HALOGENATED SOLVENTS INDUSTRY ALLIANCE, INC.
2001 L Street, N.W., Suite 506A, Washington, D.C. 20036 • (202) 775-0232 Fax: (202) 833-0381

***DATE:** December 1, 2000*

FACSIMILE COVER SHEET

From: Paul Dugard Number of Pages (incl Cover): 23

To: Dr Mary Wolfe

Subject: COMMENT ON NTP BACKGROUND DOCUMENT FOR TRICHLOROETHYLENE

Dear Dr Wolfe:

What follows is a detailed comment on the above Background Document plus the letter by Cherrie et al which is "in press". If there are other references listed that NTP requires and finds difficulty in obtaining, please let us know.

We were not sure whether these comments should have been sent to you or Dr Jameson. If the latter, I apologize and request that you pass this to him.

I have also sent the review as an attachment to an e-mail and you may find that more convenient to handle.

Sincerely,

[redacted]

HALOGENATED SOLVENTS INDUSTRY ALLIANCE, INC.

2001 L Street, N.W., Suite 506A, Washington, D.C. 20036 • (202) 775-0232 Fax: (202) 833-0381

e-mail: pdugard@hsia.org

November 30, 2000

Dr. Mary S. Wolfe
Executive Secretary
NTP Board of Scientific Counselors
P.O. Box 12233, A3-07
Research Triangle Park, NC 27709

Re: Proposed Listing of Trichloroethylene As a Known Human Carcinogen In the Tenth Report on Carcinogens

Dear Dr. Wolfe:

The Halogenated Solvents Industry Alliance, Inc. (HSIA) appreciates the opportunity to comment on the Background Document provided to the National Toxicology Program (NTP) Board of Scientific Counselors Report on Carcinogens Subcommittee in support of the proposed classification of trichloroethylene as Known to be a Human Carcinogen in the Tenth Report on Carcinogens. 65 Fed. Reg. 61352 (Oct. 17, 2000). HSIA represents the producers and users of chlorinated solvents, including trichloroethylene.

Detailed comments on both the epidemiology studies and the animal data summarized and discussed in the Background Document follow. (References are to the papers as listed in the References to the Background Document; details of unlisted references are enclosed and less accessible documents will be supplied). At the outset, however, we note several unusual aspects of the proposed classification which should be taken into account by the Report on Carcinogens Subcommittee.

First, trichloroethylene was only classified as Reasonably Anticipated to be a Human Carcinogen in the Ninth Report on Carcinogens, published earlier this year. One of the two epidemiology studies relied upon in support of the proposed Known Human Carcinogen classification in the Background Document was addressed in the Ninth Report on Carcinogens. This study, Henschler et al. (1995), was also considered by the International Agency for Research on Cancer (IARC) when it classified trichloroethylene as Probably Carcinogenic to Humans (Group 2A) in 1995. IARC (1995e). Henschler et al. (1995) was considered by the IARC Epidemiology Working Group to be unsuitable for purposes of its review because the study was initiated in response to observation of a cancer cluster and the cohort included the subjects who comprised the cluster. IARC (1995e); Weiss (1996).

Second, the other epidemiology study cited in support of the proposed classification, Vamvakas et al. (1998), is a case-control study of renal cell carcinoma. This study is discussed extensively below and, as noted in the Background Document, has been the subject of published criticism. Green and Lash (1999). The criticism of Vamvakas et al. (1998) (and Henschler et al. 1995) has been expanded with the recent submission to the J. Cancer Res. Clin. Oncol. (in press) of the enclosed letter by Cherrie, Kromhout, and Semple. These authors identify three major concerns with the exposure assessment used by Vamvakas et al. First, the physicians who carried out the interviews (Vamvakas et al. was a hospital-based case-control study) with the subjects were aware of their status as either cases or controls, likely leading to both interviewer and responder bias. As also noted by Green and Lash (1999), the potential for bias in these exposure assessments must be carefully evaluated before reliance is placed on this study. Second, the exposure rating system used in the study was based entirely on self-reported pre-narcotic symptoms, which are prone to bias. Third, it is peculiar that younger cases with more recent and therefore lower exposures apparently showed a much higher risk than older cases, again suggesting recall or interviewer bias. Most significantly, Cherrie et al. (2000) compare the exposures in Henschler et al. (1995) and Vamvakas et al. (1998) to those in the study by Blair et al. (1998). Cherrie et al. conclude that the likely long-term exposure levels in all these studies are not sufficiently dissimilar to have caused the differences in observed risk; Blair et al. reported no significant increase for kidney cancer.

Third, the Background Document refers to three published reviews of the carcinogenicity studies for trichloroethylene. Two of these, Weiss (1996) and McLaughlin and Blot (1997), concluded that the epidemiology studies available at the time of the reviews provided at most weak associations between trichloroethylene exposure and human cancer. Specifically, McLaughlin and Blot (1997) concluded that there was "no credible evidence of an association between risk of renal-cell cancer and TCE." The Background Document places emphasis on the more recent review by Wartenberg et al. (2000) which considered all of the available studies. It is significant, however, that even Wartenberg et al. (2000) appear to agree with the assessment of IARC (1995e) and Weiss (1996) that this evidence is not sufficient to support a Known Human Carcinogen classification.

Fourth, as will be seen from the discussion below, the Background Document supports classification of trichloroethylene as a Known Human Carcinogen only by finding "strong patterns" in the results of cohort studies (supplemented by Vamvakis et al. (1998)) that the authors (with the exception of Henschler et al. (1995)) conclude do not support a causal relationship between trichloroethylene exposure and cancer. It is remarkable that the Background Document thus "reinterprets" the conclusions of no fewer than four groups of well-known authors all of whom have published epidemiology studies on trichloroethylene in the past two years. The studies concerned are those reported by Blair et al. (1998); Morgan et al. (1998); Boice et al. (1999); Ritz (1999). These authors concluded that their studies did not support a strong association between trichloroethylene exposure and kidney cancer. The characterization of these data as "a large and generally consistent body of evidence indicating that TCE is a human

carcinogen," primarily on the basis of two German studies that appear to arise from a known cluster, calls into question the objectivity of the Background Document.

Fifth, the earlier epidemiology studies of trichloroethylene reached findings consistent with Blair et al. (1998), Morgan et al. (1998), Boice et al. (1999), and Ritz (1999). Indeed, the earlier studies provided such a compelling case for the absence of an association between trichloroethylene and human carcinogenicity that the American Conference of Governmental Industrial Hygienists (ACGIH) classified trichloroethylene as A5 in its classification scheme, defined as follows:

> Not Suspected as a Human Carcinogen: The agent is not suspected to be a human carcinogen on the basis of properly conducted epidemiologic studies in humans. These studies have sufficiently long follow-up, reliable exposure histories, sufficiently high dose, and adequate statistical power to conclude that exposure to the agent does not convey a significant risk of cancer to humans; OR, the evidence suggesting a lack of carcinogenicity in experimental animals is supported by mechanistic data.

As of 2000, trichloroethylene continues to be classified by ACGIH in Category A5.

Sixth, as noted in HSIA's comments of June 2, 2000 on this proposal, NTP's failure to provide background information in support of the proposed re-classification of trichloroethylene makes this rulemaking invalid because the public failed to receive sufficient opportunity to comment on the substance of the proposal. While this may not directly affect the consideration by this peer review committee, neither RG1 nor RG2 had the opportunity to review public comment on the Background Document (which was only issued in October) at the time of their reviews. Even so, RG2 voted 4-3 against a motion to list trichloroethylene as a Known Human Carcinogen.

HSIA recommends that the Background Document should be withdrawn and any consideration of the classification of trichloroethylene should be deferred until an adequate document has been prepared. The reasons for this recommendation are as follows:

1. Much of the information in the Background Document is out of date and information from a number of highly relevant references has been omitted.

2. Considerable weight is placed upon the epidemiology studies of Henschler et al (1995) and Vamvakas et al. (1998) despite severe published criticisms and IARC's decision not to consider Henschler et al. (1995).

3. The Background Document ignores the interpretations of the results of the most reliable, large and recent cohort studies as presented by their authors, and substitutes a harsh interpretation not supported by the evidence.

4. The Background Document distorts the considered opinions of the authors of reviews of epidemiology studies, not one of whom considers the evidence to show,

conclusively, an association between trichloroethylene and cancer, let alone a causal relationship.

5. The presentation of data from animal carcinogenicity studies is poor and uninformative.

6. The treatment of biochemical and mechanistic aspects is superficial and the contrasting opinions are not carried through to the Summary Statement to provide a balanced display of current scientific opinion.

7. Overall, the Background Document does not provide the Board of Scientific Couselors Report on Carcinogens Subcommittee with the accurate, balanced information that they have a right to expect.

COMMENTS ON SPECIFIC SECTIONS OF THE BACKGROUND DOCUMENT FOR TRICHLOROETHYLENE

Summary Statement

For the reasons discussed below, epidemiology study result do not come anywhere near supporting the conclusion of "known human carcinogen" and there are good reasons to believe that the tumor incidences in animals do not indicate carcinogenicity in man.

The review of epidemiology by Wartenberg et al (2000) cannot be called a "meta-analysis". When judged impartially, the patterns of incidence of specific cancers are typical of the situation where there is no association. Only Vamvakas et al (1998) has been discussed here, but the case control studies as a class provide very limited evidence relative to the large cohort studies.

The tumors in rats, and mice show species, sex and strain specificities. Only the lung and liver tumors in mice and kidney tumors in rats require consideration and for each of these, there is growing evidence that the underlying mode of induction is irrelevant to humans. This should be acknowledged.

The significance of dichloroacetic acid as a TCE metabolite has diminished greatly and may be irrelevant to man. DCVC has been associated with kidney toxicity in the rat but only at doses three orders of magnitude greater than the highest levels achieved in rat TCE bioassays. The hypotheses regarding the roles of DCVC in rat kidney toxicity and carcinogenicity are being re-evaluated and alternative hypotheses are gaining support from experimental data. These factors should be acknowledged.

The link between high levels of TCE exposure and the type of VHL mutations reported is implausible and requires confirmation in independent studies. As discussed below, the evidence is growing that activation of the products of TCE glutathione conjugation is not responsible for responses of rat kidneys and the greater sensitivity of man is not broadly

supported by information from such studies as Bernauer et al (1996) that are more reliable than Birner et al (1993).

The Summary Statement should reflect the conclusions stated in the Genotoxicity Section, 5.5 Summary.

Although the general text of the Background Document gives some coverage of conflicting information, hypotheses, and opinion, this does not appear in the Summary Statement. The Statement should an unbiased review of information and this has not been achieved,

Section 2: Human Exposure

Section 2.1 The use of TCE as a chemical feedstock is now a major application (for production of non-ozone depleting CFC alternatives).

2.2 There are two US producers of TCE.

2.4 Natural production of TCE is substantially greater than indicated, see Gribble (1992), for example.

2.4.1 Environmental Occurrence – Air Trichloroethylene emission data are available in EPA's Toxic Release Inventory (TRI) for 1998 (TRI98 2000). According to this latest TRI information, 600 U.S. facilities reported emissions of trichloroethylene. Of these, 429 reported releases to the atmosphere of more than 2,000 lbs. Among these, 129 released 2,000 to 10,000 lbs, 235 released 10,000 to 50,000 lbs, 59 released 50,000 to 200,000 lbs, and 6 released more than 200,000 lbs. The total amount of trichloroethylene reported to have been released to the atmosphere in 1998 was 13,054,796 lbs.

2.4.3 Environmental Occurrence – Soil The total releases of trichloroethylene to land and underground injection wells in 1998 were 800 lbs and 593 lbs, respectively. (TRI98, 2000)

2.6.1 Environmental Exposure – Air US EPA (2000) has recently completed a comprehensive (county-by-county) assessment of ambient concentrations of trichloroethylene as part of its National Air Toxics Assessment (NATA) program. According to the NATA analysis, mean trichloroethylene concentrations in air range from 0.02 ppb (0.10 ug/m^3) in rural areas to 0.04 ppb (0.20 ug/m^3) in urban and suburban areas. Median concentrations of trichloroethylene ranged from 0.016 ppb (0.084 ug/m^3) in rural areas to 0.024 ppb (0.133 ug/m^3) in urban and suburban areas. Monitoring data available from stations in major urban areas in California indicate that the mean trichloroethylene concentration declined from 0.115 ppb in 1990 to 0.034 ppb in 1996. (ARB, 2000)

The Total Exposure Assessment Methodology (TEAM) data on personal air concentrations are more than 10 years and likely do not reflect current levels. While

HSIA is not aware of more recent data, the decline in ambient concentrations of trichloroethylene combined with a decline in the use of trichloroethylene in consumer products (see comment on section 2.6.3) would suggest that personal air concentrations will have dropped significantly as well.

2.6.3 Environmental Exposure – Consumer Products The California Air Resources Board (ARB, 2000) conducts an annual survey of manufacturers of consumer and commercial products to determine the quantity of volatile organic compounds (VOCs) like trichloroethylene that will be emitted into the atmosphere. According to the survey data collected for 1997, very little trichloroethylene is currently used in consumer products. While trichloroethylene is used in some specialty cleaning (e.g., electric motor cleaners) and automotive products, the ARB survey data indicate that it is not longer used in typewriter correction fluids, paint removers, strippers, adhesives, spot removers, or rug cleaning fluids.

Table 2-2 Year for data? Heading for first column?

Table 2-3 There must be more recent data readily available for occurrence and levels in drinking water than the information shown in this table.

2.8, Table 2-5 Regulations There is no good reason why a year 2000 review should not be conducted. This information is of little value if not up to date.

Section 3: Human Evidence

3.2 Recent Cohort Studies

1. Blair et al. (1998)

This study is an extension of the Spirtas et al. (1991) investigation of workers engaged in aircraft maintenance at Hill Air Force Base, Utah that was considered by IARC. The total cohort now includes 5,727 deaths. Of these subjects, 2,813 were judged to have been exposed to trichloroethylene. This investigation was carried out by a team of experienced epidemiologists from the National Cancer Institute. As in the earlier phase of the study, small increases in relative risk were found for liver cancer, non-Hodgkins lymphoma, and kidney cancer plus several other tumor types in comparison with workers at the base not exposed to chemicals. The relative risks, however, were inversely related to cumulative exposure to trichloroethylene. The authors concluded that "[t]hese findings do not strongly support a causal link with trichloroethylene because the associations were not significant, not clearly dose related, and inconsistent between men and women."

2. Morgan et al. (1998)

This updated study of a Hughes Aircraft Company cohort is now reported in a peer reviewed publication. Exposures to trichloroethylene were primarily the result of vapor degreasing operations. The full cohort includes 4,052 deaths. Of these subjects, 917

were considered to have been exposed to trichloroethylene. The study found no evidence of an association between trichloroethylene and liver cancer or non-Hodgkins lymphoma when compared with the U.S. population. A very slight increase in kidney cancer (8 observed vs. 6.1 expected, SMR 1.32) showed a deficit in the low exposure group and a somewhat higher, non-significant, SMR in the high exposure group. The number of kidney cancer cases was too small, however, to allow conclusions regarding any dose relationships, and the Cox Proportional Hazards Model used to differentiate exposures and to adjust for the healthy worker effect was deemed unsuitable for use with such low incidences. The authors concluded that "[t]he recent IARC review of TCE carcinogenicity considered positive findings from three occupational studies for liver/biliary cancer and non-Hodgkin's lymphoma as suggestive of TCE carcinogenicity," but "[o]ur results and the meta-SMRs do not indicate strong effects on cancer risk for these outcomes."

3. Boice et al. (1999)

This extremely large cohort study explored cause of death among employees of the Lockheed Martin aircraft manufacturing facilities in California. The study included 20,236 deaths overall. Of these subjects, 1,110 were considered to have been exposed to trichloroethylene. The levels of exposure were considered by the authors to be generally lower than those in the Blair et al. (1998) and Morgan et al. (1998) investigations. The study showed no association between trichloroethylene exposure and liver or kidney cancer and the incidence of non-Hodgkin's lymphoma was close to the expected value (14 observed, 11.9 expected). Although the SMR for non-Hodgkin's lymphoma increased slightly with cumulative exposure to trichloroethylene, this was not statistically significant and the authors put this into context with other studies suggesting that trichloroethylene was not responsible for the marginal increase. Boice et al. reviewed the evidence from the previous cohort studies in relation to their own findings and concluded that "our investigation provides little evidence that exposure to trichloroethylene in the aerospace industry has resulted in a measurable increase of any cancer."

4. Ritz (1999)

Most important in the context of the Background Document is the absence of any association between TCE and kidney cancer. The cohort (80% exposed to TCE) actually showed a deficit of kidney cancer. It should be reported that Ritz found no association between TCE exposure and hematopoietic plus lymphopoietic cancers after adjustment for exposure to cutting-fluid exposure.

The conclusion derived from the interpretation of Ritz that "Liver cancer showed a strong exposure-response relationship and increased with exposure duration" is not valid. The cohort as a whole included 8 deaths from liver or biliary cancer and four of those were in the TCE exposed group. Thus the relationships supporting the conclusion are based on three cancers in the low exposure and only one in the moderate TCE exposure. The mathematical manipulation of such low incidences is simply not appropriate and cannot support the firm conclusions stated by Ritz and repeated in the Background Document.

The listing of the incidences that were elevated in the total cohort versus the general US rates is irrelevant for TCE: Ritz clearly listed all tumor types elevated in the TCE-exposed group.

5. Henschler et al. (1995)

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 trichloroethylene-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 Republic of Germany). The study was of a small group of 169 workers in job areas regarded as involving exposure to trichloroethylene: locksmith's area, electrician's area, and board machine area. The control group consisted of 190 workers presumed not to have had exposure to trichloroethylene. There were 5 cases (0.628 expected based on the Danish cancer registry) of kidney cancer in the exposed versus none in the non-exposed (0.648 expected). This result appears to be spectacular at first sight. However, certain modifiers apply: One of the 5 cases was a urothelial cell cancer of the renal pelvis, and this is histopathologically distinct (more akin to bladder cancer) from renal cell cancer that the Henschler group considers mechanistically linked with trichloroethylene. Although the registry combined the two types of cancer, there is no reason to do so in this study. Of the remaining 4 subjects, one was exposed to trichloroethylene for three years only in an area (electrician's) where levels would be expected to be lower -- although potentially still possible, it is unlikely that this case can be associated with trichloroethylene. Taking the cohort as a whole, the expected incidence is slightly above one -- and this expected case could appear with almost equal probability in the exposed or the unexposed group. Thus the excess incidence may be only two cases. Another factor that plays into the number of cases detected is that abdominal sonography was employed to find tumors and this is clearly not the basis for incidence in cancer registries.

Much has been made of the "very high levels of exposure" in the cardboard factory. In particular workers in the board machine area were said to have been severely exposed based on the reporting of pre-narcotic symptoms. However, the procedures used to clean the machines were employed periodically, not daily. Exposures to high levels probably occurred for 8 to 10 hours per month making the average exposure similar to those of many in the Blair et al. (1998) and Morgan et al. (1998) cohorts discussed below. The exposures in the locksmith's and electrician's areas are likely to have been comparable to those in the two U.S. cohort studies. Despite the assumption that the very high levels in the board machine area played a part in the incidence in this factory, only one of the four renal cell carcinomas was in a worker from this area.

Henschler et al. (1995) have been strongly criticized (Bloemen and Tomenson (1995), Swaen (1995)) and Henschler et al. (1995) have responded. Interestingly, it is the Henschler group's own response (copy enclosed) that most effectively rules this study out of consideration for the NTP classification process. Henschler et al. acknowledge (as do Vamvakas et al. (1998)) that this is a study of a pre-recognized cluster of cases. In their response, the claim is made that clusters are "useful," and sometimes they are. However,

it is an immutable rule in the science of epidemiology that an incidence having the status of a cluster of this type can only be used in "hypothesis setting" and nothing more. [Cite] This was the reason that Henschler et al. (1995) was not given any weight in the deliberations of the IARC Working Group, and it is the reason that NTP reviewers should not use this study as contributing to the weight of evidence in any way.

3.3 Recent case-control studies

1. Vamvakas et al. (1998)

This case-control study was conducted by members of the same German group responsible for Henschler et al. (1995). At first sight, it might be thought to be capable of addressing the hypothesis raised by the cluster study. However, significant methodological concerns are apparent. The selection of control subjects in case-control studies is critical. In this study, the criteria for selection are not fully described but it is possible to recognize that not only were the controls from different hospitals and drawn from different time periods but appear to have had very different "life experience." The last problem arises largely because of a distinct age difference between the cases and the controls. Another concern in the design and conduct of case-control studies is the manner in which information is obtained at interview. In this study the nature of the interviews, although conducted by a single individual, was different since a number of the cases were deceased and all of the controls were alive, and the interviewer was fully aware of whether interviewees represented cases or controls. There are concerns regarding the exposure assessments employed in the study since additional information collected was for cases rather than controls. Many of the methodological concerns with this study have been presented by Green and Lash (1999), which stimulated a recent response from Vamvakas et al. (2000). Although the differences in findings between this study and others (an odds ratio of 10.8 versus no or marginal elevations) is claimed to be the result of very high exposures, the exposures in the cohort studies of Blair et al. (1998) and Morgan et al. (1998) appear to be generally comparable.

It should be noted that the authors conclude that an "association" has been demonstrated, not a causal relationship. Clearly, the apparent findings reported in this study require careful review before they can be given an appropriate weighting in any classification process -- they cannot be taken as definitive evidence that trichloroethylene causes kidney cancer.

3.4 Reviews

Lost in the detail is the fact that not one reviewer (including IARC) considered that a definite association between TCE exposure and an elevation of cancer of any type had been established. Words like "suggestive", "no excess", "need for additional studies", "limited evidence" do not describe evidence sufficiently strong to support a classification of known human carcinogen. Since the most recent review (Wartenberg et al, 2000) finds "....evidence more strongly suggested an association..." with liver and kidney, it is worth reviewing some aspects of this paper. For kidney cancer, Wartenberg et al

included the Henschler et al (1995) in the Tier I cohort studies. As discussed above, this study has no place in Tier I and, without it the statistical treatment of kidney cancer incidence would include the null point within the confidence interval. Since the pattern of kidney cancer across the acceptable cohort studies shows relative risks reasonably close to the null point, some values above, some below, and none showing statistical differences from the null, it is exactly what would be expected where there is no association. The patterns for liver cancer and non-Hodgkins lymphoma are similar.

3.6 Summary

Not even the discussion in the Background Document could be considered supportive of the conclusion of "...strong patterns..." in the results of epidemiology studies. The conclusion that "...there are strong data supporting a causal relationship between TCE exposure and human cancer" is not supported by the evidence, the arguments presented, nor the opinions of any other reviewers.

Section 4: Studies of Cancer in Experimental Animals

The history of rodent carcinogenicity studies is complex with a variety of routes of administration, dose levels and stabilizers being employed. In addition, results show, species, sex and strain specificity. Despite the difficulties, a much clearer and coherent presentation of the results should have been possible. The descriptions and the Summary do not give a true picture of the findings in the animal studies, and the significant tumor endpoints have not been identified. Specifically:

4.1 It is surprising that the gavage studies are described in detail and virtually no information is given regarding the more reliable inhalation studies. For example, the NCI (1976) gavage study has been criticized (Henschler et al 1977) because genotoxic stabilizers were used in the TCE. The so called "four strain" rat study (NTP 1988) and the earlier F344 rat study (NTP 1983) have been judged "inadequate" by NTP reviewers on the basis of poor survival and deficiencies in the conduct of the tests.

4.1.2 It is meaningless to quote incidences in dosed groups without showing control incidences and including statistical analyses.

4.2 Several tumor types are given full weight in the summary even though they were seen in strains of rats or mice where high and variable background rates occur or the tumor type is specific to the particular strain. The reasons for not including these tumor types are as follows:

The incidence of lymphomas was elevated in female (not male) NMRI mice in an inhalation study (Henschler et al 1980). This neoplasm is strain-specific and displays a high background incidence. Henschler et al (1980) did not consider this observation to be indicative of carcinogenic potential. Thus lymphomas should not be used as supporting evidence of carcinogenicity in mice or humans.

If given any consideration at all, the interpretation of the results of the "four strain" study (NTP, 1988) has to be undertaken with care. The poor survival and experimental notebook comments stating that rats usually had to be "revived" after dosing indicate that the maximum tolerated dose had been exceeded. Under, these circumstances, an increase in benign testicular interstitial (Leydig) cell tumors seen at the top dose of TCE in the Marshall strain which displays a very high and variable spontaneous incidence has minimal significance as evidence of carcinogenicity. An increase in benign testicular interstitial cell tumors was also reported by Maltoni et al (1988) in inhalation studies employing Sprague Dawley rats. However, this strain is especially susceptible to developing these benign tumors that are not life- threatening. Since rats appear to develop Leydig cell tumors as part of the aging process (100% incidence in the F344 strain) it likely that they can be induced by mechanisms not related to carcinogenicity in other organs in rats or any organs, including the testes, in other species. Considering that these tumors are very rare in man, their occurrence in rat studies is of limited or no concern for humans.

Mention is made of mesotheliomas that showed an elevation only in the low dose in male F344 rats in a gavage study. Given the lack of dose related pattern, the absence of a similar finding in other studies in F344 rats or other strains, this cannot be considered an effect of TCE and should be removed from this section.

Despite reference to "In rats, TCE-induced cancers.....possibly leukemias..." in the Summary Statement, no mention of this appears in the text of Section 4. This relates to inhalation studies in Sprague Dawley rats reported by Maltoni et al (1988). The incidence of leukemia was not dose related or statistically significant, and the primary neoplasm was immunoblastic lymphosarcoma which is known to have a high and variable background incidence in the Sprague Dawley rat. It is unlikely that TCE was responsible for an increase in leukemia incidence.

Overall, the rodent tumor types to be considered in relation to human carcinogenicity are mouse lung and liver tumors and kidney tumors in rats. Even in these cases, the patterns of species, sex, and strain specificity should be spelled out.

Section 5: Genotoxicity

Generally a balanced review and appropriate conclusions.

Section 6: Other Relevant Data

6.5 Molecular Changes in Human Tumors It should be noted that the same human subjects were included in both the Bruning et al (1997) study and the more intensive investigation reported by Brauch et al (1999). This means that these studies cannot be used to demonstrate reproducibility of findings linking TCE and mutations of the VHL tumor suppressor gene. This is particularly important because the results appear to be very significant and are highly improbable. This concern was captured by the comments regarding these studies provided to the German BK TOX group by C. Walker (University

of Texas, MD Anderson Cancer Center), an internationally recognized expert on the VHL gene and its mutations. We quote:

> "The finding of multiple mutations within a single VHL allele is paradoxical in light of the fact that similar multiple mutations have not been documented previously for other well characterized human tumors arising as a result of known carcinogenic exposures, such as lung cancer from tobacco exposure. In fact, the principles of clonal selection , which are known to drive the process of tumorigenesis, are not consistent with multiple mutations within a single allele having a selective advantage. This also unlikely to occur on the stochastic level, as mutations are generally induced with a frequency of 10-4 or 5, and doses required to induce concurrent, biologically relevant mutations within a single gene would be so high as to exceed toxic and/or lethal thresholds.
>
> The second paradox resides in the observation of high frequency point mutations. The nucleoside 454 "hot-spot" which was reported would not have been predicted based on our knowledge regarding mechanisms of TCE genotoxicity from in vivo animal studies and human cell lines studied in vitro. Data from these in vivo and in vitro assays clearly show that TCE does not cause such point mutations but may act like an aneugen."

Because the Brauch et al (1999) findings are intrinsically implausible, it is critical that the results are repeated in independent studies. The study by Schraml et al (1999), although limited, does not improve the confidence in the findings of Brauch et al and thus the relevance of VHL mutations to TCE remains an open question.

6.6 Mechanism of Carcinogenesis

6.6.1 Liver cancer, First paragraph, last sentence. ".....however, the actual mechanisms of carcinogenesis may be only loosely associated with peroxisome proliferation." Considering the current sophisticated understanding of events surrounding peroxisome proliferation and their probable relationship to cancer induction, this sentence is inappropriate. See Chevalier and Roberts (1998) for a comprehensive analysis.

Second paragraph, last sentence. ".....limiting the maximum levels of TCE to below....." Should be "....TCA....", not TCE.

Third paragraph. "Exposure to peroxisome proliferators induces a much weaker response in humans compared to mice". This statement is too weak because, even for highly potent peroxisome proliferators, the end product responses of interaction with PPARa are not seen in humans. There have been no reports of PPAR-related adverse effects in humans treated for many years with hypolipodemic drugs that are potent peroxisome proliferators in rodents. Chevalier and Roberts (1998) provide a perspective on a complex topic with an extensive literature. The explanation appears to be that humans possess PPAR receptors, but the subsequent gene expression is very low (hence the

absence of peroxisome proliferation, and associated phenomena such as cell proliferation).

Fourth paragraph. Barton et al (1999) and Merdink et al (1998) hold a view that is gaining general support: that DCA plays little or no part in the induction of mouse liver tumors by TCE and is irrelevant in human responses to the solvent. Aberrations in analytical techniques led to an overestimate of DCA production from TCE and, in turn, an exaggeration of the role of DCA in the induction of mouse liver tumors. If the role of DCA is insignificant in responses in the mouse, it is even less important for humans exposed to TCE because much less DCA is produced in man. Although variations in metabolism may provide, at least, a partial explanation for strain and species differences in rodent liver tumor induction, man simply will not respond to TCA in ways leading to liver tumors.

Fifth paragraph. The paper by Bull (2000) contains significant speculation and tends to over-emphasize the role of DCA. This paragraph should not be the conclusion to Section 6.1.1. A more appropriate summation would include:

"The evidence is accumulating to support the view that TCA induces mouse liver tumors via a non-genotoxic mechanism involving PPARa and gene expression leading to specific responses such as cell proliferation. The role of DCA is now considered to be minor to negligible in the induction of mouse liver tumors by TCE. There is growing evidence that man does not respond to peroxisome proliferators in a manner that might lead to liver tumors. Therefore, the probability is high that the liver tumors induced in mice by TCE are not indicative of potential human carcinogenicity. (This is the view held by European and Canadian regulatory authorities and US FDA)."

6.6.2 Lung cancer

Second paragraph. The biological and biochemical basis for considering the TCE-induced mouse lung tumors as not being considered relevant to human carcinogenicity is discussed most thoroughly in Green et al (1997a) and Green (2000). For example, the evidence for species differences in the number, structural and biochemical properties in lung Clara cells is fully displayed. Information from these references should be used and cited in the latter part of this paragraph.

6.6.3 Kidney cancer

Second and fifth paragraphs. Evidence for DCVC-related products in human urine, and the most reliable quantitative information, may be found in the report of controlled TCE exposures by Bernauer et al (1996). This human volunteer study showed that DCVC is an extremely small of part of TCE metabolism in man (ratio between the P450 path and the GST path was 3000:1 at 40 ppm and 7000:1 at 160 ppm). There was evidence of the onset of saturation of the GST path between 40 and 160 ppm. This critical information must be included in the Background Document.

Second paragraph. Although DCVC is nephrotoxic at sufficiently high doses in the rat, the work of Green et al (1997b) shows that the level of DCVC generated from TCE in vivo is three orders of magnitude below the no observed effect level for DCVC nephrotoxicity. Thus TCE metabolism to DCVC does not explain the nephrotoxicity seen in rats.

The contrast between rats and mice regarding DCVC derived from TCE and DCVC itself was explored in depth in studies sponsored by US EPA and reported in Eyre et al (1995a and b). The results of these acute duration studies provide further evidence that activation of DCVC in the kidney does not explain the nephrotoxicity or renal tumorigenicity of TCE in the rat; this information should be included in the Background Document.

Third paragraph. In contrast to DCVC, Green et al (1998) have shown that the level of urinary formic acid from bioassay dose levels of TCE does induce renal damage of the type and severity seen in repeat dose studies with TCE; this is in the absence of any contribution from DCVC.

Fourth paragraph. "……there is no evidence that formic acid produces renal tumors." This is equally true for DCVC and, in fact, the limited direct evidence for DCVC at high doses (Terracini and Parker, 1965) suggests that
DCVC is not a rat kidney carcinogen.

This paragraph from "Renal effects...." onward is speculation and the opinions expressed are contrary to the evidence. This section should be deleted from the Document.

Sixth and seventh paragraph. The work reported by Bruning et al (1996 and Bruning and Bolt, 2000) does not provide reliable evidence of kidney toxicity. The parameters considered to indicate toxicity (urinary GSTa and microglobulin) vary widely in the normal population and are affected by age, sex, drugs and many lifestyle factors. The likelihood of detecting meaningful changes in a small population 20 years after exposures to TCE ceased is remote, particularly if (known) confounding factors are not controlled.

Eighth paragraph. The statement that "....the site and histopathological characteristics of the tumors observed in patients and experimental animals were identical...." is, almost certainly incorrect. The great majority of renal cell carcinomas in man are of the clear cell type associated in a high percentage of cases with alterations in the Von Hippel Lindau (VHL) tumor suppressor gene. In contrast, renal cell carcinomas in the rat are usually of the non-clear cell type associated with changes in the tuberous sclerosis 2 tumor suppressor gene. Although the hypothesized molecular mechanism referred to in Dekant et al (1986) was biologically plausible, the accumulated evidence now indicates the GST-conjugation and activation of DCVC in the kidney does not explain kidney toxicity or tumor induction by TCE in rats and this mechanism is even less likely to be active in man.

Once again, Bernauer et al (1996) provides more reliable information than Birner et al (1993) on TCE metabolites in man. There is absolutely no basis for the statement that humans are more sensitive than rats in developing the primary biochemical lesion.

In sum, the once plausible hypothesis that kidney toxicity and tumors in rats exposed to TCE arose through renal activation of DCVC is no longer tenable now that more is known of the biological effects and potency of DCVC, and the levels of its production from TCE.

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[redacted]

Paul H. Dugard, PhD
Director of Scientific Programs

Letter to Editor of J. Cancer Res. Clin. Oncol. (In Press)

Sir,

The importance of reliable exposure estimates in deciding whether trichloroethylene can cause kidney cancer

There is currently controversy over whether exposure to trichloroethylene can cause kidney cancer. The review considers evidence from both human and animal studies, but puts great weight on two epidemiological studies of workers in Germany where increased risks of renal cell carcinoma were seen (Vamvakas *et al*, 1998 and Henschler *et al*, 1995). In contrast there are a number of large-scale cohort studies of workers exposed to trichloroethylene in the United States of America that essentially show no statistically significant risk of kidney cancer (Blair *et al*, 1998; Boice *et al*, 1999; Morgan *et al*, 1998). It is argued that the discrepancy between these two groups of studies results from long-term and exceptionally high trichloroethylene exposure levels amongst the German subjects.

We have recently tried to make comparisons between the exposures in one of the US studies (Blair *et al*, 1998) and those of Vamvakas *et al* (1998) and Henschler *et al* (1995). The Henschler *et al* paper contains a fairly comprehensive description of the exposure conditions in the factory under study. In common with most retrospective epidemiological studies there are no measurements of historical exposure to trichloroethylene. The main analysis of risk focuses on the classification of workers into either "exposed" or "unexposed" groups. On the basis of the descriptions of workers and observations of the factory the authors concluded that exposed workers inhaled "high concentrations of trichloroethylene over long periods of time". They further suggest that during the cleaning of the machinery with trichloroethylene, which was undertaken every two weeks, the concentration inhaled would have likely been well above 50ppm.

Using the information provided in the paper and making some assumptions about the factory environment and the usage of trichloroethylene we have used a simple mass balance model to estimate the maximum likely concentration during cleaning of the machinery. In addition, we have used a structured subjective assessment method, as described by Cherrie (1999), to

provide an alternative assessment of the likely exposures. Our calculations suggest the trichloroethylene exposure level during cleaning would have been approximately 1,800 to 4,000ppm for the maximum usage 23,000l per year (assuming that all trichloroethylene was used for cleaning purposes at a rate of 2-3 litres per minute). With the minimum volume of trichloroethylene used (i.e. 2,600l per annum) the air concentration would have been correspondingly lower, i.e. between about 200 and 1,200ppm. However, these cleaning activities were only carried out for between 8 to 10 hours per month and we estimate the long-term average exposure of these workers would have been lower, probably between about 10 and 225ppm. The other exposed workers in the factory were involved with fairly continuous use of trichloroethylene in cold cleaning and based on our structured subjective assessment their long-term exposure level was probably about 100ppm.

We also investigated whether dermal exposure could have been a significant contributor to overall exposure using a model of uptake described by Semple *et al* (in press). These calculations showed that the dermal route would have probably contributed less than 10% of the total exposure received by the subjects.

The study reported by Vamvakas *et al* has poorer descriptions of the exposure scenarios than the preceding work because it is a population-based case-control study. Nevertheless, for each subject the paper details the total duration of exposure (in hours), frequency of exposure, self-reported pre-narcotic symptoms on a categorical scale (0 to 3) and a brief description of the work activity involving trichloroethylene. In most cases exposure occurred on between one to three occasions per week (12 cases), with the remaining cases (8 people) exposed daily. The authors report their results for "exposed" and "unexposed" workers and by categorical exposure level (+ to +++) derived from the self-reported symptoms and the frequency of exposure.

We have three major concerns about the exposure assessment for this study. First, and most important, the physicians who carried out the interviews with the subjects were aware of their status as either cases or controls. This will

most likely have led to interviewer bias in addition to responder bias that generally plays a role in hospital-based case-control studies. The authors do not properly address the issue of bias from these sources. The second area of concern is the exposure rating system used in the study, which is entirely based on self-reported pre-narcotic symptoms. Exposure rating systems based on symptoms, although sometimes used in epidemiological studies, are prone to bias and we believe researchers should always attempt to substantiate their findings using more objective measures (Fohn *et al*, 1993; Myers 1989). There are more objective measures available to the authors, for example the "total time of exposure" or "handling of trichloroethylene". Third, looking at the presented data it also becomes clear that younger cases (< 50 years) were more likely to be classified as exposed than older cases (80% versus 28%). This explains the relatively large impact of adjustment for age on presented odds ratios (Table 5). Given the fact that occupational exposures generally have decreased over the last three decades (Symanski *et al.* 1998a, b) it is rather peculiar that in this study younger cases with most likely more recent and therefore lower exposures apparently showed a much higher risk. An alternative explanation for this phenomenon might be either recall or interviewer bias.

It is more difficult to be definitive about the exposure of subjects in a hospital-based case-control study when compared with an industrial cohort study. However, the descriptions of the work are not dissimilar to other situations where trichloroethylene was used in the past. For example, Hicklsh *et al* (1956) describes measurements of trichloroethylene in the vicinity of a small open-top degreasing tank, heated by a gas burner and fitted with cooling coils. They found that the concentration of trichloroethylene in the vicinity of the tank was between 400 and 600ppm. We consider that it is likely that the highest exposure levels in the Vamvakas *et al* study were around these levels, with lower exposure levels where metal components were cleaned with rags soaked in trichloroethylene. Because of the intermittent nature of the work the long-term average trichloroethylene exposure in the exposed subjects may have been around 100ppm.

We have compared the cumulative exposure, expressed as ppm-hours, based on our exposure assessments with the categories used by Vamvakas *et al* and found little evidence of increasing exposure being related to the categorical assignment (Figure 1). In addition, many of the subjects probably had quite low cumulative exposure, e.g. between 100 and 1000ppm-hours.

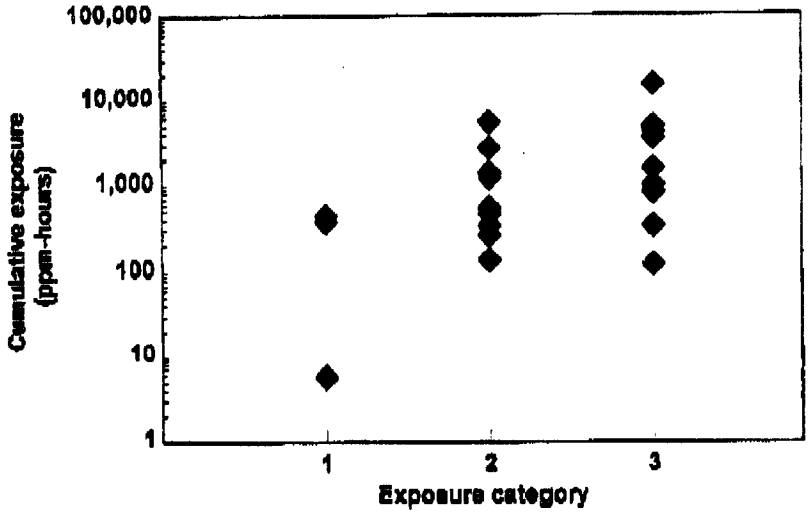

Figure 1 Comparison of assessed cumulative exposure with the categorical assignment used by Vamvakas *et al* (1998)

Finally, we consider the study by Blair *et al* (1998) where aircraft maintenance workers were either exposed during vapour degreasing or from benchwork involving wiping components with trichloroethylene soaked rags. The descriptions of the exposure scenarios are comparable with the Henschler *et al* and the Vamvakas *et al* studies. For example, Stewart *et al* (1991) say "Aircraft mechanics used large amounts of solvents when spraying and wiping hydraulic lines" and "short but high exposures occurred when mechanics worked in the semiconfined space of the aircraft wings". Also, in the 1950's and 60's "degreasers were not well controlled", some having missing lids others with faulty cooling coils. Stewart *et al* (1991) provide a semi-quantitative exposure assessment based on the estimated frequency, duration and intensity for each activity. Although not explicitly said it is assumed that the peak exposures ranged from 200 to 600ppm, based on

limited monitoring data available to the authors. The estimated long-term exposure for those involved in vapour degreasing during the 1950's and 60's was probably about 50ppm and for those involved in benchwork around 10ppm.

The data from the US aircraft maintenance workers (Blair, 1998) are therefore not markedly different from the two German studies. The likely long-term exposure levels in the former study was probably around 50ppm, with short-term excursions up to 600ppm, and in the latter the long-term exposure levels were probably around 100ppm, with short-term levels up to about 600ppm in the Vamvakas *et al* study and in excess of 2000ppm in the Henschler *et al* study. We suggest that while there might have been differences in the intensity and temporal pattern of exposure to trichloroethylene between the US and German studies, these differences were unlikely to have caused the differences in observed risk. The potential for bias in the exposure assessments undertaken by Vamvakas *et al* should be carefully evaluated.

Yours sincerely,

John W Cherrie
Department of Environmental and Occupational Medicine,
University of Aberdeen and
Institute of Occupational Medicine, Edinburgh, UK.

Hans Kromhout
Environmental and Occupational Health Group, Utrecht University, The Netherlands.

Sean Semple
Department of Environmental and Occupational Medicine,
University of Aberdeen and
Institute of Occupational Medicine, Edinburgh, UK.

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