995, personal communication at TCE Workshop, Williamsburg, Virginia).

> perfectly consistent, strong patterns emerge. In particular, associations with TCE exposure generally were observed for kidney cancer, liver cancer, non-Hodgkin lymphoma, multiple myeloma, and prostate cancer. Particular aspects of design or implementation may limit the usefulness or interpretation of individual studies, but, by and large, these studies were well designed and executed. Viewed from the perspective of Hill's aspects of causation (Hill, 1965), several of the criteria are fulfilled.

This summary from the *Draft Report* organizes the task before us. For each of the types of cancer listed in this summary as having associations with TCE, we begin by summarizing the IARC review[2] of the human evidence for that cancer type (in part because the IARC review seems to have been a basis of the *Draft Report).* Second, we examine evidence that has been published since the IARC review,[2] to see whether the new evidence ought to modify the conclusion reached by IARC.[2] Third, we examine the pattern of evidence, which *The Draft Report* characterizes as "strong," presumably with the meaning that the pattern strongly suggests a causal relation. Finally, we interpret the evidence for each cancer type, considering the evidence that had been gathered at the time of the IARC review, the new evidence, and the total pattern of results. Examining the primary epidemiologic studies, we arrive at a very different conclusion than the *Draft Report.* For each cancer type, we find that the overall pattern does not strongly support the causal hypothesis, and sometimes strongly supports the null hypothesis. The evidence clearly fails to establish TCE as a cause of human cancer.

Moreover, we have found that the *Draft Report* obscures — rather than fairly weighs — the epidemiologic evidence as a whole. The two tables (tables 3-1 and 3-2 on pages 31-35 of the *Draft Report*) in which the epidemiologic studies are summarized present only those relative measures of effect that exceed 1.2, and none of the similar measures that are less than 1.2, including ratios that are smaller than 1.0. Clearly a tabulation of only positive results cannot serve as a fair representation of all of the results. Readers of the *Draft Report* who may be unfamiliar with the primary epidemiologic literature on TCE would be seriously misled by the selective treatment given in this Report.[a]

The *Draft Report* argues that strong patterns of evidence emerge to support the causal hypothesis for TCE and the types of cancer it lists. This conclusion is said to be based on summary results from literature syntheses. Are such patterns in fact apparent in the epidemiologic data? Let us look. In particular, for each of the cancer types at issue, let us plot the accumulated results graphically. For each cancer type, we have sorted the published epidemiologic results (SMRs, RRs, and/or ORs) in ascending order. We then

[a] In this regard, it is odd and unfortunate that the authors of this *Draft Report* are anonymous. The title page notes only that the document was prepared by Technology Planning and Management Corporation, an organization that does not, judging from its website, do epidemiologic or toxicologic work or analysis, but instead seems to specialize in "Information Technology Consulting," "Software Engineering," "Web Application/eBusiness Solutions," and other, non-biological fields of endeavor. Perhaps the omissions and misinterpretations in *The Draft Report* merely reflect the scientific inexperience of the anonymous authors.

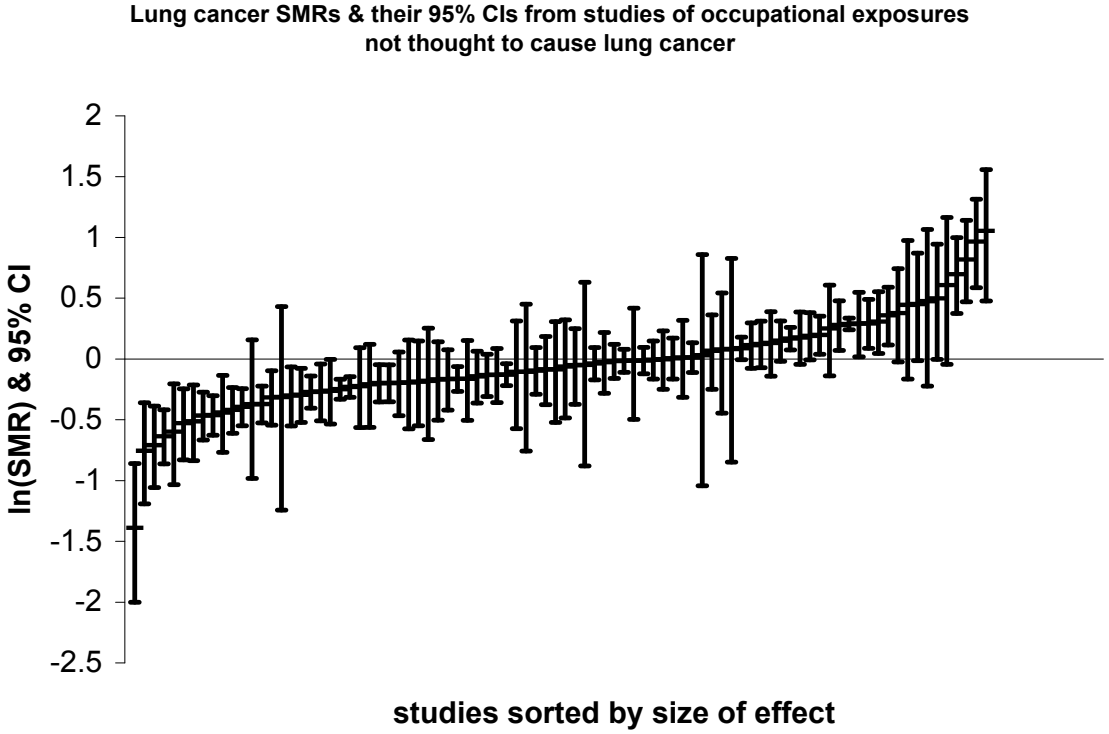
plot the results and their 95% confidence intervals, with distinct symbols representing cohort and case-control studies. These plots describe the patterns of evidence associating TCE exposure with the particular cancer type. If the pattern of evidence suggests a null association, the following characteristics of the plot are expected:

- The pattern of results from cohort studies should be approximately equally distributed below and above the null. Retrospective occupational cohort studies often examine a wide range of diseases. They are expensive and time consuming undertakings, so usually are published once completed regardless of the result being null, causal, or protective.

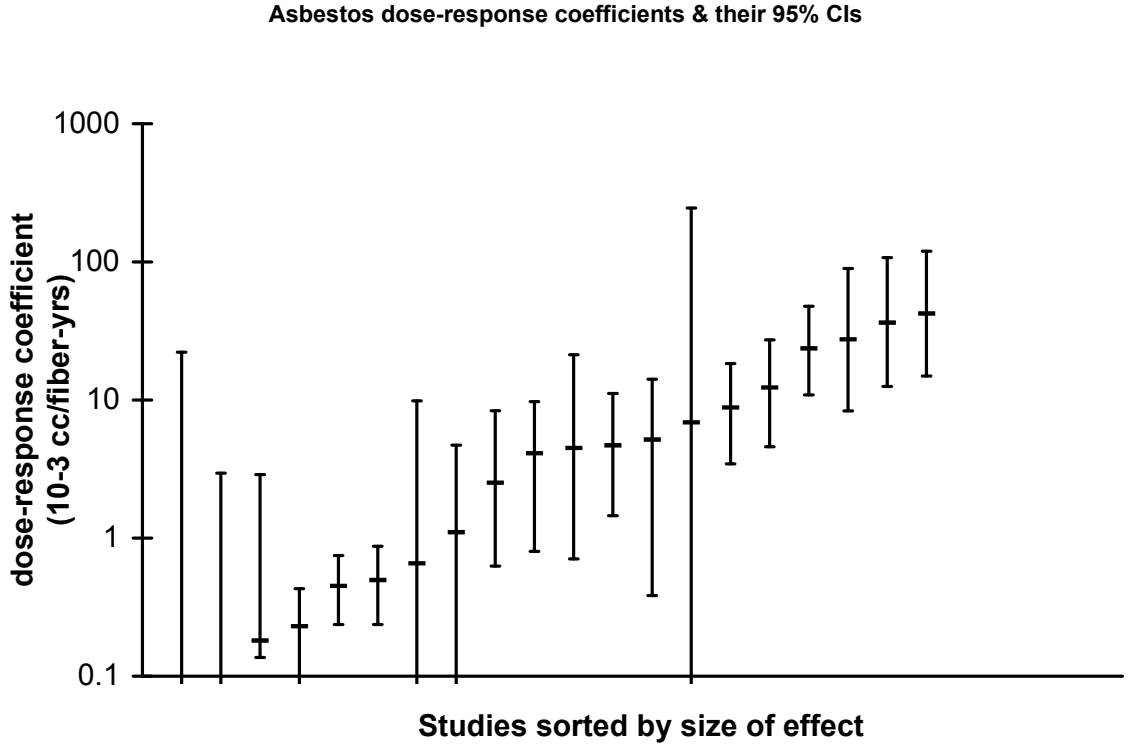
- If case-control studies of the association have been conducted, they may tend to concentrate above the null. Case-control studies often examine a number of exposures associated with a single disease. The exposures that prove to be positively associated with the disease tend to be those published or emphasized in publications. Null associations are not so often published or emphasized in publications. Thus, for a truly null association, studies that spuriously suggest a causal direction are more likely to be published than studies that spuriously suggest a protective direction, because the causal association has a stronger prior expectation. For cancer types that have been studied by case-control design, we would therefore expect that the case-control studies would tend to concentrate in the section of the plot above the null, and that this effect would shift the entire distribution towards a positive effect.

- The intervals about estimates of effect that show a strongly protective or strongly causal association will be wider than estimates of effect that suggest a null association. Thus, the widest intervals will be on the left side and right side of the plot, while narrower intervals will surround the estimates of effect near the null at the center of the plot. This pattern is expected because estimates of effect based on small numbers are more likely to deviate from the truth and will have wider intervals. Methods have been suggested to correct for this phenomenon[6] and have been applied in other settings,[7] but have not been applied here because the pattern is an important clue to discern a true null effect.

The following plot provides an example of the pattern expected for a null distribution of cohort studies. The data derive from a published series of SMRs associating lung cancer risk with various occupations thought *not* to cause lung cancer.[8] The SMRs and their intervals are plotted (here and throughout these comments) on the logarithmic-scale, so that the distribution is symmetrical about the null (0 on the log scale). Note that the SMRs in this distribution ranged from 0.25 to 2.87, suggesting that the range of observed SMRs for null associations can deviate substantially from a narrow range around 1.0.



Plots of this type can also provide strong visual evidence of a truly causal association. For example, the next plot shows the slope of the dose-response coefficient relating cumulative exposure to asbestos and relative risk of lung cancer.[9] The publication from which this distribution derives is attached. Note that all but two of the twenty estimates of effect exceed the null (a slope of 0), that the intervals seldom cover the null, and that the width of the intervals is not dependent on the size of the effect. That is, the widest intervals are not at the left and right sides of the plot.



3. Cancer types

Our review of the association between trichloroethylene exposure and the cancers at issue follows.

3.1. Kidney and renal cell carcinoma

3.1.1. Summary of IARC 1995 review

The IARC 1995[2] review dismissed cohort studies of dry cleaning workers because they were not relevant to trichloroethylene exposure *per se*, given the extensive exposure of dry cleaners to other solvents. Cohort studies of workers whose exposure to trichloroethylene was documented by biologic monitoring were given the most emphasis, although cohort studies of workers in other industries were given consideration as well. In its review of two studies with biologic monitoring to document trichloroethylene exposure, IARC[2] made no mention of the kidney cancer findings, although the findings were available for review. In its description of four of the five cohort studies of miscellaneous manufacturing industries, IARC[2] similarly made no mention of [the] kidney cancer findings. The fifth cohort is the cohort of cardboard manufacturing in Germany, [10] which we discuss at length below in section 3.1.3.1. In addition to the issues we raise in that section and the criticisms published elsewhere,[3–5, 11] IARC[2] noted that measurements of exposure to trichloroethylene were not available, and workers were classified as exposed or unexposed on the basis of job

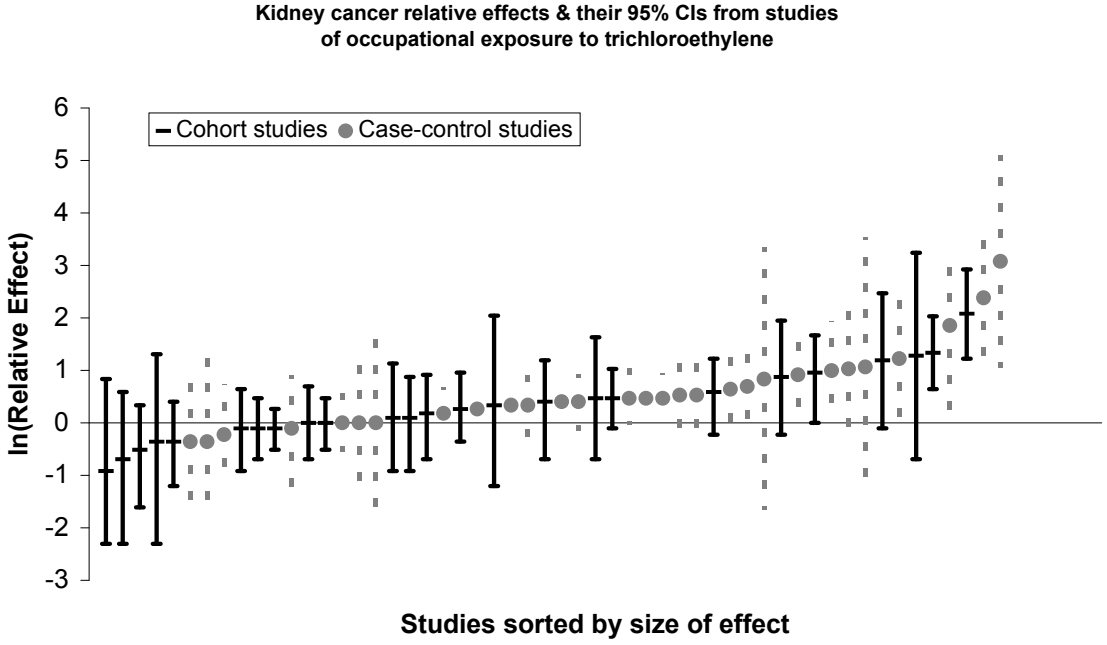
categories. This classification could be subject to differential misclassification given that the outcomes and hypothesis were known before the investigation began.

In its review of case-control evidence associating trichloroethylene exposure with renal cell carcinoma, IARC[2] reviewed a single case-control study of exposure to degreasing solvents. This study was not specific to trichloroethylene exposure.

In it summary of the human carcinogenicity data, IARC[2] stated that the occurrence of cancer of the kidney was not elevated in the cohort studies, except for the single study from a cardboard box-making plant introduced above.[10] They gave limited credence to that study because it had been initiated after the observation of a cluster. IARC said that the case-control data were discordant and not specific to trichloroethylene. They did not list the kidney cancer among the types of cancer with even limited epidemiologic evidence of elevated risks associated with trichloroethylene exposure.

3.1.2. Summary of new evidence

Since the IARC 1995 review, five cohort studies and five case-control studies have examined the association between occupational exposure to trichloroethylene and the risk of kidney cancer in general, or renal cell carcinoma in particular.[5] Some of the cohort studies are updates of earlier investigations. In these new cohort results, the relative risks of kidney cancer associated with occupational exposure to trichloroethylene have ranged from 0.7 (95% CI 0.3–1.5)[12] to 3.6 (95% CI 0.5–25.6).[13] The latter result applied to women only. The same study found an SMR of 0.4 (95% CI 0.1–2.3) among men. In the new case-control results, the relative risks of kidney cancer associated with occupational exposure to trichloroethylene have ranged from 0.7 (95% CI 0.2–3.6)[14] to 10.8 (95% CI 3.4–34.8).[15] The Figure shows results of all of the studies of the association between occupational exposure to trichloroethylene and kidney cancer or renal cell cancer.[5] As can be seen, the distribution is what one would expect for a truly null association. That is, the



results from cohort studies are centered about the null, and the studies with the widest intervals are nearer the left and right sides of the distribution. Case-control studies more often are towards the right side of the distribution, reflecting the tendency to publish or emphasize exposures with positive findings from case-control research. Given this tendency, the entire distribution seems somewhat shifted towards causal associations, but this is best viewed as an artifact of the aforementioned publication bias. In contrast to the opinion expressed in the *Draft Report,* no "strong pattern" evoking causality is evident.

There are four results with substantially elevated associations at the far right side of the plot. Two of these results are the cohort[10] (SMR = 8.0, 95% CI 3.4–18.6) and case-control[15] (OR = 10.8, 95% CI 3.4–34.8) studies generated by a German research group. Were these results valid representations of the effect of occupational exposures to trichloroethylene on kidney cancer risk, then we should see a much more consistently positive result from other occupational investigations (assuming roughly equal levels of TCE exposure). Instead, the consistent result favors the null hypothesis. This discrepancy begs for an explanation, and we present one alternative hypothesis for this select set of findings below in section 3.1.3.1.

The third of these studies is a case-control study with exposure classification defined as solvents,[16] so is not specific to trichloroethylene. The estimate of effect is restricted to women. The estimate of effect for the same exposure definition in males yielded a relative risk of 1.5 (95% CI 0.9–2.4).

The fourth of these studies is a case-control study in which the exposure was defined as occupational exposure to trichloroethylene and solvents, so again is not specific to TCE.[17]

3.1.3. Interpretation

Before interpreting the studies of the association between trichloroethylene and kidney cancer or renal cell cancer, we present an alternative hypothesis that may explain some or all of the observed association in the German cohort[10] and case-control[15] studies. We also discuss limited data associating Von Hippel Lindau mutations in kidney cancer patients with occupational exposure to trichloroethylene. We conclude with our interpretation of the literature.

3.1.3.1. Alternative hypothesis

A minority of recent analyses[10, 15] suggest that occupational exposure to trichloroethylene may cause renal cell carcinoma. These observations require an explanation for the disparity between the majority of the published results of the association between occupational exposure to trichloroethylene and the risk of renal cell cancer — which support a null association — and this limited subset of studies that suggest a strong association. We propose an alternative hypothesis to explain why most studies are null, but a limited subset might be positive. Until this hypothesis is further investigated, one should not conclude that trichloroethylene *per se* causes kidney cancer in humans. The hypothesis is this.

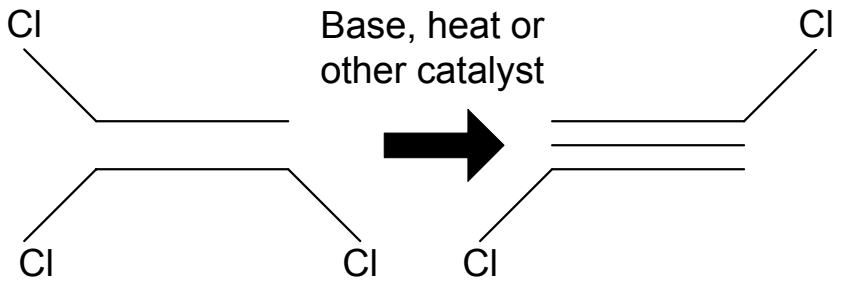
- Under specific, physical and chemical conditions, TCE decomposes *via* dehydrochlorination to the compound dichloroacetylene (DCAene).

- This decomposition of TCE to DCAene has occurred in certain, limited occupational settings, and during specific anaesthetic uses of TCE, but *does not* occur in most occupational settings, *cannot* occur in environmental settings — such as in contaminated water or ambient air — and *cannot* occur *in vivo via* metabolism

- DCAene is a potent nephrotoxin in laboratory rodents, as well as a potent cause of renal cell carcinoma in both sexes of two species, mice and rats.

- To the extent that occupational cohorts using TCE may have been at increased risk of kidney cancer, the increase is more plausibly due not to TCE *per se* but instead to chronic exposure to low but toxicologically significant levels of DCAene that formed inadvertently.

The evidence supporting this hypothesis is as follows. For much of the 20th century, TCE has been used as an inhalation anaesthetic and analgesic agent.[18–21] Anesthesia is typically induced by levels on the order of 5,000–10,000 parts TCE per million parts air,[19] and reversed without incident upon cessation of exposure. Occasionally, however, not only reversible narcosis but also neuropathy results, with distinct, toxic effects on the patient's trigeminal nerve. The circumstances and causes of this toxicity are of interest both with respect to the nervous system and, more relevant for this commentary, with respect to kidney toxicity and kidney cancer.



The cause of the trigeminal neuropathy is not TCE *per se*, but instead the dehydrochlorination breakdown product of TCE — namely, dichloroacetylene (DCAene; see Figure). TCE, like other inhalation anesthetics, can be administered in one of two ways: (a) in a re-breathing circuit, the purpose of which is to deliver to the patient oxygen and anesthetic gases, and eliminate exhaled carbon dioxide (typically *via* soda lime absorption); or (b) in a non-rebreathing circuit. For TCE, only the second method is safe. As became evident early on, use of soda lime in a re-breathing circuit is a dangerous way to administer TCE, since the sodium hydroxide catalyzes dehydrochlorination of TCE to form the potent toxin, DCAene.[20–25]

Moreover, as in operating rooms, use of TCE in factories can sometimes involve conditions under which dehydrochlorination is catalyzed. Case reports of trigeminal or other facial nerve damage in workers exposed to breakdown products of trichloroethylene parse into

two categories of exposure. First, industrial exposure to trichloroethylene vapors that have been heated or passed over fine metal shavings can involve generation of toxicologically significant quantities of DCAene. Second, in other cases, workers have inhaled TCE through face masks or other absorbers in place to reduce their exposures to carbon dioxide. Unfortunately, the alkaline absorbers (soda lime or its equivalent) served also to catalyze the formation of DCAene, thereby causing toxicity rather than preventing it.[26–32]

Nervous system toxicity aside, DCAene is also a specific nephrotoxin in laboratory animals, as well as a potent cause of renal cell cancer in these animals. Bioassay data show DCAene to be a potent inducer of kidney tumors in mice and rats of both sexes.[33] TCE, in contrast, is a weak inducer of kidney tumors in male rats alone. It fails to induce kidney tumors in female rats[a] or in mice of either sex (see Table 1, below). The difference in carcinogenic potencies is striking: comparing TD_{50}'s, one finds that DCAene is at least 65 to 1,600 times more potent an inducer of kidney tumors than is TCE.

Table 1: TD_{50}'s[b] (in mg/kg-day) for kidney tumors in laboratory rodents administered TCE or DCAene.

	Rats		**Mice**	
	Males	Females	Males	Females
Trichloroethylene[c]	1700	NSR[d]	NSR[d]	NSR[d]
Dichloroacetylene[e]	26	12	12	11

Of course, if exposures to TCE necessarily or often involve exposures to DCAene, the practical distinction between the two might be unimportant. That is, if DCAene often forms from TCE, the distinction between the two chemicals might be more academic than otherwise. Importantly, this is not the case. Instead, DCAene formation is rare, is catalyzed by specific, physical and chemical conditions, persists only under certain conditions, and is not a metabolite of TCE or other compounds in any species. In the environmental setting, the chemical conditions required for TCE to breakdown to DCAene, *and* for DCAene to persist once formed, are not those that accompany domestic uses of water or air contaminated with TCE, however heavily. Even in the occupational (and anesthetic) setting, absent strong alkali, heat, and/or catalytic metal surfaces (or other

[a] The *Draft Report* implies that TCE is known to cause kidney cancer in both female and male rats, but this is incorrect.

[b] The TD_{50} is the dose at which chronic administration of the chemical throughout the standard life-span of the species halves the probability of the animals remaining tumor-less. In cases in which the tumor type occurs in 0% of control animals, the TD_{50} is simply the dose of the chemical that induces tumors (of a specified type) in 50% of dosed animals. The *inverse* of the TD_{50} is a measure of the carcinogenic potency of the test chemical — that is, the smaller the TD_{50}, the more potent the chemical as a carcinogen.

[c] *Sources*: Maltoni, *et al.*, 1986; National Toxicology Program (NTP), 1988; NTP, 1990.

[d] NSR = No significant response.

[e] *Source*: Reichert *et al.*, 1984.

conditions conducive to solid-phase dehydrochlorination), the generation of DCAene from TCE is the exception, not the rule.[34–38]

With this understanding of the chemistry and toxicology of DCAene[a] (and the quite different toxicology of TCE), one begins to understand why the vast majority of epidemiologic studies of TCE-exposed workers fails to find an elevation in risk of kidney cancer, even as the epidemiologic results from a small minority of these investigations[10, 15] seem to indicate a sizable elevation in kidney cancer risk. There are, as published elsewhere,[3–5, 11] significant methodologic weaknesses in the apparently positive studies, such that the odds ratios are strongly biased away from the null. The point here, though, is that if there is some actually elevated risk of kidney cancer for the workers therein studied, the risk is more plausibly due to DCAene, and implausibly due to TCE. Moreover, it is in exactly the workplace settings studied by Vamvakas[15] and Henschler[10] -- because of the simultaneous presence of strongly alkaline materials, such as cardboard starches made up in 50% NaOH -- that DCAene formation would be predicted.

3.1.3.2. Von Hippel-Lindau mutations

The Draft Report discusses (section 6.5) recent studies in which mutations were analyzed in the Von Hippel-Lindau (VHL) genes of patients with renal cell carcinomas. Two studies by the same group report unusual patterns of VHL mutations in renal cell carcinoma (RCC) patients with prior TCE exposure, compared to RCC patients without such exposure.[39, 40] As the *Draft* also notes, a similar investigation by Schraml *et al.*[41] failed to find any differences in VHL genes between TCE-exposed and unexposed patients. We have several comments on these studies.

- Because the patient populations studied by Bruning *et al*.[39] and Brauch *et al*. [40] evidently overlapped substantially, the findings of these related studies need to be evaluated in another population.

- Most patient numbers and ages at diagnosis listed by Bruning *et al*. [39] appear in Brauch *et al*.'s [40] population; however, not all subjects examined by Bruning *et al*. are also studied by Brauch *et al*., and no reason for the discrepancy is given. Some ages at diagnosis disagree.

- Bruning *et al*. [39] used no concurrent controls.

- In Brauch *et al*. [40] only TCE-exposed patients and controls were given questionnaires exploring various disease risk factors. Such information was not gathered from unexposed renal cell carcinoma patients or controls.

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[a] This well-known breakdown product of TCE, namely DCAene, is not even mentioned in *The Draft Report*, let alone analyzed with respect to its toxicity. The best one finds therein is the partially correct statement (on page 3), "In the presence of moisture and light, TCE decomposes by forming hydrochloric acid." This is rather like saying, "Rome burns, forming water." Hydrochloric acid is the *leaving group*, of course, in the breakdown of TCE; it is not the toxic material of interest; DCAene is.

- Whether familial VHL disease occurred in any of the patients was not discussed.

- In each patient population, ages at diagnosis range from 38 to 84. There is no discussion about whether RCC mutations may vary with age, and controls or comparison populations are not identified as to age.

- Sexes of patients are not given, nor is there any discussion of whether this variable may be important. The sex distributions of comparison populations are not specified.

- There is no discussion of smoking history in the Bruning *et al*. study.[39] In the Brauch *et al*. paper,[40] 58% of TCE-exposed patients with VHL mutations were said to be smokers. No definition of "nonsmoker," the only other category, is given. It is unclear how former smokers would be classified. Smoking histories of the whole population are not given, nor is there any discussion of the possible significance of smoking to the occurrence of VHL mutations. Cigarette smoking is an established cause of renal cell cancer.[42]

- The methods used by Brauch *et al*.[40] to analyze DNA from tumor and normal kidney tissue (and from lymphocytes) are very unclear. In particular, it is unclear whether tumor samples had been preserved by the same method in each of the three study groups (one exposed, two unexposed). Tissue from exposed RCC patients had been formalin-fixed and embedded in paraffin; DNA from such samples is likely to be highly damaged. Tissues preserved in this way are not comparable to fresh tissue or cells. It is also unclear whether tumor tissue from 73 unexposed patients was analyzed in the same manner as tumor tissue from exposed patients.

- There was no positive control for the method used to identify nt454 mutations. Thus, failure to find such mutations may be due to experimental error.

- Controls were underutilized by Brauch *et al*.[40] Lymphocyte DNA (taken as indicative of germ-line VHL status) was analyzed only for the mutation at nucleotide 454, and not for any other VHL mutation. Analyses of tumor DNA from unexposed patients are designated as unpublished, and given in a summary fashion in Table 4. Only a subset of unexposed patients (73/107) was completely assessed for VHL mutations, and no explanation is given for the absence of such data for the remaining 34 subjects.

- An internet database of VHL mutations (www.umd.necker.fr) indicates that a nt454 hotspot had not previously been identified. Brauch *et al*.'s findings of multiple mutations in the gene are also highly inconsistent with previous data. We wonder whether Brauch *et al*.'s findings are artifacts of their methods.

- Brauch and others have recently presented evidence suggesting that VHL mutations are more frequent with advanced cancer stage (Brauch *et al*., 2000).[43] However, tumor stage was not identified in the TCE-exposed patient populations assessed by Brauch *et al*. and the comparison groups.[40]

- The descriptions of the TCE exposures experienced by the most highly exposed cases suggest frankly toxic exposures – concentrations apparently high enough to induce narcotic symptoms. Also, as noted above, these cases may have been exposed to the potent nephrotoxin, dichloroacetylene.

How are these data to be interpreted? Cautiously, we suggest, given the flaws and uncertainties noted above. Certainly, hypotheses other than TCE-induced mutation must be considered. For example, the TCE used industrially contains stabilizing chemicals, which may be or are known to be mutagenic. Alternatively, industrial exposure to TCE may apply selective pressure to cancerous (or pre-cancerous) kidney cells and give a survival advantage to cells with particular VHL mutations, independent of any mutagenic effect of TCE. Such a hypothesis has recently been proposed for lung cancers in smokers and the p53 tumor suppressor gene (Rodin and Rodin, 2000). Finally, the biologic plausibility of TCE-induced mutation must be questioned, since the putative mutagenic metabolite, chlorothioketene, is unstable in aqueous environments and is not expected to react with DNA.[44]

3.1.3.3. Conclusions

There have been important new results published since the IARC review[2] regarding the association between occupational exposure to trichloroethylene and the risk of kidney and renal cell carcinoma. Most of these studies are consistent with the literature published before 1995. That is, the distribution of results is consistent with a null association. There are four discrepant results that suggest a causal association. Two of these derive from case-control studies in which the exposure definition may have included trichloroethylene, but were certainly not specific to trichloroethylene.[16, 17] One of them,[17] and two others with exposure classifications more specific to trichloroethylene,[10, 15] derived from occupational settings in which trichloroethylene may have dehydrochlorinated to form dichloroacetylene. Dichloroacetylene is a potent nephrotoxin, and a far more potent kidney carcinogen than trichloroethylene in both sexes of laboratory rats and mice. The epidemiologic data as a whole suggest both that trichloroethylene *per se* is not a cause of kidney cancer in humans, and that dichloroacetylene may be such a cause.

3.2. Liver and biliary tract

3.2.1. Summary of IARC 1995 review

In its review of two studies with biologic monitoring to document trichloroethylene exposure, IARC[2] reported SMRs for liver cancer of 1.4 (95% CI 0.38–3.6) and of 1.9 (95% CI 0.86–3.6). The SMR was higher in the latter study for men with higher exposure and after twenty years latency. In its review of four cohort studies of miscellaneous manufacturing industries, IARC[2] made no mention of liver cancer findings in two although the findings were available for review, and reported SMRs of 0.94 (95% CI 0.4–1.9) and 2.2 (95% CI 0.96–4.4) in the other two.

IARC[2] also reviewed three case-control studies, all of which considered exposure to mixed solvents. No case-control study specific to trichloroethylene was reviewed.

With this evidence, the review concluded that the cohort studies consistently indicate an excess relative risk for cancer of the liver and biliary tract. They recognized that the case-control studies of mixed solvents, with very few subjects reporting exposure to trichloroethylene, were of little value. They concluded that there was limited evidence of an association between trichloroethylene exposure and liver cancer in the epidemiologic results.

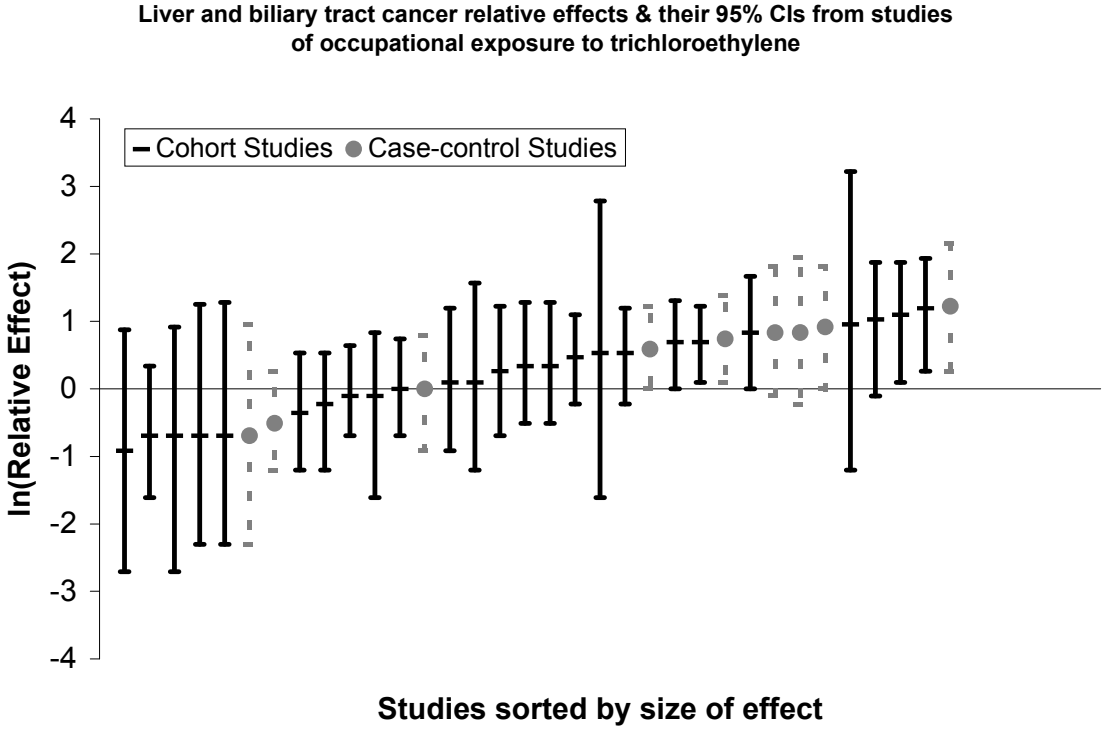
3.2.2. Summary of new evidence

Since the IARC review,[2] four cohort studies and one case-control study have examined the association between occupational exposure to trichloroethylene and the risk of liver or biliary tract cancer.[5] The first cohort study is an update of an earlier investigation. The results from the update are an SMR of 1.7 (95% CI 0.2–16.2)[13] for liver cancer mortality and an SMR of 2.6 (95% CI 0.3–25) for liver cancer incidence among men.[13] The SMR for liver and biliary tract cancer mortality was 1.3 (95% CI 0.5–3.4) and the SMR for incidence among men was 1.1 (95% CI 0.3–4.8). This result derived from a cohort exposed to trichloroethylene and other organic solvents, as described in the published title. Boice *et al*. reported an SMR for liver or biliary tract cancer mortality of 0.5 (95% CI 0.2–1.4),[45] Morgan reported an SMR for liver or biliary tract cancer mortality of 1.0 (95% CI 0.5–2.1),[46] and Ritz reported an SMR for liver or biliary tract cancer mortality of 1.7 (95% CI 0.8–3.3).[12] The majority of evidence accumulated since the IARC review[2] supports the null hypothesis.

In the new case-control study, the relative risk of liver cancer associated with occupational exposure to dry cleaning solutions equaled 0, as there were no exposed cases.[14]

There is no new evidence published since the IARC review[2] that would lead one to conclude that trichloroethylene should be "upgraded" from a probable to a known cause of liver cancer in humans.

The Figure shows all of the studies of the association between occupational exposure to trichloroethylene and liver or liver and biliary tract cancer.[5] The distribution is what one would expect for a truly null association. That is, the results from cohort studies with the widest intervals are nearer the left and right sides of the distribution. Case-control studies more often are towards the right side of the study, reflecting the tendency to publish or emphasize exposures with positive findings from case-control research. Given this tendency, the entire distribution seems somewhat shifted towards causal associations, but this is best viewed as an artifact of the aforementioned publication bias.



3.2.3. Interpretation

There is no new evidence to suggest that trichloroethylene is a cause of human liver cancer. In fact, the new evidence most strongly supports the null hypothesis. The complete distribution of results is as expected for a truly null association.

3.3. Non-Hodgkin Lymphoma

3.3.1. Summary of IARC 1995 review

In its review of two studies with biologic monitoring to document trichloroethylene exposure, IARC[2] reported SMRs for non-Hodgkin lymphoma (NHL) of 1.6 (95% CI 0.51–3.6) and 1.8 (95% CI 0.78–3.6). In its review of four cohort studies of miscellaneous manufacturing industries, IARC[2] made no mention of NHL findings in three although the findings were available for review, and reported an SMR of 2.9 (95% CI 0.78–7.3) for women in the fourth. The SMR for men and women combined in the fourth cohort was 1.3 (95% CI 0.68–2.1).

IARC[2] also reviewed one case-control study of NHL, which considered exposure to mixed solvents. Although TCE specific data were available, only a crude result was reported. No case-control study specific to trichloroethylene was reviewed.

With this evidence, the review concluded that the cohort studies consistently indicated a modest excess relative risk for NHL. They concluded that there was limited evidence of an association between trichloroethylene exposure and non-Hodgkin lymphoma in the epidemiologic results.

3.3.2. Summary of new evidence

Since the IARC review,[2] three cohort studies and one case-control study have examined the association between occupational exposure to trichloroethylene and the risk of non-Hodgkin lymphoma.[5] The first cohort study is an update of an earlier investigation. The results from the update are an SMR of 2.0 (95% CI 0.9–4.6)[13] for NHL mortality, an SMR of 1.0 (95% CI 0.3–2.9) for NHL incidence among men, and an SMR of 0.9 (95% CI 0.2–4.5) for NHL incidence among women.[13] This result derived from a cohort exposed to trichloroethylene and other organic solvents, as described in the published title. Boice *et al*. reported an SMR for NHL mortality of 1.2 (95% CI 0.7–2.0)[45] and Morgan reported an SMR for NHL mortality of 1.0 (95% CI 0.5–1.7).[46] The majority of evidence accumulated since the IARC review[2] supports the null hypothesis.

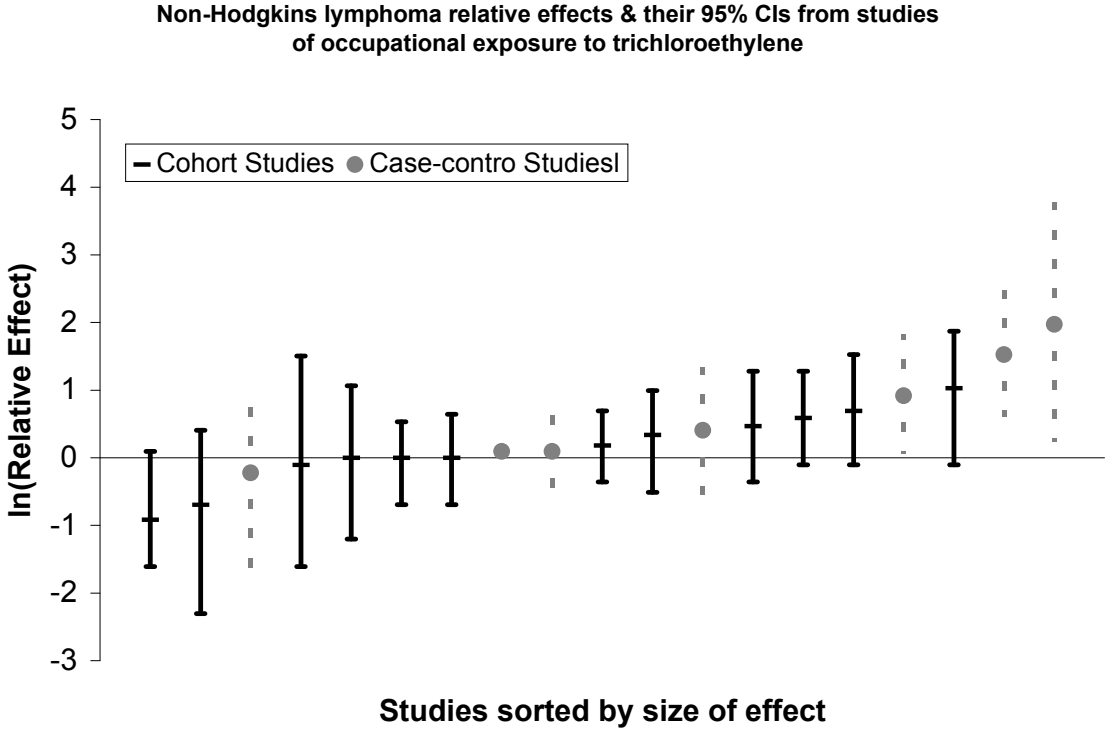
In the new case-control study, the relative risk of NHL mortality associated with occupation as an aircraft mechanic, as described on the death certificate, equaled 2.5 (95% CI 1.1–6.0).[47] This definition of exposure is not specific to trichloroethylene.

There is no new evidence published since the IARC review[2] that would lead one to conclude that trichloroethylene is a known cause of NHL in humans.

The Figure shows all of the studies of the association between occupational exposure to trichloroethylene and non-Hodgkin lymphoma.[5] The distribution is what one would expect for a truly null association. That is, the results from cohort studies with the widest intervals are nearer the left and right sides of the distribution. Case-control studies more often are towards the right side of the study, reflecting the tendency to publish or emphasize exposures with positive findings from case-control research. Given this tendency, the entire distribution seems somewhat shifted towards causal associations, but this is best viewed as an artifact of the aforementioned publication bias.

3.3.3. Interpretation

There is no new evidence to suggest that trichloroethylene is a cause of non-Hodgkin lymphoma. Instead, the new evidence supports the null hypothesis. The complete distribution of results is as expected for a truly null association.



3.4. Multiple myeloma

3.4.1. Summary of IARC 1995 review

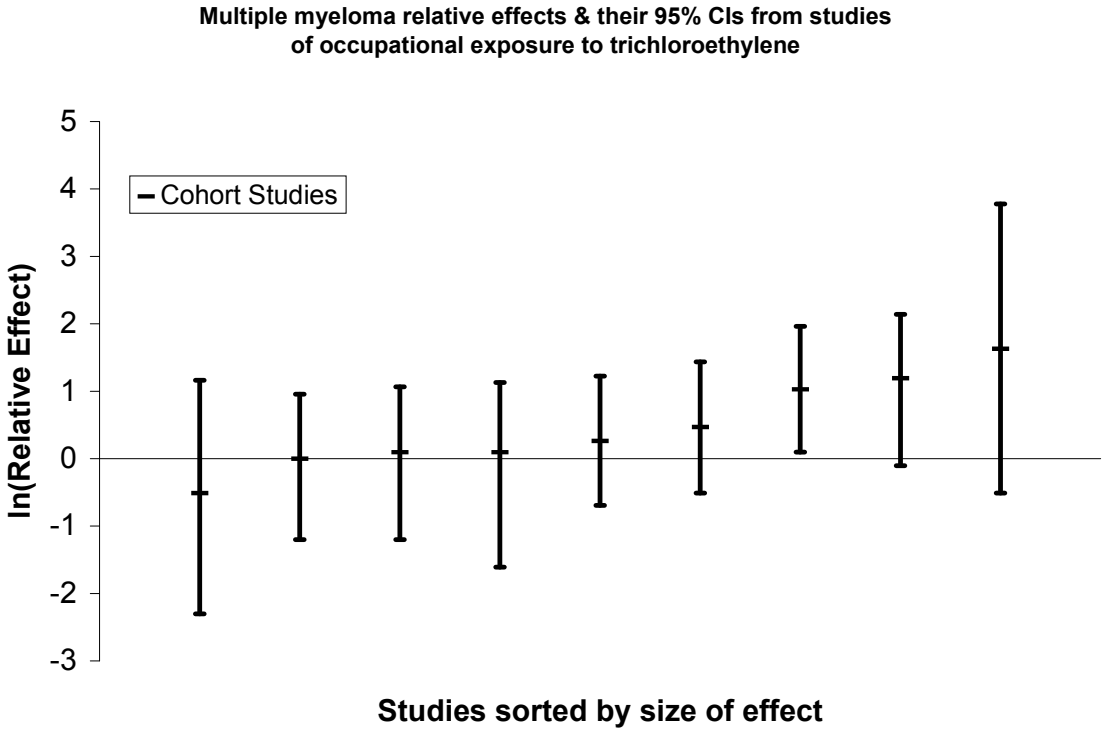
In its review of two studies with biologic monitoring to document trichloroethylene exposure, IARC[2] reported no SMRs for multiple myeloma. In its review of four cohort studies of miscellaneous manufacturing industries, IARC[2] made no mention of multiple myeloma findings. Findings were available for review, but not discussed.

IARC[2] reviewed no case-control studies of the association between trichloroethylene exposure and the risk of multiple myeloma.

With no evidence reviewed, IARC[2] offered no conclusion about the strength of the evidence associating trichloroethylene exposure with the risk of multiple myeloma.

3.4.2. Summary of new evidence

Since the IARC review,[2] two cohort studies have examined the association between occupational exposure to trichloroethylene and the risk of multiple myeloma.[5] The first cohort study is an update of an earlier investigation. The results from the update are an SMR of 1.3 (95% CI 0.5–3.4)[13] for mortality attributed to multiple myeloma, and an SMR of 5.1 (95% CI 0.6–43.7 for multiple myeloma incidence among men.[13] This result derived from a cohort exposed to trichloroethylene and other organic solvents, as described in the



published title. Boice *et al*. reported an SMR for mortality attributed to multiple myeloma of 2.8 (95% CI 1.1–7.1).[45] While the new evidence suggests a potential association, the accumulated evidence is too unstable to warrant a conclusion that the association is established as causal. This is particularly true in light of the evidence that preceded these recent results — evidence upon which that IARC[2] did not comment. That evidence suggests a null association between trichloroethylene exposure and the risk of multiple myeloma.

The Figure shows all of the studies of the association between occupational exposure to trichloroethylene and multiple myeloma.[5] The distribution is what one would expect for a truly null association. That is, the results from cohort studies with the widest intervals are nearer the left and right sides of the distribution. The most stable estimates concentrate about the null, and only one result's 95% confidence interval excludes the null.

3.4.3. Interpretation

While recent evidence suggests that there may be an association between trichloroethylene exposure and the risk of multiple myeloma, that evidence derives from a very small number of cases. Preceding evidence suggests no association. Taken together, the results do not establish that trichloroethylene causes multiple myeloma.

3.5. Prostate cancer

3.5.1. Summary of IARC 1995 review

In its review of two studies with biologic monitoring to document trichloroethylene exposure, IARC[2] reported an SMR for prostate cancer of 1.3 (95% CI 0.84–1.8) from one

study. For the second study, IARC reported an overall SMR of 1.4 (95% CI 0.73–2.4), an SMR of 0.68 (95% CI 0.08–2.4) for men with the highest exposure, and an SMR of 3.6 (95% CI 1.5–7.0) for men with a 20–year latency. In its review of four cohort studies of miscellaneous manufacturing industries, IARC[2] reported no prostate cancer SMR for two, an SMR of 0.80 (95% CI 0.5–1.2) for a third, and an SMR of 0.93 (95% CI 0.60–1.4) for the fourth.

IARC[2] reported an odds ratio of 1.8 (95% CI 0.7–4.7) associated with at least five years of exposure at a presumably medium or high concentration and frequency from one case-control study.

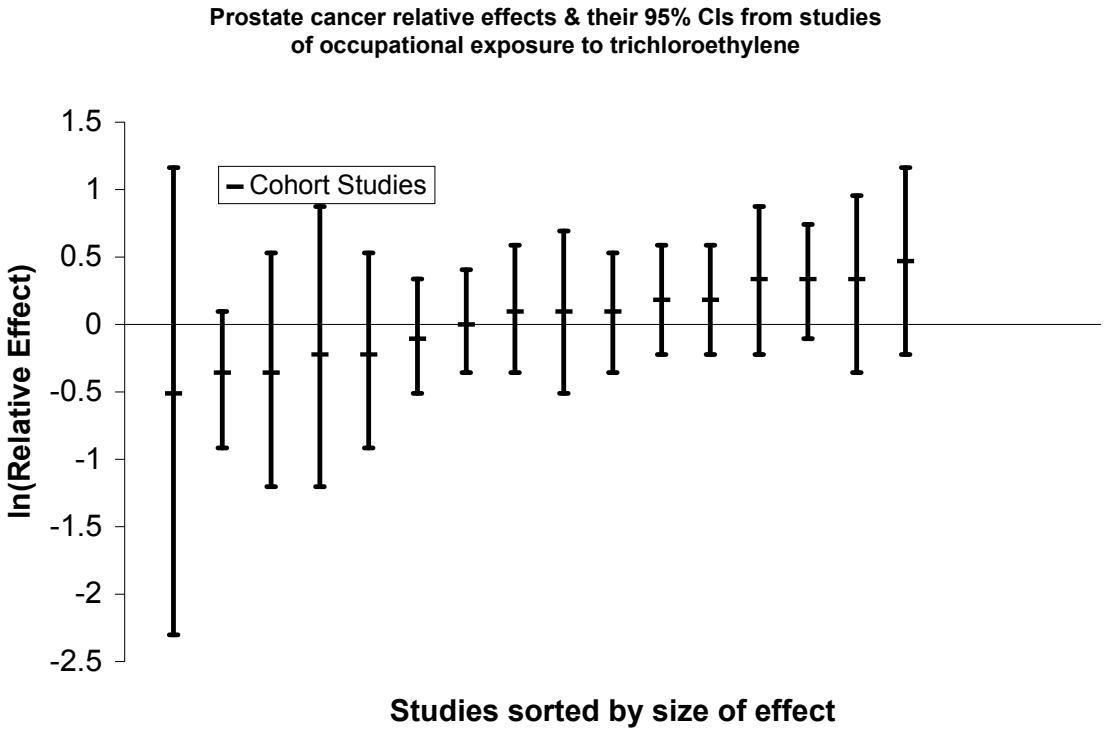
The prostate cancer associations were not mentioned in the summary section of IARC,[2] in which it was concluded that there was limited human evidence to suggest that trichloroethylene was carcinogenic.

3.5.2. Summary of new evidence

Since the IARC review,[2] four cohort studies have examined the association between occupational exposure to trichloroethylene and the risk of prostate cancer.[5] The first cohort study is an update of an earlier investigation. The results from the update are an SMR of 1.1 (95% CI 0.7–1.8)[13] for prostate cancer mortality and an SMR of 1.2 (95% CI 0.8–1.8) for prostate cancer incidence among men.[13] Boice *et al*. reported an SMR for prostate cancer mortality of 1.0 (95% CI 0.7–1.5),[45] Morgan reported an SMR for prostate cancer mortality of 1.2 (95% CI 0.8–1.8),[46] and Ritz reported an SMR for prostate cancer mortality of 1.4 (95% CI 0.9–2.1).[12] The majority of evidence accumulated since the IARC review[2] supports the null hypothesis.

There is no new evidence published since the IARC review[2] that would lead one to conclude that trichloroethylene causes prostate cancer.

The Figure shows all of the studies of the association between occupational exposure to trichloroethylene and prostate cancer.[5] The distribution is what one would expect for a truly null association. That is, the results from cohort studies are centered about the null with the widest intervals nearer the left and right sides of the distribution.



3.5.3. Interpretation

Results published since the IARC review[2] regarding the association between occupational exposure to trichloroethylene and the risk of prostate cancer are consistent with the findings published before 1995. That is, the distribution of results appears as one would expect for a null association.

4. Additional flaws in the *Draft Report*

- Sections 5.3 and 6.6.4 present information on vinyl chloride and other compounds "similar" to TCE (termed "structural analogues"). This "arguing by analogy" is highly inappropriate, and should be removed entirely from the *Draft Report*. Just as no sensible analyst would, for example, discuss methanol toxicology and epidemiology in a monograph on ethanol, no one writing about TCE should rely on the toxicology and epidemiology of vinyl chloride. This is especially true given the marked qualitative and quantitative differences in metabolism, mutagenicity, and other central aspects of the compounds at issue.

- The *Draft* frequently cites papers indirectly – for example, "Jaffe *et al*., 1985, Vamvakas *et al*., 1992, both cited in Vamvakas *et al*., 1993." Surely the *Draft* authors should have gathered and read the original papers, especially those published in readily available journals.

5. Conclusion

Neither the *Draft Report* nor the primary epidemiologic and toxicologic information on trichloroethylene provides compelling evidence that the chemical is a cause of human cancer. As a matter of public health *policy*, we might wish to regard TCE as if it were a risk factor for human cancer. Since the 1970's, U.S. EPA and others have been doing just that. But public policy decision making is not scientific decision making, and conflating the two processes makes for neither good policy nor good science. As the above analysis makes plain, the scientific evidence cannot be fairly judged as implicating TCE as a *bona fide* cause of cancer in humans — not even for those most likely to have been most highly exposed in the workplace, let alone for others.

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TRICHLOROETHYLENE and THE VHL TUMOR SUPPRESSOR GENE: COMMENTS IN RELATION TO US EPA'S AUGUST 2001 DRAFT "TRICHLOROETHYLENE HEALTH RISK ASSESSMENT: SYNTHESIS AND CHARACTERIZATION"

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This commentary is written in response to EPA's draft Trichloroethylene Health Risk Assessment: Synthesis and Characterization" report dated August 2001. My comments are restricted to aspects of the report involving a possible relationship between Trichloroethylene (TCE) exposure and VHL tumor suppressor gene mutations and animal models, since the role of VHL in renal tumorigenesis is my major area of expertise. This commentary is submitted in conjunction with the Halogenated Solvents Industry Alliance, Inc. for submission to US EPA. My affiliation and contact information are included at the end of this report.

1. Background on Human Renal Cancer and the VHL Tumor Suppressor

The classification of human renal cell carcinoma (RCC) is complicated and is based on histologic and cytologic evaluation.

i) Clear cell RCC account for about 75% to 80% of all cases. The term "clear cell" was derived because these RCC cells store excess lipid and glycogen and appear as nucleated cells with an empty cytoplasm after standard tissue processing with organic solvents for diagnosis.

ii) Chromophilic RCC account for about 15% of all cases and often have a papillary growth pattern, characterized by vascularized stalks of connective tissue surrounded by neoplastic cells. These tumors have historically been called papillary RCC in the literature.

The genetics of human RCC correlates well with histology and indicate that clear cell RCC and papillary RCC may have distinct genetic origins. Patients with von Hippel-Lindau disease (an inherited human cancer syndrome) have VHL gene mutations (chromosome 3p25.5) in the germ line and are strongly predisposed to developing clear cell RCC (reviewed in Gnarra 1996). The mechanism of tumorigenesis in VHL patients appears to be through deletion (loss of heterozygosity) of the chromosome arm 3p carrying the inherited wild type VHL allele and retention of the chromosome arm 3p carrying the inherited mutant VHL allele. There is strong evidence supporting a "gatekeeper" role for the VHL tumor suppressor in renal tumorigenesis.

Hereditary papillary RCC (HPRC) has been described (Zbar et al 1995) and these patients have germline activating mutations of the MET proto-oncogene on chromosome 7 (Schmidt et al 1998). HPRC patients show no involvement of the VHL tumor suppressor. Mechanisms of tumorigenesis in HPRC patients include amplification of the copy of chromosome 7 that carries the activated MET allele. Thus, HPRC is one of the few hereditary cancers (MEN2 with the RET proto-oncogene being another) involving proto-oncogene activation rather than tumor suppressor gene inactivation.

In addition to playing a role in inherited disease the VHL tumor suppressor is somatically inactivated in probably about 70% to 80% of sporadic clear cell RCC cases. Mechanisms of

somatic VHL inactivation include mutation (microdeletions or insertions leading to frameshift mutations or non-conservative amino acid substitutions) of one VHL allele and loss of heterozygosity of the other VHL allele. Somatic VHL mutations are seen in about 50-60% of clear cell RCC cases. Different studies that analyzed VHL mutations in RCC cases have reported varying percentages of tumors with VHL mutations, ranging from 33% to 57% (Foster et al 1994; Gnarra et al 1994; Shuin et al 1994; Whaley et al 1994; Brauch et al 2000). There are at least two reasons for this variance among studies. First is the issue of diagnosis. Studies that employed multiple pathologists to review tumor histology in a blinded manner tended to show greater VHL mutation rates, probably because they segregated cases into clear cell or papillary histologies more accurately. The second source for variance involves methods of DNA extraction from tissues and mutational analyses. RCC tumors are highly vascular and a tissue sample may contain a greater number of lymphocytes than tumor cells. Therefore, studies in which DNA was extracted from whole pieces of tissue tended to show lower VHL mutation rates because of the large number of contaminating normal cells. On the other hand studies employing clear cell RCC-derived cell lines or tissue microdissection tended to show higher VHL mutation rates. In addition, direct sequencing of the entire VHL gene has proven to be more accurate than analyses using single strand conformation polymorphism (SSCP) gels, which was used as a primary screen in very early studies. In up to about 20% of clear cell RCC cases the VHL gene is hypermethylated with consequent transcription silencing (Herman et al 1995; Brauch et al 2000). The conclusion is that probably up to 80% of all clear cell RCC cases involve VHL tumor suppressor gene activation (mutation or methylation-induced gene silencing). The remaining ~20% of clear cell RCC cases do not yet have a clear genetic basis.

It has also been shown that familial clear cell RCC (FCRC) occurs and is independent of the VHL tumor suppressor. The et al (1997) and Clifford et al (1998) analyzed several kindreds and failed to demonstrate linkage of FCRC families with the VHL locus. To date the genetic basis for these tumors is unknown (Woodward et al 2000). It is likely that when the FCRC gene is described we will also gain an understanding for the genetics on non-VHL related sporadic clear cell RCC.

While VHL clearly plays a “gatekeeper” role in the majority of clear cell RCC, it has been suggested that additional genes on chromosome arm 3p may also play a role in tumorigenesis. The fact that we commonly see loss of heterozygosity of loci on 3p12-21 in renal cancer, as well as many other malignancies such as lung cancer, indicates that additional tumor suppressors may map to these loci. Martinez et al (2000) studied a number of clear cell RCC with or without inactivation of VHL. They showed that both VHL-negative and –positive clear cell RCC showed a similar high frequency of 3p12-21 loss of heterozygosity, but VHL-positive clear cell RCC showed less frequent loss of heterozygosity at 3p25. Their data support the possibility that loss of VHL alone may be insufficient for renal tumorigenesis and that loss of additional tumor suppressor(s) more centromeric on chromosome arm 3p may be important for RCC development. This is supported by the observation that loss of chromosome arm 3p heterozygosity uniformly occurs in tumors from VHL patients. A situation in which a second mutation occurring in the inherited wild type VHL allele, without chromosome arm 3p loss of heterozygosity, has not been reported.

In summary, RCC in humans is a complicated disease with varying histologies and the probable involvement of multiple genes. Loss of VHL tumor suppressor activity is clearly important for the development of clear cell RCC, but additional genes perhaps also on chromosome arm 3p may also play a significant role in tumorigenesis.

2. Animal Models

The development of animal models for RCC has not been straight-forward. VHL knockout mice have been independently derived and analyzed by two separate laboratories (Gnarra et al 1997; Haase et al 2001). In each study, VHL null embryos died at about day 10 of gestation, indicating that the VHL protein is required for normal development. VHL heterozygotes developed normally and when maintained on a C57BL/6 background showed no increased incidence of tumor formation in the kidneys or in other organs (Gnarra et al 1997). Haase et al (2001) developed conditional VHL knockouts on a BALB/c background and found that VHL heterozygotes animals developed cavernous hemangiomas of the liver. No renal lesions were noted. Therefore, whether VHL plays a role in renal tumorigenesis in mice is unclear.

The Eker rat model has proven to be very useful in the study of the etiology of RCC. Eker rats have a germline mutation in the tuberous sclerosis 2 (TSC2) tumor suppressor gene (reviewed in Walker 1998). These animal develop spontaneous multiple, bilateral renal tumors at high frequency and exhibit heightened sensitivity to treatment with a variety of carcinogens (Walker 1998). Chemical induction of renal tumors in other rat strains has also been extensively analyzed. Rat RCC tumors (Eker or other strains) are not typically of the clear cell type, but rather tend to be chromophobic. Chromophobic RCC are seen in about 5% or fewer of human cases. Several groups have analyzed a large number of rat renal tumors for VHL and TSC2 mutations. The TSC2 tumor suppressor is a common target for inactivation in rat RCC, while VHL mutations have not commonly been observed (Walker 1998). The situation is probably similar for the mouse, since TSC2 knockouts show RCC susceptibilities and histologies similar to the Eker rat. One group identified 8 rat RCC with a clear cell histology from a large group of tumors induced by N-nitrosodimethylamine (NMDA) (Shiao et al 1998). They classified clear cell RCC in rats as "rare" and did not indicate exactly how many tumors were analyzed to find these 8 samples. Three of the 8 rat clear cell RCC tumors showed VHL mutation, while 40 other NMDA-induced rat tumors of other histologies did not have VHL mutations. This supports the involvement of VHL in formation of clear cell RCC. However, it is clear that conclusions regarding susceptibility to developing carcinogen-induced renal cancer between laboratory animals and humans must be made with a great deal of caution. The fact that rats (and probably mice) and humans appear to have different target genes for RCC tumorigenesis (TSC2 versus VHL) and different RCC phenotypes (chromophobic versus clear cell) complicates the translation of carcinogenesis data. It is not yet clear whether a carcinogen that targets TSC2 and induces renal tumors in rats will similarly target VHL and induce renal tumors in humans. Therefore, any animal data relating TCE and renal tumors must be cautiously interpreted pending evaluation of tumor histology and genetic mutations in the TSC2 and VHL genes.

3. Comments on the Findings of Brauch et al (1999)

The report from Brauch et al (1999) that extends the study by Bruning et al (1997), had many unique and interesting findings, but also raised some questions:

3.1 Multiplicity of VHL Mutations

Brauch et al (1999) identified a high frequency of multiple VHL mutations within individual tumors along with an association between multiplicity of VHL mutations and TCE exposure levels.

The lack of a precise classification of TCE exposure levels of the study population, as well as the presence or absence of other potential risk factors, is a weakness. It is difficult mechanistically to account for multiple VHL mutations present in a clonal population of tumor cells. Multiple mutations within the VHL gene would not be likely to contribute a selective advantage to the transformed cells. It will be critical to confirm these findings in independent laboratories and with the same and additional RCC samples from TCE-exposed patients

3.2 VHL Mutational Hot-Spot and TCE

A VHL mutational hot-spot was identified in clear cell RCC from TCE-exposed patients. This is the first study to report such a hot-spot, and similar results have not been reported previously in other VHL gene mutation studies. To confirm the significance of this hot-spot, it will be necessary to identify and evaluate other RCC patients with similar chemical exposure to determine whether these findings can be reproduced.

3.3 Summary

The report by Brauch et al (1999) indicating that the VHL gene may be a target of TCE is potentially very significant. The fact that these patients with clear cell RCC have VHL tumor suppressor gene mutations is expected, given the well-described involvement of VHL in renal tumorigenesis. Issues regarding exposure levels of the study population, the presence of co-existing multiple VHL mutations, and the potentially conflicting data presented by Schraml et al (1999) indicate that caution should be used in interpreting these findings. Additional studies on other TCE-exposed human populations are warranted to confirm these data as well as mechanistic studies to determine if TCE is a renal carcinogen at relevant exposure levels.

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Attachment 6

Comments on the Quantitative Analyses of Epidemiological Data in the EPA's Trichloroethylene Health Risk Assessment: Synthesis and Characterization

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January 16, 2002

Comments on the Quantitative Analyses of Epidemiological Data in EPA's Trichloroethylene Health Risk Assessment: Synthesis and Characterization

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Abstract

In the EPA's Trichloroethylene (TCE) Health Risk Assessment Synthesis and Characterization (EPA/600/P-01/002A, August 2001), the EPA calculates a number of risk estimates based on epidemiological studies (Anttila et al., 1995, Cohn et al., 1994, Henschler et al., 1995). These estimates are used in the document to support a summary range of risk estimates that also includes the agency's linear risk estimates based on mouse liver tumors, as well as to suggest that risks could be much higher than the summary range. However, none of these epidemiological studies are strong enough quantitatively to support such definitive conclusions. In particular, the highest EPA risk estimates were obtained from a Finnish cohort (Anttila et al., 1995), for tumor endpoints that were characterized in the original publication as negative (no statistically significant increase associated with exposure to TCE). Unfortunately, the EPA's analysis of this study is inadequately documented, and only a portion of the unpublished data they used in their analysis is provided, so that it is not possible to determine exactly how the agency's risk estimates were obtained or to verify their results. Thus a major factor in the EPA risk assessment for TCE can not be adequately evaluated.

Discussion

In their TCE Health Risk Assessment Synthesis and Characterization (EPA/600/P-01/002A, August 2001), the EPA describes quantitative dose-response analyses performed by the agency with three different epidemiological studies (Anttila et al., 1995, Cohn et al., 1994, Henschler et al., 1995). These analyses play a significant role in the agency's risk assessment for TCE. The risk estimates obtained by the agency for the Cohn et al. (1994) and Henschler et al. (1995) cohorts serve, along with linear risk estimates based on mouse liver tumors, as the primary basis for their summary range of risk estimates. The risk estimates obtained by the agency from

the Anttila et al. (1995) cohort are used to support the suggestion that risks could be even higher than those in the proposed summary range.

In the case of the first two epidemiological studies (Cohn et al., 1994, Henschler et al., 1995), the EPA presents risk estimates in their TCE Health Risk Assessment Synthesis and Characterization using simplistic calculations based on the published study results. However, both of these studies are inadequate to support a quantitative dose-response analysis for TCE. In one case (Henschler et al., 1995), no exposure data was provided, and an East German regulatory exposure limit was used by the EPA as a surrogate for the air concentrations of TCE to which the workers were actually exposed. In the other case (Cohn et al., 1994), all of the cancer outcomes reported in the publication were attributed by EPA to TCE in the drinking water in spite of the fact that the agency admits in the Synthesis and Characterization that: "The residents were exposed to other drinking water contaminants, so that attributing all risk to TCE can overestimate the risk from TCE." Thus neither of these studies provides an adequate basis for quantitative dose-response modeling of TCE carcinogenicity.

In the case of the Anttila et al. (1995) cohort, the EPA obtained the unpublished data from the authors and performed their own analysis, which is documented only in the TCE Health Risk Assessment Synthesis and Characterization. Unfortunately, the brief description in the document is not adequate to fully document the analysis, and only a portion of the unpublished data is provided,[1] so that it is not possible to determine exactly how the agency's risk estimates were obtained or to verify their results. Surprisingly, the highest risks estimated by the EPA for TCE were obtained from their own analysis of this Finnish cohort, for tumor endpoints that were characterized in the original publication (Anttila et al., 1995) as negative. Specifically, the EPA calculates risks for TCE-induced kidney tumors, liver tumors, and non-Hodgkins lymphoma, but in the original analysis reported by Anttila et al. (1995) no statistically significant increases in any of these tumor endpoints were associated with exposure to TCE.

[1] The EPA included a footnote in the TCE Health Risk Assessment Synthesis and Characterization that provides data for each cancer case for the three tumor endpoints analyzed. These data include average urinary TCA, age at first TCA measurement, age at last TCA measurement, and age at end of follow-up. However, the similar data for the other members of the cohort are not provided.

Conclusions

The risk estimates obtained by EPA from their inadequately documented analysis of the Anttila et al. (1995) cohort range from 0.07 to 7 $(mg/kg/day)^{-1}$, well above the risks they estimate from other human and animal data. The EPA uses these results to support the reasonableness of their proposed slope factor range of 0.02 – 0.4$(mg/kg/day)^{-1}$: "Because they are supported by diverse studies and do not reflect the highest estimates (from the Anttila study) or the lowest estimates (from the rat studies), these remaining estimates constitute a middle range of risk estimates where confidence is greatest." Considering the potential impact of the extremely high risk estimates obtained by the EPA from their analysis of the Anttila et al. (1995) cohort, it is reasonable to expect that the methods used to obtain them should be clearly described, and that the data on which they are based should be made available to the public, so that the agency's results can be verified and critically evaluated. In similar situations with other chemical risk assessments, the agency has published a technical support document as an agency report. Such a support document is needed in this case, containing a detailed description of the methods used in the analysis of the Anttila et al. (1995) cohort, the assumptions made in the analysis, and the models applied, along with a complete listing of the unpublished data used in the analysis.

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Attachment 7

Comments on the Role of DichloroaceticAcid in the Production of Mouse Liver Tumors from Exposure to Trichloroethylene, and the Implications for a Trichloroethylene Risk Assessment

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January 9, 2002

Introduction

The United States Environmental Protection Agency (USEPA) recently released a Health Risk Assessment Synthesis and Characterization for trichloroethylene (TCE) (USEPA 2001). With regard to the mode of action (MOA) of TCE-induced rodent liver tumors and relevance of that MOA to human health, a major focus of the USEPA risk assessment is the potential role of the oxidative metabolites of TCE, trichloroacetic acid (TCA) and dichloroacetic acid (DCA). Based on their interpretation of several state-of-the-science papers (Bull 2000, Chen 2000, Lash 2000), the USEPA concludes that DCA may play a major role in the formation of TCE-induced rodent liver tumors. The USEPA also concludes that TCA may contribute to the formation of TCE-induced rodent liver tumors. However, the agency's emphasis on DCA and its apparent peroxisome-proliferation-independent MOA provides much of the basis upon which they conclude that the MOA for formation of TCE-induced liver tumors is relevant to humans. The purpose of this review is to evaluate the conclusions drawn by the USEPA regarding the proposed MOA for TCE-induced liver tumors and the relevance of the MOA to human health, with particular focus on the evidence for a role for DCA in the formation of TCE-induced liver tumors in rodents and in humans.

Mode of Action Considerations

The USEPA (2001) concludes that the available experimental evidence supports a MOA hypothesis for the formation of TCE-induced liver tumors that involves the actions of TCA and/or DCA. The two primary modes of action that they suggest could be associated with the formation of TCE-induced liver tumors are activation of the peroxisome proliferator-activated receptor (PPARα) by TCA[1] and effects on glycogen

[1] In their risk assessment, the USEPA distinguishes the older hypothesis that TCA-induced liver tumors were linked to its ability to induce peroxisome proliferation, resulting in oxygen stress and subsequent DNA damage, which the agency refers to as a peroxisome proliferation MOA, from an MOA involving PPARα-mediated alterations in cell signaling that arise parallel to the induction of peroxisome proliferation. In either case the initiating event is still the binding and activation of PPARα. It has been reported that that genetically altered PPARα-knock-out mice lose the ability to respond to peroxisome proliferators and also become resistant to the induction of liver tumors by this class of compounds (Bull 2000).

metabolism by DCA, with both MOAs proposed to alter cell-signaling systems that control rates of cell division and death (Bull 2000). Based on the evidence for the MOAs of TCA and DCA, and based on the similarities of some of the pleiotropic effects elicited by TCE to those of either TCA or DCA, the USEPA concludes that both TCA and DCA are likely to contribute to the formation of TCE-induced tumors. Unfortunately, although there are indeed certain similarities that can be identified between TCE-induced rodent liver effects and those induced by either TCA or DCA, many of the parallels that can be drawn are mutually contradictory. For example, the USEPA points out that subsets of TCE- and DCA-induced tumors tended to be similar with regard to certain genetic markers, whereas tumors expressing these markers tended to be absent following TCA exposure. However, the agency also notes that TCE and TCA both induce tumors only in susceptible strains of mice, whereas DCA induces tumors in both mice and rats. Thus these comparisons are not as straightforward taken as a whole as they may seem when considered individually. The available mechanistic data simply does not provide an adequate basis for determining the relative contribution of TCA and DCA to TCE-induced liver tumors. In particular, there is no unambiguous basis for positing a significant role for DCA.[2]

It is clear, on the other hand, that TCA-mediated effects occur following administration of TCE. Specifically, the activation of PPARα and the stimulation of peroxisome proliferation that are observed following exposure of mice to TCE are

[2] While the USEPA acknowledges that TCE, TCA, and DCA are not considered competent genotoxic agents based on the state-of-the-science report by Moore and Harrington-Brock (2000), the USEPA suggests that there were certain similarities between DCA- and TCE-induced tumors that support the conclusion that DCA contributes to the formation of TCE-induced liver tumors. Bull (2000) reports that whereas DCA- and TCE-induced tumors show a low frequency of mutation, as compared to spontaneous tumors, TCA-induced liver tumors show a frequency of mutation almost identical to spontaneous tumors. DCA- and TCE-induced tumors also reportedly share nucleotide sequences within codon 61 of h-ras that are different from those observed in spontaneous tumors, thus further suggesting a commonality between the tumor types. In contrast, nucleotide sequences in TCA-induced tumors were similar to those observed in spontaneous tumors. The numbers of c-jun+ tumors observed when liver tumors were induced by TCE or DCA were also similar, as were the numbers of tumors that did not display a mutation to c-jun. In contrast, TCA tumors do not display mutations in c-jun, and thus, do not label c-jun+. The USEPA suggests that the most plausible explanation for these similarities between liver tumors induced by TCE and DCA, is that TCE-induced tumors are the result of DCA effects, and thus that these similarities support an inference that DCA is an important contributor to the formation of TCE-induced liver tumors. This evidence is, however, highly circumstantial, and other factors associated with the TCE exposures, including effects from the corn oil vehicle, could result in the above noted differences between tumors produced by corn-oil gavage with TCE and drinking water exposure to TCA.

primarily associated with the effects of TCA. DCA has been shown to be a weak peroxisome proliferator that only produces transient increases in peroxisomes. TCE itself has been reported to lack activity *in vitro* at PPARα (Zhou and Waxman 1998), and thus, it is evident that the peroxisome proliferation and other PPARα-mediated effects associated with exposure to TCE are predominantly the result of production of the metabolite TCA. As acknowledged in both the USEPA risk assessment and in the review by Bull (2000), the exact mechanism underlying the formation of liver tumors induced by peroxisome proliferators has not been completely elucidated. Based on this uncertainty, the USEPA concludes that a PPAR-mediated MOA for tumor formation in humans can not be ruled out.[3] However, as pointed out by Bull (2000), a number of expert panels "…have generally concluded that peroxisome proliferators, per se, are unlikely to represent a carcinogenic hazard under anticipated conditions and levels of [human] exposure…"

The effects of TCA appear to mimic those of a classic tumor promoter; in particular, its effects are consistent with the "negative selection" hypothesis that has been proposed to explain the activity of other mitogenic tumor promoters (Bull 2000). While

[3] Humans have also been shown to be less responsive, if not totally resistant, to the effects of peroxisome proliferators, and expression of PPARα messenger RNA has been reported to be significantly less in human liver when compared to susceptible murine species (Bull 2000). While the USEPA acknowledges that there are species and strain differences with regard to the pleiotropic responses resulting from activation of PPARα, it maintains that there is not sufficient evidence to support the existence of qualitative differences between rodents and humans (USEPA 2001). Consequently, the USEPA concludes that a PPAR-mediated MOA for TCE-induced liver tumors would be relevant in decisions regarding human health. In support of this conclusion, the USEPA cites the studies conducted by Maloney and Waxman (1999), in which it was reported that no species differences were noted in the magnitude of PPARα activation in response to exposure to TCA and DCA. However, in other studies, DCA and TCA readily induced palmitoyl CoA oxidation in rodents, whereas DCA and TCA had no effect on palmitoyl CoA oxidation in human hepatocytes, thus illustrating qualitative species differences (Walgren et al. 2000). The USEPA also cites the review by Bull (2000), which states that, although peroxisome proliferators failed to elicit a PPAR-mediated response in human tissue, there does exist an analogous protein in human tissue and that cis-acting peroxisome proliferator-responsive elements (PPREs) have been identified in the 5'-flanking regions of relevant genes in humans. However, the review by Bull (2000) does not fully support the conclusion of the USEPA that PPAR mediated responses are relevant to humans, with regard to the formation of liver tumors. Bull (2000) goes into much greater detail on the mechanism of PPAR-mediated responses, the whole of which suggests that the quantitative differences in PPARα expression between rodent and human liver could effect the formation of PPARα and PPARγ heterodimers, thus changing the qualitative influence of the receptors. Bull (2000) concludes that PPARs may be important to developmental biology in all species, including humans. However, the discussion stops short of stating that liver tumors arising via PPAR-mediated pleiotropic responses are relevant to human health. In summary, the conclusion of the USEPA regarding the relevance of a PPAR-mediated MOA to humans is not supported by the preponderance of the experimental evidence, and does not accurately reflect the discussion in Bull (2000).

TCA has been found to depress replication rates of normal hepatocytes, experimental evidence suggests that TCA irreversibly promotes the expansion of a subgroup of cells, with the subsequent rate of replication for this subgroup being unaltered by termination of TCA treatment or re-exposure. TCA has also been reported to irreversibly modulate the expression of *c-myc* via demethylation, which has been suggested to result in an increase in cell replication. The relationship of the irreversibility in tumor promotion and the irreversibility of *c-myc* activation is not known; however, as proposed by Bull (2000), it is likely to be a property of the particular clone of cells promoted by TCA.

Unlike TCA, the effects of DCA are best described as insulin-mimetic (Bull 2000).[4] DCA treatment results in significant accumulation of glycogen in extrafocal hepatocytes accompanied by decreases in plasma glucose concentrations and plasma insulin levels. There are no reported effects on glycogen accumulation or plasma glucose or insulin levels resulting from exposure to TCA. There also have been no reported TCE-induced effects on glycogen accumulation. In contrast to the effects of DCA, TCE has been reported to have no effect on plasma insulin levels in rodents (Arai 1989) and to slightly increase plasma insulin levels in humans (Goh et al. 1998). TCE has also been reported to decrease plasma glucose concentrations, but unlike DCA, this decrease was

[4] In normal hepatocytes in mice ingesting DCA (~ 0.5 g/L) in water, DCA was reported to inhibit pyruvate dehydrogenase kinase and increase glycogen accumulation (glycogenosis), which in turn inhibited glycogen synthase. The subsequent decrease in glycogen synthesis was speculated to be responsible for the observed DCA-induced decrease in expression of insulin receptors in normal hepatocytes. DCA was also reported to decrease plasma insulin concentrations independently from a DCA-induced decrease in plasma glucose (Bull 2000). The net effects of DCA exposure on normal hepatocytes were glycogenosis (accompanied by cytomegaly), decreased cell replication, and a proposed decrease in apoptosis (USEPA 2001, Bull 2000). The decrease in cell replication and decreased rate of apoptosis was said to be the result of the decrease in insulin receptor expression coupled with a decrease in plasma insulin concentrations and a direct inhibitory effect of DCA on normal hepatocyte replication (Bull 2000). Accumulation of glycogen was reported to be a common characteristic noted in normal cells prior to pre-neoplastic development in livers from rodents and humans and was also reported to be inversely related to cell replication (Bull 2000, USEPA 2001). The dose-response for glycogenosis in normal hepatocytes was reported to be in the same range (0.5 g/L when administered in water) where DCA induced liver tumors (Bull 2000). The conclusion drawn by the USEPA and by Bull (2000) was that glycogenosis and DCA-induced tumors may be the result of modifications of the same cell signaling pathway. The most notable issue regarding the metabolic and cell signaling effects of DCA with regards to the formation of rodent liver tumors, was related to the reported resistance of certain pre-neoplastic cell populations to DCA-induced down-regulation of insulin receptors. These resistant cell populations responded to DCA treatment by slightly over-expressing insulin receptors. Because insulin is mitogenic in hepatocytes, the resistant cell population gained a proliferation advantage over the normal hepatocytes. Further, over-expression of insulin receptors was reported to be associated with a decrease in apoptosis, giving the pre-neoplastic cell populations a further growth advantage over the extrafocal hepatocytes (Bull 2000).

attributed to a decrease in renal re-absorption of glucose accompanied by glycosuria (Arai 1989).

As with TCA, experimental evidence suggests that DCA elicits a differential effect on normal and susceptible cell groups. In normal hepatocytes, DCA increases glycogen accumulation, decreases insulin receptor expression, and decreases cell replication and apoptosis. In contrast, DCA has no effects on glycogen storage in pre-neoplastic cells, it slightly increases the expression of insulin receptors, and at high doses, has been reported to stimulate cell replication in a select group of cells. Unlike TCA, DCA-mediated tumor promotion appeared to be reversible, with cessation of neoplastic progression occurring upon termination of treatment. Similarly, DCA induces a reversible hypomethylation which returned to control status upon termination of DCA exposure. Furthermore, DCA exerts a mitogenic effect on groups of cells that label for *c-jun* rather than *c-myc*. Consequently, Bull (2000) concludes that the tumor promoter properties of DCA are unlike those of TCA, primarily due to the fact that DCA and TCA promote different groups of cells.

Despite these differences between the MOAs for TCA and DCA, Bull (2000) concludes that:

> "If DCA- and TCA-induced mechanisms can be assumed to be good models for TCE-induced carcinogenesis, it would appear that tumors are only induced by doses of the compounds that result in significant downregulation of normal control mechanisms in normal cells. Apparently it is this negative selection process that is active at low doses of both metabolites. If sufficient perturbation has to be produced in normal cells for downregulation to be observed, then it is probable that the tumorigenic response has an effective threshold."

That is, whether the mode of action for the induction of mouse liver tumors by TCE is associated with activation of the PPAR receptor by TCA or effects on the insulin receptor by DCA or a combination of the two, the resulting carcinogenic process is inherently nonlinear, with negligible risk at doses which fail to produce profound effects on cell-signaling systems. As stated by Bull (2000): "Since the activity of cell-signaling systems is constantly modified as part of normal physiological function, it seems unlikely

that perturbations that do not take them outside their normal operating limits are going to have irreversible and cumulative effects."

Dosimetry Considerations

In addition to comparison of the qualitative MOA information for TCE with that for TCA and DCA, it is also important to establish whether an adequate quantity of either or both of the metabolites is produced following administration of TCE to explain the observed increase in liver tumors. This issue is particularly important with regard to the implication of DCA as a driving force in the formation of TCE-induced liver tumors. The USEPA suggests that DCA would be produced in sufficient amount to induce liver tumors in the TCE bioassays. To support the suggestion that DCA would be produced in sufficient quantities to induce liver tumors in the TCE bioassays, the USEPA cites the results of Barton et al. (1999). Based on this report, the USEPA concludes that "concentrations of DCA in mice produced from exposures experienced in the TCE gavage bioassay are of sufficient quantity to induce hepatic tumors." In stark contrast to the conclusion reached by USEPA, the conclusion reached by Barton et al. (1999) was that the levels of DCA produced in mice resulting from a tumorigenic dose of TCE would be insufficient to account for the observed liver tumors resulting from the TCE exposure.[5]

[5] The USEPA conclusion was based on the results of the Barton et al. (1999) modeling that predicted DCA concentrations in the blood would be equivalent to approximately half the levels measured in blood samples taken from mice ingesting DCA at a concentration of 0.5 mg/L. The USEPA suggests that this level of DCA production is comparable to concentrations reported to result in an increased incidence of hepatocarcinoma based on reports from Daniel et al. (1992) and DeAngelo et al. (1999). The study conducted by Daniel et al. (1992) reported a significant increase in liver tumors at the 0.5 mg DCA/L concentration. In the study conducted by DeAngelo et al. (1999), significant increases in hepatocarcinomas were noted in mice ingesting DCA at a concentration of 1.0 mg/L. However, DeAngelo et al (1999) also tested 0.05 and 0.5 mg DCA/L concentrations and found no significant increase in the incidence of liver tumors at these concentrations. In the report by Barton et al. (1999), estimated exposure to DCA resulting from a 1000 or 2000 mg/kg dose of TCE were estimated to be 0.25 and 0.31 mg-hr/L, respectively, assuming conservatively that all non-trichloro-metabolites were DCA. These concentrations were reported to be "between those concentrations in blood estimated for the 0.05 g/L and 0.5 g/L drinking water exposures to DCA" (Barton et al. 1999). Barton et al. (1999) commented that the 0.05 g/L dose of DCA was not associated with an increase in the incidence of liver tumors, but that the modeled doses of TCE were associated with large increases in liver tumor incidence in male mice, and smaller, but significant increases in female mice. Barton et al. (1999) concluded that the blood levels of DCA produced "from oral TCE exposure is similar to that estimated for the 0.05 g/L DCA drinking water exposure which was not associated with an increase in prevalence of liver cancer."

As an additional argument to support their conclusion, the USEPA risk assessment cites a report by Gonzales-Leon et al. (1999) which states that the elimination half-life of DCA was increased upon repeated exposure. The USEPA also cites the reports by Tong et al. (1998) and Tzeng et al. (2000) regarding the tendency of DCA to inhibit its own metabolism. However, the increase in the elimination half-life for DCA and the increase in DCA levels resulting from metabolic inhibition were associated with exposure to high concentrations of DCA (2 g/L) and would not be likely to occur at the much lower concentrations of DCA that could be expected as a result of TCE metabolism.

The conclusion of Barton et al. (1999) was further supported by the experimental data generated in the studies conducted by Merdink et al. (1998, 1999) who reported that the concentrations of DCA in blood, urine and feces collected from mice and rats following intravenous administration of TCE, CH, and TCA at doses on the order of 100 mg/kg were below the detection limit of 2 uM, even in animals pretreated with 2 g/L DCA for two weeks to address the potential inhibitory effects cited by the USEPA. A recent study conducted by Bloemen et al (2001) also failed to detect DCA in urine samples collected from human volunteers exposed to TCE, TCE and TCA or in urine samples collected from workers exposed to TCE occupationally.

The results generated with a biologically-based model employed by Chen (2000) were also used by the USEPA to support the suggestion that DCA plays a quantitatively important role in the production of liver tumors in the TCE bioassays. Indeed, Chen (2000) suggests that the mode of action of DCA represents the common mechanism underlying the formation of TCE-, TCA- and DCA-induced liver tumors. However, as previously discussed, DCA-induced tumors and TCA-induced tumors are phenotypically distinct, and as such are unlikely to arise from a common mechanism or cell population. Further, if DCA were the principal carcinogenic component of TCA exposure, and taking into the account the reported linearity of DCA production from TCA (Lash 2000), then one would expect the dose-response curves in the two bioassays to be similar. However, this is not the case, as the dose-response curve for TCA is gradual and linear whereas the dose-response curve for DCA is steep and non-linear (Bull 2000).

Moreover, the results generated with the Chen (2000) model were reported to be highly variable depending on the data used as input. Chen (2000) used data generated by the physiologically based pharmacokinetic (PBPK) model developed by Fisher (2000), which modeled concentrations in the liver. However, Chen acknowledged that the goodness of fit of the model changed significantly if the input data were drawn from the results of the PBPK model developed by Clewell, and that the results using that input data would imply a significant contribution from both TCA and DCA (Chen 2000). In fact, while the empirical, quasi-"biologically-based" modeling performed by Chen (200) is a useful exploratory tool for hypothesis generation, it does not represent a true biologically-based model with parameters derived from experimental data, and its conclusions must be viewed with caution.

A final argument put forth by the USEPA in support of the possibility that DCA is produced in sufficient quantities to account for the formation of TCE-induced tumors was the observation that similar numbers of *c-jun*+ tumors were observed when liver tumors were induced by TCE or DCA. Also, the numbers of tumors that did not display a mutation to *c-jun* were also similar in mice treated with TCE and DCA. TCA tumors tended not to display mutations in *c-jun*, and also tended not to label *c-jun*+. The USEPA implies that the most plausible explanation for these similarities between liver tumors induced by TCE and DCA was that TCE-induced tumors were the result of DCA effects, and thus, DCA must be produced in sufficient quantities to induce liver tumors. However, another equally plausible explanation for these similarities is that other factors associated with the administration of TCE in corn oil effect similar cell signaling pathways, or promote the clonal expansion of the same groups of cells, as DCA.

In contrast, there is little doubt that TCA is produced in concentrations sufficient to account for its possible role in the formation of TCE-induced liver tumors. TCA-induced tumors are observed in the same dose-range as other TCA-mediated effects, including peroxisome proliferation, suppression of hepatocyte replication, tumor promotion, and hypomethylation. Both measured concentrations of TCA in pharmacokinetic studies of TCE dosing and the observation of TCA mediated effects such as peroxisomal proliferation at TCE bioassay doses provide clear evidence that TCA

is present in adequate concentrations to account for the mouse liver carcinogenicity of TCE.

Conclusions

Although there is circumstantial evidence supporting a role for DCA in the induction of mouse liver tumors by TCE, the best available dosimetry information does not support the use of a mode of action that relies on the presence of tumorigenic concentrations of DCA following the administration of TCE. Moreover, as a result of the significant uncertainties associated with the dosimetry estimates for DCA, it does not seem appropriate to perform a quantitative risk assessment using these data. There is also substantial evidence supporting a role for TCA in the induction of mouse liver tumors by TCE, and there is no question that sufficient levels of TCA are produced in the positive TCE bioassays. Therefore, any quantitative dose-response assessment for TCE-induced liver tumors should be based on the dosimetry for TCA.

Finally, whether the mode of action for the induction of mouse liver tumors by TCE is associated with activation of the PPAR receptor by TCA or interaction with the insulin receptor by DCA, the mode of action is inherently nonlinear, as discussed by Bull (2000), and is consistent with an effective threshold below which cancer risk is negligible. As concluded by Bull (2000), the assumptions underlying the use of a linear risk estimate do not apply in the case of TCE-induced mouse liver tumors. Therefore, the only appropriate risk assessment approach for these tumors is a margin of exposure approach based on tissue exposure to the metabolite TCA.

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Appendix: Potential Sensitive Populations to the Effects of DCA

A concern of any risk assessment is the issue of sensitive subpopulations. The USEPA proposes that diabetics and those with glycogen storage disease may be at significantly increased risk from the liver carcinogenicity of TCE. The support for this conclusion was primarily based on parallels that could be drawn between some of the observed characteristics of these disease states and observed effects in the liver of rodents associated with DCA exposure.

The USEPA states that the results of epidemiology studies indicate that diabetics may be at increased risk for developing hepatic tumors. Both uncontrolled diabetes and DCA-induced liver toxicity have been reported to involve disturbances in carbohydrate metabolism and excessive storage of glycogen. Hepatomegaly has also been reported as being prevalent in both uncontrolled diabetics and rodents treated with DCA. Based on these parallels, the USEPA concludes that there is evidence to suggest that the mechanism of DCA-induced liver tumors and the mechanism underlying an increased risk of liver tumor formation in uncontrolled diabetics may be the same. Thus, the USEPA implies that exposure to concentration of DCA sufficient to cause glycogenosis and hepatomegaly could exacerbate these effects in diabetics.

While there is a considerable body of literature that has explored the possible relationship between diabetes and the formation of liver tumors, there is no direct evidence to suggest that any relationship would exist between diabetes and the formation of TCE-induced tumors. The one study located that evaluated the effect of diabetes on chemical induced hepatotoxicity reported no effect on TCE-induced heptotoxic responses (Hanasono et al. 1975). Also, no evidence has been presented to suggest that a disruption in carbohydrate metabolism is a characteristic of TCE exposure. TCE has been reported to have no effect on plasma insulin levels in rodents (Arai 1989), and to significantly increase plasma insulin levels in humans (Goh et al. 1998). In contrast, DCA treatment (0.2-2 g/L) reliably decreases plasma insulin (Lingohr et al. 2001, Kato-Weinstein et al. 2001), and is associated with a decrease in plasma glucose and glycogen metabolism. Although TCE has been reported to decrease plasma glucose, this effect was attributed to a decrease in glucose re-absorption in the kidney accompanied by increased glycosuria,

and not to an effect on insulin or carbohydrate metabolism (Arai 1989). Finally, in the most recent epidemiology report in the literature, diabetes was associated with an increased risk of liver cancer only when the disease state was combined with other risk factors such as alcoholism or cirrhosis (El-Serag et al. 2001). Thus, the relationship between diabetes *per se* and liver tumor formation in the absence of other factors appears to be questionable. Consequently, there is little evidence to support the assertion that livers of diabetics would be more sensitive to the direct effects of TCE.

The USEPA also suggests that individuals with glycogen storage disease would be at a significantly increased risk of developing liver tumors following exposure to TCE, when compared to the general population. According to the USEPA, there is a striking similarity between glycogenosis preceding the development of tumors in individuals with glycogen storage disease and glycogenosis in extra-focal hepatocytes in DCA-treated animals. In addition, a similar trend to develop multiple hepatocellular neoplasms has been reported in both individuals with glycogen storage disease and DCA-treated mice. According to the USEPA, the observed similarities seen in the livers of individuals with glycogen storage disease and DCA-treated mice provide a basis to conclude that the mechanisms underlying liver tumor formation for both may be the same. Thus, the USEPA concludes that individuals with glycogen storage disease may be considered a sensitive population to DCA exposure.

However, there is no conclusive evidence to support an inference that the mechanism of tumor formation underlying TCE-induced liver tumors, and that of an increase in hepatic tumor risk associated with glycogen storage disease would be the same. Nevertheless, if indeed an interaction did exist, the sensitivity of individuals with glycogen storage disease to TCE-induced liver tumors would be dependent on the production of DCA in sufficient amounts to significantly disrupt carbohydrate metabolism. Even in sensitive individuals, the available dosimetry evidence suggests that the required concentrations of DCA would not be formed for any reasonably anticipated human exposures to TCE.

National Toxicology Program (NTP) Board of Scientific Counselors' Meeting;
Review of Nominations for Listing in the 10th *Report on Carcinogens*
December 13-15th Washington DC.

Proposed upgrade of Trichloroethylene to known human carcinogen based on recent published data that indicate an excess of kidney cancers in workers exposed to trichloroethylene.

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Summary

The possible modes of action of trichloroethylene as a potential rat and human renal toxin and carcinogen are reviewed. Exposure levels, toxicity, genotoxicity and metabolic activation are seen as critical aspects of the mode of action. The following points are noted.

Human kidney cancer is reported to occur, in the German studies, following exposure to very high nephrotoxic doses of trichloroethylene. Data to substantiate the magnitude of exposure and evidence for renal toxicity in humans is lacking. Anecdotal reports of exposure do not correlate with cancer incidence.

Trichloroethylene is a weak renal carcinogen in the rat in a single valid study. It did not cause renal cancer in the mouse or hamster, or in the rat in two studies. Renal cancer has never been seen in the absence of toxicity.

The DCVC pathway is a very minor pathway, less than 0.01% of the dose and 7000 fold less than the cytochrome P-450 pathway in humans exposed to 160 ppm.

The amounts of DCVC formed from trichloroethylene are 3 orders of magnitude lower than the renal NOEL for DCVC in the rat.

More DCVC is formed from trichloroethylene in the mouse than the rat, and DCVC itself is more toxic in the mouse than the rat.

There is no evidence that DCVC is either mutagenic or carcinogenic in vivo.

Evidence of a hot spot mutation in the human VHL gene in a single study has not been reproduced in a similar study.

Alternative modes of action have been proposed to explain the kidney damage in the rat.

Overall, the trichloroethylene data is weak, key aspects are absent, many of the results are not reproducible between studies and a mode of action has not been established. The data are inadequate to classify trichloroethylene as a human carcinogen.

Introduction

The evidence being used to support the proposed upgrade of trichloroethylene comes from:-

- Epidemiology; principally studies in Germany where small populations were reportedly exposed to be uniquely high concentrations of trichloroethylene.

- Rat cancer bioassays where the same tumour type was seen.

- A common mode of action in rats and in humans.

In this submission, the question is asked, has a mode of action been established that is consistent with the development of kidney tumours in rats and humans?

A number of different aspects of trichloroethylene toxicology are relevant to an understanding of its mode of action as a renal carcinogen. Kidney tumours are reported to be increased in rats and humans only after exposure to very high nephrotoxic dose levels of trichloroethylene. The magnitude of exposure, its characterisation, and the accompanying toxicity, are therefore relevant. A metabolic pathway has been proposed which leads to metabolites which are toxic to the kidney and mutagenic in bacteria. Evidence of mutations has also been reported in human kidney tumours taken from individuals exposed to trichloroethylene. Thus, metabolism and potential genotoxicity are the other critical aspects of the mode of action

A careful analysis, given below, of each of these areas reveals a lack of data, and also inconsistent data, which leads to the conclusion that the evidence available to classify trichloroethylene as a human carcinogen is inadequate.

Human Exposure

The remarkably high incidence of kidney cancer in a small population of 169 individuals in a cardboard factory in Germany is attributed, by the authors, to the uniquely high concentrations of trichloroethylene which occurred in that factory (Henschler et al.1995). The same conclusion is reached in the study reported by Vamvakas et al. (1998).

The populations in these studies were small, <200 people, compared to the large epidemiology studies with total cohorts of about 30,000 individuals.

Neither atmospheric monitoring, nor biological monitoring of exposure was undertaken. Assessment of exposure is based on recollection of physical symptoms which occurred at the time. This assessment was made 20-30 years after the factory closed and exposure ended.

The incidences of cancer did not correlate with exposure. Most of the cancers were seen in individuals employed as locksmiths and electricians and not in the highest exposed group in the Henschler study.

The claim that these exposures were uniquely high is untenable. Trichloroethylene has been in commercial production for almost 70 years and was used without controls or knowledge of adverse long term health effects for decades. Consequently, comparable exposures have occurred elsewhere. It is also particularly difficult to rationalise why people employed as locksmiths and electricians in Germany should be exposed to concentrations of trichloroethylene which did not occur elsewhere.

Conclusion: There are no measurements of exposure to trichloroethylene or other possible confounding factors. There is lack of a correlation between exposure and cancer incidence. Finally, the Henschler study is a cluster study that should not be considered as definitive but as 'hypothesis generating' and should not be used to make a causal inference.

The Significance of the Animal bioassays

There have been seven lifetime cancer bioassays in rats, seven in mice and one in hamsters. There has been no site concordance between the species and none of the tumours have occurred reproducibly in all studies, even within the same species.

Of the studies in rats, four were by gavage and three by inhalation. Because bioassays were conducted at maximum tolerated doses, dose levels between the studies were comparable, around 500-600 ppm by inhalation and up to 1000 mg/kg by gavage. Three studies, two by

inhalation (Henschler et al 1980; Fukuda et al. 1980), and one by gavage (NCI, 1976) did not find any increase in kidney cancer. Two studies conducted by NTP (1988; 1990) using the gavage route did find a sporadic increase in kidney tumours whose incidences were neither dose, sex, nor strain related. Both studies were judged (by NTP) to be inadequate for the purpose of determining carcinogenicity due to poor survival (NTP TR 243, 273) and deficiencies of the conduct of the studies (TR 273). In the inhalation study reported by Maltoni et al (1988), a small increase in kidney tumours was seen in male rats (4/130) at the top dose level of 600 ppm. Kidney toxicity was a common finding in all of the studies.

Kidney cancer has not been seen in mice in seven studies, nor in hamsters in a single study.

Conclusion: The kidney tumour incidence in the rat is low and frequently did not achieve statistical significance. Most studies exceeded the MTD, in one, the conduct of the study was inadequate. The finding of an increase in kidney cancer in the rat is not reproducible and these tumours have never been seen in mice or hamsters. Kidney tumours have only been seen at the highest dose levels and have never been seen in the absence of kidney toxicity. The evidence for renal cancer is, therefore, limited and confined to the rat.

Metabolic Activation

In addition to the major cytochrome P-450 pathway, trichloroethylene is also metabolised by conjugation with glutathione (Goeptar et al. 1995). The derived cysteine conjugate, S-(1,2-dichlorovinyl)-L-cysteine (DCVC; also present as the 2,2- isomer), is known to be nephrotoxic following activation by the renal enzyme β-lyase. Although this pathway has been identified in rats and humans, a link between this pathway and the development of kidney cancer has not been established for the following reasons.

The DCVC pathway has been assessed in vivo by measurements of N-acetyl DCVC in urine. Between 50-73% of a dose of DCVC itself is excreted in this way in the rat (Goeptar et al. 1995). The DCVC pathway is a very minor pathway for trichloroethylene in both rats and humans, typically less than 0.01% of the dose (Birner et al. 1993; Goeptar et al. 1995; Green et al. 1997). In human volunteers, the amount metabolised by this pathway was 7000-fold

lower than that by the cytochrome P450 pathway at exposures of 160 ppm (Bernauer et al. 1996).

There is no evidence to suggest that the DCVC pathway becomes a major pathway at the high dose levels associated with renal cancer. In the human volunteer study reported by Bernauer et al (1996), urinary N-acetyl DCVC levels decreased with increasing dose relative to metabolites from the cytochrome P-450 pathway (ratio P450:GST, 3292:1 at 40ppm and 7163:1 at 160ppm). Over the range of dose levels used (40-160 ppm) cytochrome P-450 metabolism was essentially linear (3.74 fold increase in metabolism for a 4-fold increase in dose) whereas GST metabolism only increased 1.7-fold.

The amount of DCVC formed by the metabolism of trichloroethylene in vivo is three orders of magnitude lower than the NOEL for kidney damage in rats dosed with DCVC itself (Green et al. 1997).

More DCVC is formed in the mouse than the rat following exposure to equivalent dose levels of trichloroethylene. Furthermore, DCVC is 5-10 fold more toxic in the mouse than the rat and caused an increase in renal cell division in the mouse, but not the rat (Birner et al. 1993; Eyre et al. 1995a,b; Green et al. 1997)

There is no evidence whatsoever to show that DCVC when formed as a minor metabolite of trichloroethylene is causally related to the development of either kidney toxicity or kidney cancer. DCVC has not been tested in a full cancer bioassay, in fact, the limited evidence available suggests that DCVC may cause liver cancer in rats rather than kidney cancer (Terracini and Parker, 1965). Equally, although a bacterial mutagen, its ability to cause genetic damage in vivo, including in the rat kidney, has not been established.

Recently alternative explanations for trichloroethylene induced kidney damage in rats have been proposed. It has been reported that trichloroethylene interferes with folate metabolism causing a chronic acidosis which results in kidney damage in rats (Green et al. 1998; Dow and Green, 2000). This mechanism, unlike DCVC activation, is consistent with kidney damage only occurring as a result of chronic high exposure to trichloroethylene.

Conclusion: Although it has been assumed that DCVC is responsible for the renal effects of trichloroethylene, evidence to support these claims is lacking. In fact, the evidence suggests that DCVC is not responsible. The DCVC hypothesis fails to explain the species differences in renal carcinogenicity between rats and mice. The amounts of DCVC formed from trichloroethylene are more than three orders of magnitude lower than a toxic dose of this material. There is no evidence to suggest that the DCVC pathway becomes a major pathway at high dose levels and, DCVC has not been shown to be either carcinogenic or mutagenic in vivo. Even Henschler and co-workers in a recent review of this area (Dekant and Henschler, 1999) conclude that "the present data do not permit a definite assessment of risk of renal tumour formation by trichloroethylene in humans on the basis of available mechanistic or epidemiological data".

Alternative explanations are starting to emerge and the mode of action of trichloroethylene as either a nephrotoxin or rat renal carcinogen, and its relevance to humans, have yet to be established.

Toxicity

In animals, renal cancer has only been seen in the presence of life shortening renal toxicity and it is assumed that toxicity is the fundamental cause of the renal tumours reported in the Henschler study. A number of studies have looked at the same German populations used in the cancer studies and claim to have detected kidney damage (Bruning and Bolt, 2000). These studies have several inherent weaknesses:

Kidney toxicity has been measured more than 20 years after exposure ended.

As with the German cancer studies, exposure levels are unknown.

The reported toxicities do not reflect the magnitude of exposure as assessed by job description.

The parameters used, urinary GST alpha and microglobulin excretion, vary widely in the normal population and are affected by age and sex, and a wide range of drugs, environmental

and lifestyle factors (Yu et al. 1983; Waller et al. 1989; Gan et al. 1994). The populations exposed to trichloroethylene in these studies are small, < 50 exposed individuals, and the studies do not have the power to detect any change, particularly more than 20 years post exposure. The large number of potential confounding factors which may have affected the outcome of the study since exposure to trichloroethylene ended have not been considered.

Conclusion: There is no meaningful evidence of renal toxicity in the populations exposed to trichloroethylene in Germany 20-30 years ago.

Genotoxicity

Recent major reviews of the potential genotoxicity of trichloroethylene have been consistent in concluding that trichloroethylene is unlikely to cause cancer by chemically induced somatic mutations (Fahrig et al. 1995; Moore and Harrington-Brock, 2000).

Although DCVC is mutagenic in bacteria, it has not been shown to be genotoxic in the rat either in the kidney, or at any other site, in vivo. In addition, although chlorothioketene, the proposed reactive intermediate formed from DCVC is DNA reactive in inert solvents, DNA adducts could not be detected under physiological conditions (Volkel and Dekant, 1998). It seems unlikely therefore that DCVC will be genotoxic in the rat kidney in vivo.

Brauch et al (1999) described a “hot spot” mutation at nucleoide 454 of the VHL gene which was only present in renal tumours from individuals exposed to trichloroethylene during metal degreasing. This study suffers from the same fundamental weakness as the cancer and toxicity studies, namely the poor characterisation of the study population with respect to exposure. No direct measurements were available and categorisation of exposure relied on recall of physical symptoms. In some instances this information was obtained from the relatives of deceased members of the study population. It also seems unlikely that a small electrophile such as the chlorothioketene derived from DCVC should cause a specific single mutation of this type. A second similar study by Schraml et al (1999), also carried out in Germany, similarly analysed tumour tissue from trichloroethylene exposed patients for mutations in the VHL gene. This study found no unique phenotype, genotype, or mutation pattern in the VHL

gene from renal tumours of trichloroethylene exposed patients and, in particular, none of the hot spot mutations reported by Brauch.

Conclusion - There is no evidence to show that DCVC is an in vivo mutagen. A single report describing a hot spot mutation in tumours from populations exposed to trichloroethylene appears not to be a reproducible finding.

Comparisons with other human carcinogens

Analogy has been drawn (by NTP) between vinyl chloride, vinylidene chloride, trichloroethylene and perchloroethylene in that they all cause liver cancer in mice. This is an entirely inappropriate analogy since vinyl chloride is a multi-species liver carcinogen with an accepted genotoxic mode of action which involves a reactive epoxide and the formation of DNA adducts. Vinylidene chloride, the closest structural analogue to vinyl chloride, did not cause liver cancer in any species and tri- and perchloroethylene cause liver cancer in mice, but not rats, by non-genotoxic mechanisms involving peroxisome proliferation and increased cell division. It would be more appropriate to compare the biological properties of vinyl chloride, a known human carcinogen, with those of trichloroethylene.

Vinyl chloride is multi-species carcinogen causing liver cancer reproducibly in all rat, mouse and hamster studies. The tumours are induced at low dose levels and following limited exposures. It has a define mode of action based on the formation of a reactive epoxide which is genotoxic and alkylates DNA in vivo. There is clear unequivocal evidence of human cancer from multiple epidemiology studies with known exposure levels. Trichloroethylene on the other hand gives tumours in animals only after exposure to maximum tolerated dose levels. There is no site concordance between rats and mice and poor reproducibility between studies in the same species. All of the evidence suggests that trichloroethylene does not cause cancer by somatic mutation, but by toxicity and increased cell division induced by high dose levels. The evidence of human cancer is weak with an increase in renal tumours seen only in a small poorly characterised cluster study with no measurements of exposure. Evidence of kidney toxicity in humans is similarly confounded by poor characterisation of exposure and the fact that some of the assessments were made 20 years after exposure had ceased.

Thus, for vinyl chloride there is consistency in the animal studies, a clearly defined mode of action and an increase in human cancer linked to exposure. None of this consistency is present for trichloroethylene.

Overall Conclusions

There is a marked lack of consistency in all of the data sets, a poorly defined mode of action, and a general lack of any single unifying observation to suggest that trichloroethylene should be included alongside those carcinogens which have been proven unequivocally to cause cancer in exposed human populations.

The Henschler and Brauch studies suggest areas of further investigation and follow-up but at the present time these studies are flawed by a lack of exposure data and confounded by the fact that the findings are not reproduced in other studies. At the present time a causal relationship cannot be made between exposure to trichloroethylene and increased incidences of human kidney cancer.

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November 29th 2000

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EPA TRICHLOROETHYLENE HEALTH RISK ASSESSMENT

Synthesis and Characterization

Commentary of the Role of Dichlorovinylcysteine (DCVC) in the Development of Renal Toxicity and Carcinogenicity in Rats Exposed to Trichloroethylene.

by
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EPA infer that a likely mode of action, based on the metabolism of trichloroethylene to DCVC, exists to explain the development of renal cancer in rats and humans. It is also proposes that gene mutation forms part of that mode of action.

EPA themselves have established and published in their Draft Risk Assessment Guidelines a generic list of the data requirements needed to support an acceptable mode of action for chemical carcinogens. In this commentary I wish to point out that in the case of trichloroethylene, few if any of these data requirements are fulfilled, and that the DCVC mode of action can, at best, only be considered to be a hypothesis.

DCVC formation as a "mode of action"

Metabolism of trichloroethylene by conjugation with glutathione results in the formation of the cysteine conjugates S-1,2- and S-2,2-dichlorovinylcysteine (DCVC). These conjugates are metabolised by renal β-lyase to reactive intermediates which are nephrotoxic in vivo and are mutagenic in salmonella typhimurium in vitro. The pathway was first detected in rats exposed to trichloroethylene and, given the known properties of DCVC, was proposed as a possible explanation for the development of the rat kidney tumours. Subsequently, the same

pathway was found in humans and the increased human kidney cancer incidences became 'biologically plausible' on the basis of a common mode of action in rats and humans.

In vivo, the pathway is detected by the presence of the mercapturates, the N-acetyl-S-dichlorovinylcysteine conjugates in urine. There is no direct measure of the amount metabolised by the β-lyase pathway in vivo. Commandeur et al (1990) reported that rats dosed intraperitoneally with the 1,2 and 2,2 isomers of DCVC excreted 50% of the 1,2 isomer and 73% of the 2,2 isomer in urine as the respective N-acetyl cysteine conjugates. Thus, urinary N-acetyl DCVC concentrations are a reasonably accurate estimate of the total amount of DCVC formed in rats dosed with trichloroethylene. Enzyme kinetic measurements show that the amount of DCVC metabolised by β-lyase is at least 100-fold lower than the amount metabolised to N-acetyl DCVC. Assays of N-acetyl DCVC in urine are therefore an over-estimate of β-lyase metabolism.

Quantitation of the DCVC pathway

There are three main sources of data on DCVC formation from trichloroethylene; in vitro studies in liver and kidney cells and fractions, in vivo studies in rats and humans exposed under controlled conditions, and in vivo data from humans occupationally exposed.

In vitro studies enable comparisons of the activities in rats and humans but tells us little about the overall extent of metabolism by this pathway, particularly when the in vitro studies are conducted in the absence of the high affinity, and major, cytochrome P-450 pathway.
The occupational studies have limited information concerning exposure, they are somewhat variable and inconsistent, and serve mainly to show that the pathway is a very minor one.
The single human volunteer study reported by Bernauer et al (1996) is the most valuable data source comparing the DCVC and cytochrome P-450 pathways under controlled exposure conditions at three exposure concentrations. Rats were simultaneously exposed in the same chamber. The principal findings from this study were that the pathway is very minor, the ratio of the P-450 pathway to the DCVC pathway was 7163:1 in humans at the highest exposure concentration (160 ppm for 6 hr). Over the dose range studied (40-80-160 ppm) the cytochrome P-450 metabolism increased linearly whereas the DCVC pathway appeared to be saturated. Hence there is no evidence to suggest that the DCVC ever becomes a major

pathway of trichloroethylene metabolism. Estimates from all of these studies indicate that the DCVC pathway accounts for approximately 0.005% of the dose.

The formation and activation of DCVC as a mode of action leading to trichloroethylene induced renal toxicity and carcinogenicity.

EPA have succinctly outlined their expectations for an acceptable mode of action and in order to best illustrate the weaknesses in the DCVC case, a comparison is made below between the EPA's expectations and the data available for DCVC.

The suggested mode of action involves metabolism of trichloroethylene to DCVC, activation by renal β-lyase, toxicity (possibly genotoxicity), increased cell replication and cancer. To support such a mode of action EPA look for information within the following framework:

- Identify key events
- Strength, consistency, specificity of association.
- Dose response relationship
- Temporal association
- Biological plausibility and coherence of the database
- Other modes of action

- ***Identify key events***

Prior to looking at the key events which lead to renal cancer it is worth noting the inadequacy of the cancer data base for trichloroethylene induced rat renal tumours. Tumours have only been seen in very low incidences at supra MTD dose levels in studies judged to be inadequate due to either exceeding the MTD or poor study conduct (NTP, 1988, 1990). The increased incidence seen in a single none peer reviewed study occurred at 159 weeks, a time point with little or no historical control data (Maltoni et al. 1988).

In terms of the key events which are believed to lead to this low incidence of renal cancer, trichloroethylene has no effect on the target organ in vivo at maximum tolerated doses during studies of up to 28 days (Stott et al. 1982; Goldsworthy et al. 1988; Green et al. 1997) or 90 days (NTP 1988) duration. Minimal effects were seen only after a re-evaluation of the kidney

sections in the NTP 13 week study conducted in Fischer rats (NTP, 1990). Overt kidney toxicity has only been seen at 104 weeks when neoplasia is present.

There are no data to show an increase in cell replication rates in the kidneys of rats exposed to either trichloroethylene or DCVC in vivo.

There is no evidence to show that either trichloroethylene or DCVC are genotoxic in the target organ. There is no evidence to show that DCVC is genotoxic in any assay using modern protocols other than bacteria. Indirect DNA effects have not been reported.

Although a range of toxicities have been attributed to DCVC, and to a considerably lesser extent to trichloroethylene, including oxidative stress, lipid peroxidation, changes in mitochondrial function etc., it is of particular note that none of these effects have been recorded in the kidneys of rats exposed to trichloroethylene in vivo under the conditions of the cancer bioassays.

Renal toxicity has been reported in humans (reviewed by Bruning and Bolt, 2000). However, the data were obtained from a number of small populations whose exposure to trichloroethylene ceased more than 20 years ago. Evidence of exposure is anecdotal, there were no direct measurements, and the study populations are too small to detect toxicity in the presence of the many potential confounding factors which can affect renal function, particularly over a 20 year period.

A single report describes a mutation in the VHL gene in the kidneys of humans exposed occupationally over 20 years ago (Brauch et al 1999). This work has not been repeated, in fact a more recent study has failed to reproduce the finding (Schraml et al. 1999). Again, the estimates of exposure, which ceased more than 20 years ago, are anecdotal.

- ***Strength, consistency, specificity of association***

There is a total lack of evidence since, as already noted, neither acute nor sub-chronic responses have been described in the target organ of rats exposed to trichloroethylene. There is no evidence for trichloroethylene induced genetic change in the rat kidney.

For DCVC

There is no evidence that DCVC is an in vivo mutagen or a kidney carcinogen. The limited evidence available suggests that higher doses of DCVC may cause liver cancer rather than kidney cancer in rats (Terracini and Parker 1965).

DCVC is more hepatotoxic than nephrotoxic following acute dosing (Green et al. 1997, .

DCVC is formed in greater quantities in the mouse than in the rat after equivalent exposures to trichloroethylene (Birner et al. 1993).

DCVC is an order of magnitude more toxic to the mouse kidney than the rat kidney yet trichloroethylene induced kidney tumours are seen in the rat not the mouse (Eyre et al. 1995a, b; Lash et al., 2000).

Concentrations of DCVC formed from trichloroethylene are a factor of 10^3 lower than the acute NOEL in the rat (Green et al. 1997).

- ***Dose response relationship***

No key events have been established in vivo that relate to the tumour incidences. Therefore no dose dependent relationships exist.

There is no evidence from the human volunteer study to show that the amount of DCVC formed from trichloroethylene increases with increasing dose, yet kidney toxicity and cancer are believed to only occur at high dose levels.

- ***Temporal association***

Again no key events have been established which precede tumour appearance.

- ***Biological plausibility and coherence of the database***

Whilst it seems plausible that the formation of a metabolite (DCVC) which is both nephrotoxic and a bacterial mutagen should relate to the development of renal cancer, there is

no evidence to support this supposition. Experimental toxicology on this subject has, in recent years, concentrated largely on assays for the formation of DCVC in various systems and on the toxicity of DCVC in a variety of in vitro systems at dose levels which are orders of magnitude higher than those formed from trichloroethylene. Unfortunately, the key studies to investigate whether DCVC is an in vivo mutagen in the kidney or a renal carcinogen have not been conducted.

Overall, these studies have failed to demonstrate that DCVC, when formed in microgram quantities from trichloroethylene, has any toxicological consequences whatsoever. Most importantly, none of these studies have established a train of key events in the kidneys of rats exposed to trichloroethylene itself. Consequently, none of the so-called key events required for a plausible mode of action exist. At the same time there is a considerable amount of contradictory data with respect to the mouse and the relationship between the DCVC and P-450 pathways and dose. Human data, where it exists is confounded by lack of exposure data and the fact that in many studies exposures ended more than 20 years ago.

- ***Other modes of action***

EPA note one other possible mode of action involving the excretion of formic acid in rats exposed to trichloroethylene. This second possibility casts further doubt on the role of DCVC.

Conclusion

The available data on the metabolism of trichloroethylene to DCVC, and on the toxicity of these two chemicals to the rat kidney, fail to meet the criteria proposed by EPA for an acceptable mode of action.

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Technical Memorandum
January 2002

Comments on Bois's Statistical Analyses of Clewell and Fisher PBPK Models

The U.S. Environmental Protection Agency (EPA) has chosen to ignore portions of the results from Clewell et al. (2000) and Fisher (2000) for the TCE risk assessment, primarily because of the results of Bois's statistical analyses of these models (Bois 2000a,b). However, as described in these comments, the analyses of Bois do not provide adequate justification for dismissing the results of Clewell et al. (2000) and Fisher (2000).

Bois's analysis is purported to rely on Bayesian principles (updating model parameter distributions) implemented by employing Markov-chain sampling (Monte Carlo) in conjunction with simulating the physiologically based pharmacokinetic (PBPK) models to reproduce the "data" that contain information about the PBPK model parameters. Monte Carlo simulations are conducted to determine which joint parameter vectors (pairs of model parameter values) are "consistent" (are within a predetermined error criterion) with the data (Bayes updating). Conceptually, the approach of coupling Bayes updating with PBPK modeling is valid; however, additional analyses are required to determine whether the results of Bois are correct. As currently presented, insufficient information is provided to make such a determination.

EPA's Justification for Not Using PBPK Modeling Results

The following quotes from the TCE Assessment highlight the EPA's justification for not using the PBPK modeling results of Clewell et al. (2000) and Fisher (2000).

1. Page 4-1: Statistical analyses improved these models by calibrating them to fit more data sets, then quantifying the uncertainty in each dose metric (Bois 2000a,b).

2. Page 4-3 – 4-4: These analyses use a Bayesian statistical framework and Markov-chain Monte-Carlo simulations to refine the models by using more data sets to estimate each model's parameters. The result is a set of calibrated models that better fits a wider range of experimental data. In some cases the calibrated parameters are quite different from the originals, thus substantial information has been gained by fitting the models to additional experimental data sets.

3. Page 4-4: Another product of the statistical analyses is a quantitative description of uncertainty in dose estimates (Bois, 2000b)… From Table 4-1 it is apparent that lung and kidney dose metrics are quite uncertain, with 95% confidence intervals spanning more than a 5,000-fold range.

4. Page 4-5: Rather than make such a large adjustment, this assessment uses default RfC dosimetry models (USEPA, 1994) when modeling lung tumors

by inhalation and the default ¾-power scaling factor when modeling kidney tumors by ingestion… In this way, uncertainty analysis is used to distinguish between uncertain applications (lung and kidney) and more robust applications (liver), so that pharmacokinetic modeling is used when results are robust and other methods are considered when there is too much uncertainty.

5. Page 4-5: Females have a significantly lower alveolar ventilation rate (beyond that explained by allometric scaling), higher TCOH body-to-blood partition coefficient, lower TCA body-to-blood partition coefficient, higher Vmax/Km ratio for TCOH glucoronidation, higher conversion of TCOH to TCA, and higher urinary excretion of TCA (Bois, 2000a).

6. Page 4-6: For liver effects, pharmacokinetic modeling (Clewell et al., 2000) was used to estimate plasma TCA as the dose metric (Barton and Clewell, 2000). For kidney effects, statistical analyses (Bois, 2000b) revealed substantial parameter uncertainty in the pharmacokinetic modeling (see Table 4-1), consequently, human-equivalent doses were based on equivalence of mg/kg ¾ - d (U.S. EPA, 1992).

7. Page 4-25: Bois' uncertainty analysis (Bois, 2000b), however, revealed the presence of substantial uncertainty in the kidney dose estimates (see Table 4-1, Section 4.2.2). Consequently, dose estimates for the kidney were based on equivalence of mg/kg$^{3/4}$-d.

As detailed below, we feel that Bois' analyses are wholly inadequate and insufficiently documented to justify dismissal of the work of Clewell et al. (2000) and Fisher (2000) for kidney and lung endpoints.

The Most Relevant Data Sources Should be Used to Update Model Parameter Values

One of the fundamental tenets of Bayes updating is that the new data must contain relevant information about the pre-existing data (or, in this case, parameters). It is therefore important to note that the pharmacokinetic (time course) data set on TCE and its metabolites in rodents and humans does not necessarily contain information pertinent to updating all PBPK model parameters.

In fact, in most cases, time-course kinetic data sets usually contain relevant information concerning comparatively few PBPK model parameters (i.e., usually less than 5% of the PBPK model parameters make up 90% of the "sensitivity" toward the kinetic/time-course data). The other model parameters play very minor roles in dictating the model predictions that correspond with the time-course data. This means that the other model parameters have little sensitivity toward the model predictions of the endpoint being simulated (i.e., large variations in these model parameters result in little change in the endpoint being simulated).

The model parameters that have little sensitivity should be updated with information specific to their values. For instance, physiological studies designed to measure the percentage of body compartments (fat, liver, kidney, etc.), *in vitro* experiments to measure partition coefficients, and *in vitro* biochemical studies designed to measure rates of metabolic conversion provide the most useful information for the values of these parameters, and thus should take priority over time-course kinetic data in terms of having information to update the model parameters. Instead, Bois has relied on gas uptake data as "the most important" information to update ALL model parameters. This is contrary to the accepted method of using the most relevant data as a source for updating specific model parameters.

Model Structure Affects How the Model Parameters Are Interpreted

When interpreting data, it is important to realize that the model structure imparts a filter of sorts. The model structure dictates the form and shape of the response simulations. Therefore, this imparts a constraint on how the model parameters might be interpreted when using the model to "optimize" parameter values by optimizing the fit between model simulations and the data being replicated.

When creating models, one must always balance the principle of parsimony (keeping the model as simple as possible) with creating a model that describes all the possible biological, chemical, and physiologic processes that govern how a compound is "handled" by the body. One cannot create a model that describes all processes with certainty. At the same time, the goal of PBPK modeling is to create a model that is not overly simplistic (i.e., one-compartment pharmacokinetic model) while at the same time attempting to capture the "important" processes in a manner that makes it amenable to predicting outside the constraints under which it was validated (e.g., in animals, inhalation, etc.). In this way, intelligent and informed predictions can be made for situations outside the realm under which the model was validated (e.g., extrapolating to humans, assessing different exposure routes, etc.). Because these models do not include all possible processes involved in the kinetics of compounds in the body, they cannot possibly simulate EXACTLY all scenarios. Therefore, they are approximations of a complex process. Thus, using a model to simulate data and hoping that the data set has enough information to tell you exactly all the possible values for every model parameter is over-stretching the power of the model, and certainly the data being used. This is exactly the trap into which Bois has fallen.

Bois's approach assumes that PBPK models are precise and that they describe all aspects of the way in which TCE is absorbed, distributed, metabolized, and excreted.

Models Typically Have Multiple Solutions

It is not unusual for models of any type to have multiple solutions. Therefore, it is always critical to make sure that the solutions obtained from model optimizations make sense. If a solution is found that is vastly, or substantially, different from perceived or expected solutions,

it should indicate to the model developer that a local minima solution has been achieved, and the true global minima has not yet been found. Dr. Bois has not tested to make sure that this phenomenon has occurred.

Results That Don't Fit With the Data

In the re-analysis of Fisher et al., Bois found a poor correlation between measured and predicted VFC (volume of fat compartment) for the human volunteers who participated in the study that Bois simulated. In fact, the consistent over-prediction of percent body fat at low observed percentages of body fat, and under-prediction of percent body fat at higher observed percent body fats, is an indication that the model, as a whole, has some potential biases that need to be controlled for. This type of result should alert Bois to the fact that there is a bias in the model, not necessarily that the Bayesian updating has identified a flaw in the technique used to measure percentage of fat among the human volunteers. This indicates Bois's bias in trusting the PBPK model to be an exact description of the physiologic and biochemical processes governing the kinetics of TCE in humans. This is another indicator that Bois's results are potentially flawed and should be explored in further detail, to ensure that his results are consistent with all that is known about TCE kinetics and all of the data sets that have been used over the years to develop these models.

Not Controlling for Unrealistic Results and Correlations

Bois did not ensure that unrealistic extremes in model parameters were achieved. For instance, the ratios of model compartment volumes and flows were not maintained within a reasonable value. For instance, allowing a small liver to be paired with a large liver blood flow will necessitate creating extreme metabolic rates to account for the large delivery of compound to the metabolic tissue. This becomes more important for small model compartments, such as the kidney and lung. As has been shown previously (Hays et al. 2000), this creates large uncertainties in Monte Carlo simulations for PBPK models and yields dramatically unrealistic tissue perfusion qualities (ratios of tissue flow to volume) and resulting values for the internal dose metrics. It is no wonder that large variations in the dose measures for these tissues were generated. A sensitivity analysis would likely show that these parameters (blood flow, volume and ratio of the two-perfusion quality) are extremely sensitive toward the internal dose metrics of interest. This should be explored to see how significantly these distributions are out of line with historical distributions obtained from focused physiology studies.

Conclusions

Bois's statistical analyses of the Clewell et al. (2000) and Fisher (2000) PBPK models attempted to use a fundamentally valid approach (Bayes updating) to assess the validity and uncertainty of these PBPK models. However, the manner in which Bois employed this method has created a false sense of uncertainty about these models, and in the process, has done a potentially dramatic disservice to the discipline of PBPK modeling in general. Either Bois's results should

be ignored, or the analyses should be re-done, with significant control measures, to ensure that the results are valid. EPA should ignore the results of Bois for the purposes of this analysis and should use the results of Clewell et al. (2000), Fisher (2000), and Barton and Clewell (2000) in their cancer and non-cancer assessments.

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Title: Trichloroethylene Health Risk Assessment: Synthesis and Characterization.

Section: 3.4.4. Immune and Lympho-Hematopoietic Systems. (Pgs 3-15 - 3-17)

Reviewed by: Michael Holsapple

Technical Leader for Immunotoxicology

Reviewed for: Bill Stott

Reviewed on: November 10 - 11, 2001.

General Comments: According to the Abstract, TCE exposure is associated with several adverse health effects, including immunotoxicity. However, one can assume that although these effects are observed, the authors of this report do not consider the immunotoxicity to be as important as effects seen on other target organs. The report devotes a little over two pages to the effects of TCE on the immune system. More importantly, as conveyed in the Abstract, no effects on the immune system were used in the risk characterization of this chemical. The calculation of the oral RfD was based on critical effects in the liver, kidney and developing fetus. The calculation of an inhalation RfC was based on critical effects in the central nervous system, liver, and endocrine system. This observation is consistent with the fact that most immunotoxicity studies that have been performed with TCE to date have incorporated very high doses. This observation is also consistent with the role that metabolism plays in the ultimate toxicity by TCE - i.e., the immune system is characterized by very poor metabolic capability. Therefore, reactive metabolites must be generated elsewhere and somehow make their way to immune organs and/or immunocompetent cells.

Specific Comments: The TCE Health Risk Assessment appears to have cited the most important papers dealing with the effects of this chemical on the immune system. Most

notably, previous risk characterizations for TCE have relied primarily on only a few of these papers for its effects on the immune system. For example the paper by Barton and Clewell, 2000 cited only the studies by Sanders et al., 1982 for the effects on the immune system. In that regard, it is interesting to note that on Line 11 on Pg 3-15, Barton and Clewell (2000) is offered to support the statement that "Females showed a greater susceptibility than males to these immunotoxic effects of TCE." In actuality, the paper by Barton and Clewell did no original studies and merely cited the work done by Sanders et al. (1982). It is important to emphasize that the laboratory of the original study is the Medical College of Virginia and was under the leadership of Dr. Al Munson at the time these studies were conducted. These investigators have always been recognized as one of the recognized leaders in the characterization and validation of approaches in immunotoxicology. Therefore, it can be concluded that these studies were well-designed, well-executed and interpreted appropriately. The principle conclusion by Sanders et al. was stated as follows: "**Although the effect of oral exposure to TCE on the immune system was not remarkable even at the highest doses employed in this study, this system is sensitive to the chemical.**" The emphasis should probably be on the phrase, " . . . **at the highest doses employed** . . . ". In reviewing this study, Barton and Clewell offered the following summary, "**Taken together, these assays are supportive of a LOAEL of 400 mg/kg/day and a NOAEL of 200 mg/kg/day**."

The paper by Aranyi et al. (1986) is frequently cited as a study supporting the fact that TCE following inhalation is immunotoxic. However, there are some inconsistencies in the results. For example, the % mortality for the control groups for the treatments with TCE was as follows: 50 ppm - 13.4%; 25 ppm - 25.2%; 10 ppm - 13.2%; 5 ppm - 13.2%

and 2.5 ppm - 5.7%. It is the control group for the 25 ppm treatment with TCE which causes the most concern - the 25.2% mortality observed in this control group is essentially equal to the 27.2% mortality observed in mice exposed to 10 ppm TCE. As such, these results make it difficult to accept that 10 ppm is the LOAEL for the change in host resistance. The authors do not address the fact that the mortality in control mice was variable. A second problem with this paper is the fact that there was absolutely no correlation between the doses of TCE that caused changes in the host resistance and the doses that caused changes in the bactericidal activity of alveolar macrophages, the presumed mechanism. As emphasized by Aranyi et al. (1986), "**Such correlated responses imply that the infectivity model is primarily dependent on the function of alveolar macrophages**." For example, exposure to 10 ppm, which was claimed to be the LOAEL for the increased susceptibility to bacterial infections as noted above, actually caused a significant increase in the number of bacteria killed by the alveolar macrophage, and exposure to 25 and 50 ppm, which caused significant changes in host resistance caused slight increases in the bactericidal activity of the alveolar macrophages, which were not statistically significant.

The paper by Khan et al. (1995) addressed the potential that exposure to TCE was capable of exacerbating autoimmune disease. The primary conclusion of the authors of this paper is stated as follows, "**These results suggest that TCE and its metabolite, DCAC, induce and/or accelerate autoimmune responses in female MRL +/+ mice**." There are a number of flaws in the study design utilized by Khan et al. First, their studies are based on a single dose of either TCE or DCAC. Second, their studies with TCE are based on an "N" of four mice (i.e., they started out with five mice per treatment group;

but one of the mice died). Third, although they set out to provide evidence for a role by a reactive metabolite in the immunotoxicity of TCE, their results are not exactly consistent with this possibility. There were marked differences in the profiles of the presence of various autoantibodies between mice treated with TCE and mice treated with DCAC. Moreover, all mice treated with DCAC developed DCAC-specific antibodies in their sera; but none of the mice treated with TCE developed DCAC-specific antibodies. Taken together, their results are not consistent with a role y the generation of DCAC in the profile of activity of TCE.

The series of papers by Griffin et al. (2000 a, b, c) offer a more compelling series of results regarding the impact of TCE on autoimmunity. Using the same model as Khan et al., MRL +/+ mice, they showed that TCE accelerated autoimmunity and caused an activation of CD4+ T-cells, which express a Th1 cytokine profile (Griffin et al., 2000 a). They subsequently showed that metabolite activation of TCE by cytochrome P450 2E1 is important for the activation of CD4+ T cells in the MRL +/+ mouse model (Griffin et al., 2000 b). As discussed above for the study by Khan et al., the latter study was also based on exposure to only a single dose of TCE, 2.5 mg/ml in the drinking water, which calculate to 400 mg/kg/day. The authors recognized that most of the early studies with TCE, including their own studies, have used very high doses. In their most recent study (Griffin et al., 2000 c), they exposed MRL +/+ mice to 0.1, 0.5 and 2.5 mg/ml which calculate to 21, 100 and 400 mg/kg/day, respectively. The authors cite the recommendation from the ACGIH that the TLV of 269 mg/m^3 (or a time-weighted average of 50 ppm for TCE) converts to a concentration of approximately 40 mg/kg/day, assuming a moderately active work environment. The results are described in the Health

Risk Assessment as follows, "**An early response to TCE exposure was a dose-related and significant increase in serum antinuclear antibodies at the lowest exposure (0.1 mg/ml or 4 mg/kg/day)**." The authors of the report have miscalculated the dose which was, as noted above, calculated by the authors to be 21 mg/kg/day and not 4 mg/kg/day. There was an early increase in serum antinuclear antibodies; but it was not entirely dose-related as the effect at the highest dose was less than the two lower doses. The authors of the report are correct that " . . . **histopathologic changes that included portal infiltration by mononuclear cells were seen at the termination of the study, 32 weeks, and were consistent with the induction of autoimmune disease in the liver**." However, they failed to point out that at 32 weeks, there were no longer any increases in serum antinuclear antibodies, as seen as the earlier time point. Despite some inconsistencies in the description of these studies in the Health Risk Assessment, the papers by Griffin et al. are some of the best work to date on the effects of TCE on the immune system, and are mostly consistent with a possible link to the onset of autoimmune disease. Nonetheless, as emphasized above, these effects on the immune system still occurred at doses higher than effects seen on other organs, and were not factored into the risk characterization for TCE.