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Dr. C. W. Jameson
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Dear Dr. Jameson:

On behalf of my colleagues Sean Hays, Michael Kelsh, Michael Garry and Lisa Yost, I am pleased to submit the enclosed report in response to the NTP proposal to list trichloroethylene as a "known human carcinogen". We appreciate the opportunity to submit these comments and hope they are helpful to the committee.

Yours sincerely,

[redacted]

Jack S. Mandel, Ph.D., M.P.H.
Vice President

Comments on the National Toxicology Program (NTP) Proposed Listing for Trichloroethylene (TCE):

A Critical Review of Epidemiologic Research and Selected Toxicological Issues on Cancer Risks due to TCE Exposure

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Summary

These comments were prepared in response to the National Toxicology Program (NTP) proposal to list trichloroethylene (TCE) as a "known human carcinogen" to replace the prior listing as "reasonably anticipated to be a human carcinogen" (Federal Register Vol. 65 pp 17889-17893). According to NTP guidelines, a chemical is identified as a "known human carcinogen" where there is "sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance, or mixture and human cancer". The prior listing "reasonably anticipated to be a human carcinogen" is used for compounds having "limited evidence of carcinogenicity in humans and/or sufficient evidence of carcinogenicity in experimental animals". In these comments, we summarize our view on the weight of the evidence available in the scientific literature at this time. Based on this review, the weight of the evidence for TCE does **not** support the listing as "known human carcinogen". The epidemiological evidence for TCE does not support this listing and the animal data is subject to question for some endpoints. We recommend a continuation of the "reasonably likely" designation, which is similar to the designation for saccharin and ethyl acrylate. This categorization better represents the weight of evidence for TCE given the many methodological problems and inconsistencies among the epidemiology studies.

The following comments are organized into two main sections; a review of the epidemiology literature and a review of the weight-of-evidence regarding the animal tumors and their potential relevance to humans. Our review of the epidemiological research emphasizes the recently published reviews and occupational studies and a critical evaluation of the recent studies of renal cell cancer in Germany that report much higher cancer risks than all of the previously published research. In addition, a discussion is provided of the role of mutations in the von Hippel-Lindau (VHL) tumor suppressor gene in human renal cell cancer (Friedrich, 1999). While the "known human carcinogen" designation is fundamentally based on the

epidemiology literature, the weight of evidence for human carcinogenic potential of TCE also relies on laboratory studies. Therefore, a review of animal research is provided to discuss the degree to which the animal data on cancer, and particularly proposed mechanisms of TCE induced carcinogenicity in animals, provides additional support for, or is contrary to, the potential for cancer development in humans exposed to TCE.

Epidemiology Review

The latest review of the epidemiology of TCE identifies a number of studies on TCE and cancer including 20 occupational cohort studies, 40 case-control studies and more than 12 community studies (Wartenberg et al. 2000). As stated by the authors, not all of these studies are equal in terms of quality and the degree of confidence in the findings regarding the carcinogenicity of TCE. The occupational studies are generally the most informative considering the strengths of the study design and the exposure assessment procedures. However, even these studies have limitations including a lack of specific quantitative exposure information, limited data on potential confounders, and other workplace exposures, whose potential health effects cannot easily be disentangled from potential TCE health effects.

Three recent reviews evaluated epidemiologic studies on TCE and cancer and reached different conclusions. In the most recent review, Wartenberg et al. concluded:

> In summary, the cohort studies provide strong evidence and the case-control studies provide supporting evidence of an association between the kidney cancer among workers exposed to degreasing agents and solvents and to those in both the iron and steel and dry cleaning and laundry work industries (Wartenberg et al., 2000).

These authors also concluded "studies of liver and bilary cancers also offer strong data in support of the carcinogenicity of TCE".

In 1996, Weiss reviewed the results on gastrointestinal cancers, lymphatic/hematopoietic cancers, urinary track cancers (kidney/bladder), respiratory cancer, prostate and breast cancer in four studies (See Table 1) and concluded:

> It is clear from a review of the data that, both in terms of the small relative increases seen and the small number of observations upon which those increases are based, the

> evidence currently available in support of a causal hypothesis is quite limited (Weiss, 1996).

In a review of renal cell cancer and TCE, McLaughlin and Blot concluded:

> In an evaluation of the seven cohort studies examined in this review, none showed an association with kidney cancer risk, except for the methodologically questionable study by Henschler et al. .... The six case-control studies of renal-cell cancer and exposure to TCE and degreasing solvent provide little support for a causal association. ... In summary there is no credible evidence to support an association between risk of renal-cell cancer and TCE (McLaughlin and Blot, 1997).

In the most recent review, Wartenberg et al. based their conclusions on a similar set of studies, yet reached a different conclusion than the previous reviews. Wartenberg et al. divided available cohort studies into three tiers: studies with known TCE exposure in which the exposure was best characterized (Tier 1), studies without certain TCE exposure in which the exposure was inferred by job categories (Tier 2), and studies with mixed solvent exposure, basically studies of dry cleaners and laundry workers (Tier 3). Conclusions were based primarily on the results of the Tier 1 studies because these were considered the strongest on which to base a causal inference. However, an examination of the methods and the studies included in the Tier 1 analysis reveal potential flaws both in the classification of studies, designation of influential studies, assessment of heterogeneity, and in the conclusions drawn from the analysis.

Kidney Cancer

The relationship between TCE exposure and cancer incidence and/or mortality has been studied in seven large occupational cohorts (Table 1): Axelson et al., 1994, Anttila et al., 1995, Blair et al., 1998, Morgan et al., 1998, Ritz, 1999 and Boice et al., 1999. The study of aerospace workers by Garabrant and colleagues (Garabrant et al., 1988) has also been included in summaries of the occupational studies (McLaughlin and Blot, 1997). These seven studies were included in Wartenberg's "Tier 1" analyses; cohort studies that were judged to have the best characterized TCE exposure. A study by Henschler et al was also included in Wartenberg's Tier 1 analysis, although as discussed below, this study did not characterize exposure well and had numerous methodological flaws and inconsistencies in the data. An examination of these eight large cohort studies (Table 1), which represent the best available human data on TCE exposure

and kidney cancer (except for Henschler et al), provides no evidence that occupational exposure to TCE causes kidney cancer. Four of the studies had incidence data available, and only one (Henschler) has a significantly elevated standardized incidence ratio (SIR), strongly influencing the average SIR estimate. For cancer mortality, only the Henschler study had an elevated SMR, which was not statistically significant.

Liver Cancer

Seven of the eight cohort studies summarized in Table 1 reported results for liver cancer. None of the studies observed a statistically significant risk.

Lung Cancers

For the seven occupational cohorts, all SMRs and SIRs were close to 1.0, five of the seven were less than 1.0. Two of the larger studies of aerospace workers (Boice et al, 1999 and Blair et al., 1998) reported significantly **lower** relative risks for lung cancer in the TCE-exposed groups (Table 1).

Lymphatic and Hematopoietic Cancers

For lymphatic and hematopoietic cancers five of the six studies did not find a significantly elevated risk. The SIR for the Finnish workers study, 1.63 with a lower confidence limit of 1.06, was the only statistically significant positive result. In fact, it was the only significant positive result of the 28 results provided for the four types of cancers (kidney, liver, lung and lymphatic/hematopoietic) in Table 1.

Critical Review of Investigations Identifying Kidney Cancer Risks

The articles by Henschler et al. and Vamvakas et al. have been prominent in the considerations of TCE carcinogenicity (Henschler et al., 1995, Vamvakas et al., 1998). The authors of these studies suggest that exposures to TCE significantly and substantially increase the risk of renal cell 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 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 that permeate these two studies.

Henschler et al.

Henschler et al. conducted a retrospective cohort study at a cardboard factory in Germany (Henschler et al., 1995). One 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 the validity of their conclusion.

This study appears to be an expanded investigation of a cluster of kidney cancer cases. If true, then a conclusion regarding causation is inappropriate. 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 suggest 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 in order to result in the median years of observation suggested by the authors. This is a highly unlikely occurrence.

Other data in Henschler's Table 1 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 Henchler et al). This is essentially the same as the expected number of deaths presented in Table 5 of Henchler et al., an unexpected 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.

The many methodological problems and inconsistencies in the data render this study almost uninterpretable. It is likely that the Henschler et al. finding is either due to chance based on a cluster investigation presented as a hypothesis testing study or to confounding. The most probable explanation is chance.

Vamvakas et al., 1998

Vamvakas et al. conducted a case-control study with cases 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 (Vamvakas et al.,1998). Cases included in an earlier study by Henschler et al. were excluded even though they might have been eligible by virtue of having undergone surgery at the study hospital (Henschler et al., 1995). 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. Cases included may not be similar to 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 during 1987 and 1992 were excluded from consideration. The 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, strong attempts are 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.

Comparing the "highest" exposure category to no exposure gives 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.

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.

It is surprising that these studies, with their many significant design and methodological flaws, are given credibility in the assessment of the carcinogenicity of TCE. These fundamental flaws greatly diminish the studies' credibility and render them of questionable value in an evaluation of the evidence on renal cell cancer and TCE.

Mutations of the Von Hippel-Lindau gene are a biomarker for renal cancer, but evidence is lacking as a marker of TCE exposure in human kidney cancer

While most of the debate regarding the mechanism of action of TCE in carcinogenicity has focused on investigations in laboratory animals, recent researchers have proposed mutations in the von Hippel-Lindau (VHL) tumor suppressor gene as having a role in human renal cell cancer. Hereditary mutations in the von Hippel-Lindau (VHL) tumor suppressor gene leading to loss of its function results in VHL syndrome, a condition characterized by vascular tumors, cysts of the kidney, liver, and pancreas, and, in 70% of patients with VHL syndrome, clear cell renal cell carcinoma (RCC) (Friedrich 1999). VHL mutations have also been detected in approximately 60% of patients with sporadic RCC (i.e., patients without VHL syndrome) (Brauch et al 1999).

Two recent molecular epidemiological studies reported an association between TCE exposure and mutations in the VHL gene of renal cell carcinoma (RCC) patients as supporting evidence

that TCE causes kidney cancer. In a short communication, Bruning et al. reported the presence of VHL mutations in 100% of tumors from 23 renal cell carcinoma patients with a history of prolonged, high occupational exposure to TCE (in a metal-processing plant)(Bruning et al., 1997). Although exposure was not well characterized in this study, it appeared to be based on patient reported symptoms and the description of working conditions. By comparison, VHL mutations have been reported in approximately 60% of patients with sporadic renal clear cell carcinoma without known TCE exposure. In a follow-up to Bruning et al., Brauch et al. recently reported that 75% of TCE-exposed RCC patients from the same metal-processing plant had a VHL mutation, with an association between the number of different mutations and the apparent dose of TCE received (Brauch et al., 1999). However, both the Bruning et al. and Brauch et al. studies suffer from a number of methodological problems that adversely impact the validity of the studies.

Bruning et al. did not attempt to characterize exposure at all, except to describe it as likely exceeding by "manyfold the… occupational exposure limit… of 50ppm." In Brauch et al., exposure levels were based on patient responses to a questionnaire regarding duration and frequency of exposure (presumably by estimating the time spent at certain jobs where TCE concentration was assumed to be high), and the severity and frequency of symptoms of solvent exposure (dizziness, headache, and nausea). However, given the description of working conditions, it is likely that the air TCE concentrations were consistently very high. All individuals were probably exposed to equally high air concentrations. Therefore, placing individuals in exposure categories based on self-reported severity and frequency of symptoms decades after exposure, is likely to introduce exposure misclassification since there would be a wide inter-individual variation in the threshold for such general symptoms, as well as in the motivation to report symptoms.

Although TCE is the only chemical mentioned, it is unclear if TCE was the only chemical to which employees of the metal working facility were exposed. The questionnaire used by Brauch et al.reportedly queried patients regarding exposure to perchloroethylene and other chemicals, but the responses were not reported or summarized. Likewise, the questionnaire investigated the presence of potential confounders, such as smoking and family and personal

history of kidney disease, but responses were not reported, nor were any adjustments made to the study results.

The analysis of tumor DNA from control RCC patients by Brauch et al. (those apparently not exposed to TCE) was not reported in a way that allows a meaningful comparison. Although data were collected both from 34 controls from the same region as the cases and 73 controls from other regions, it is the former group that provides the most appropriate comparison. However, only pooled results were presented: 58% of control tumors had a VHL mutation versus 89% in the high exposure group cases (75% for all cases combined). The number of cases occurring specifically in the control group from the TCE exposure region was not reported.

Lastly, the size, progression, or class of tumors was not reported in either study. This is important since more advanced tumors are likely to contain a greater number of mutations than smaller, less advanced tumors. As a tumor progresses, cancer cells divide and DNA is replicated in a more rapid and uncontrolled fashion than occurs in normal cells. This provides an ideal environment for mistakes (i.e., mutations) to occur in DNA replication. In addition, DNA repair mechanisms, which detect and repair most damaged DNA under normal conditions, are often damaged in cancer cells. This provides further opportunity for additional mutations to arise in the daughter cells of replicating cancer cells. Without accounting for the relative progression of the tumors from the TCE-exposed and TCE-unexposed groups, the ability to attribute differences in mutation rates to TCE exposure is severely limited.

Brauch et al. also reported the presence of a mutational hot spot at nucleotide 454 of the VHL gene in RCCs from individuals occupationally exposed to TCE. The nucleotide 454 mutation occurred in 39% (13 of 44 cases) of tumors from the TCE exposed group, but in none of the controls. In contrast, Schraml et al. reported no specific mutational patterns in the VHL gene in RCC patients occupationally exposed to TCE and other solvents relative to unexposed sporadic RCC patients (Schraml et al., 1999). The limitations of the Brauch et al study, along with the lack of association between TCE exposure and VHL mutations reported by Schraml et al., underline the need for caution in inferring a causal role for TCE in RCC through a mechanism involving VHL mutations.

Review of Animal Studies

In lifetime bioassays, TCE causes liver tumors in mice following gavage administration, liver and lung tumors in mice following inhalation exposures and kidney tumors in male rats following gavage or inhalation. While the listing of TCE as a "known human carcinogen" is primarily dependent on the epidemiologic evidence, the degree to which human cancers would be expected based on findings in animal studies is also critical to the weight of evidence. Therefore, this section presents a review of the potential mechanisms of action for tumor types found in animals and the degree to which the animal models are predictive of TCE carcinogenicity in humans. The recently issued Monograph on Trichloroethylene Toxicity published in May of 2000 as Supplement 2 of the journal Environmental Health Perspectives, which is comprised of review articles commissioned by the United States Environmental Protection Agency (USEPA), was the main resource reviewed in preparing these comments.

As described in detail below, while TCE is carcinogenic in mice and rats, the lack of mutagenic potential, and the requirement for high doses to yield cancers in laboratory animals, and the unique mechanism of action of kidney cancers in male rats, of liver cancers in mice, and of lung cancers in mice, all suggest that these findings must be interpreted carefully for application to human populations exposed at lower levels in occupational or environmental settings.

Kidney Tumors in Rats Are Not Predictive of Carcinogenic Potential in Humans

TCE induces an significantly increased incidence of kidney cancer in male rats following oral or inhalation exposure. TCE has not, however produced statistically significant increases in kidney cancer in female rats or in other species tested (i.e., mice or hamsters). Moreover, TCE does not consistently produce kidney cancer in all rat species tested (Lash et al., 2000a). This pattern of findings is consistent with a mechanism of action of TCE unique to male rats. Several different hypotheses have been developed to explain the kidney tumors observed in rats including: ability of rats to produce *S*- (1,2-dichlorovinyl)-1-glutathione (DCVG) and *S*- (1,2-

dichlorovinyl)-1-cysteine (DCVC); peroxisome proliferation, alpha-2u-globulin nephropathy, genotoxic mechanisms, and acute and/or chronic toxicity mechanisms including oxidative stress, alterations in calcium ion homeostasis, mitochondrial dysfunction, protein alkylation, alterations in cellular repair processes, gene expression and cellular proliferation.

A review of each of these potential mechanisms follows.

Rats produce a carcinogenic metabolite not readily produced in humans

All of the potential mechanisms associated with renal tumors in rats have been primarily associated with the metabolic production of DCVC (Lash et al., 2000a). DCVC is metabolically produced from DCVG (see Figure 4; Lash et al., 2000a) via β-lyase. DCVG is metabolized from TCE via glutathione S-transefase. While humans do have the metabolic capability to produce DCVG (Lash et al. 1999, in Lash et al. 2000a), humans appear to have little to no capability to produce DCVC (Kharasch et al. [1999]a,b in Lash et al., 2000a). The rat on the other hand does have the metabolic capacity to produce DCVC. Estimates of β-lyase activity in rats and humans indicate that the rat has at least a 6-fold greater rate of β-lyase activity than humans (Kharasch et al. [1999], b in Lash et al., 2000a). This information led the authors of the EHP State of the Science Chapter on kidney tumors (Lash et al., 2000a) to suggest that "use of rodent data for human health risk assessment likely overestimates the risk to humans" (Lash et al., 2000a). Therefore, the species differences between the rates of production of the metabolites thought to be responsible for the kidney tumors in rats compounded with the fact that kidney tumors are only observed in rats at very high exposure levels, suggests that kidney tumors may not be relevant in humans.

Paroxysm proliferation may have some limited role in kidney cancer in rats

Peroxisome proliferation has been proposed as a mechanism of action of liver cancer in mice and is discussed further in a later section of these comments. TCE is metabolized to chloroacetates, which produce peroxisome proliferation in the liver and this proliferation has been proposed as a mechanism of liver cancer in mice. This response is generally held to be a

rodent-specific response, with limited occurrence in humans and primates (Kluwe 1994 in Lash et al. 2000a and Lake 1995 in Lash et al. 2000a). While there is some evidence of peroxisome proliferation in the rat kidney, evidence from exposure to other chemicals resulting in peroxisome proliferation suggests that the rat accumulates lower levels of TCA in the kidney than in the liver. Furthermore, rat kidney peroxisomes are generally less responsive to peroxisome proliferators than are rat hepatic peroxisomes. Both of these lines of evidence suggest that peroxisome proliferation in the kidney is not likely to be a significant cause of kidney cancer (Lash et al., 2000a).

α_{2u}-Globulin nephropathy is unique to male rats and is a likely mechanism for kidney cancer

A well-established mechanism of action of TCE-induced kidney tumorigenesis in male rats is α_{2u}-globulin nephropathy (Lash et al., 2000a). The hypothesis underlying this mechanism of action is that renal-cell tumors are induced as a result of nephropathy and consequent cellular proliferation caused by accumulation of α_{2u} in kidneys of male rats as a result of chemical exposure. As noted in Lash et al. and supported by the National Research Council (NRC 1995), this mechanism is widely regarded as being male rat-specific and not relevant to humans because α_{2u} is a protein unique to male rats. Moreover, as stated above, this mode of action is consistent with the finding of statistically significant increases in renal tumors in male rats, but not female rats or other animal species (e.g., mice, hamsters). These findings are relevant to the assessment of the animal carcinogenicity data for TCE and should be considered by NTP in their review of the weight-of-evidence for the potential for TCE to induce cancer in animals and in humans.

Genotoxicity appears to play little role in carcinogenicity related to TCE

The authors of the State of the Science paper on the potential mutagenicity of TCE concluded that trichloroethylene and its metabolites are not mutagenic in humans (Moore and Harrington-Brock, 2000). Specifically, the authors concluded, "the weight of evidence argues that chemically induced mutation is unlikely to be a key event in the induction of human tumors that might be caused by TCE itself (as the parent compound) and its metabolites, CH, DCA and

TCA" (Moore and Harrington-Brock, 2000). The authors made this conclusion based on the evidence that these chemicals require extremely high doses to be genotoxic. The authors continued, "there is not enough information to draw any conclusions for trichloroethanol and the two trichloroethylene conjugates, DCVC and DCVG". Thus, "definitive conclusions as to whether TCE will induce tumors in humans via a mutagenic mode of action cannot be drawn from the available information" (Moore and Harrington-Brock, 2000).

The finding of a lack of evidence for mutagenicity of TCE is consistent with the finding that cancers occur at high doses and appear to occur as a result of cellular injury. Thus application of carcinogenicity data on TCE for protection of human health is most accurately represented by a nonlinear relationship between exposure and risk.

Acute/chronic toxicity likely contributes to rat kidney cancers

It has been proposed that various acute and chronic toxicity mechanisms may play a potential role in the development of renal tumors in rats. These mechanisms include oxidative stress, disturbances in calcium ion homeostasis, mitochondrial dysfunction, protein alkylation, renal repair processes and alterations in gene expression and cell proliferation (Lash et al., 2000). Such toxic effects associated with TCE exposures only arise at very high cellular concentrations and have been principally attributed to DCVC concentrations (Lash et al., 2000). Each of these potential mechanisms may have a minor contributory role in producing kidney cancer in rats (Lash et al., 2000). Because these mechanisms occur at doses much higher than those resulting from workplace levels and because they are principally attributed to a metabolite (DCVC) that is not readily produced in humans, these findings further limit the applicability of kidney cancer results from animal studies for evaluation of human populations who may be exposed to TCE in the workplace or in the environment.

TCE interaction with vitamin B_{12}, leading to formic acid excretion, may play a role in rat kidney cancer

A new mechanistic hypothesis has been developed that links TCE exposures, kidney toxicity and the observed increase in formic acid excretion in rats following exposures to TCE (Dow and Green, 2000). Dow and Green conducted a set of experiments that yielded results

indicating that TCE (as well as TCA and trichloroethanol) interact with vitamin B_{12} inhibiting both the methylmalonyl CoA and methionine salvage pathways. The result is a secondary folate deficiency due to the 'methyl folate trap' leading to a major impairment in formate metabolism and the excretion of large amounts of formic acid in urine (Dow and Green, 2000). The implications for humans are currently unknown. The fact that these experiments were conducted at very high doses (in excess of 1 g/L in drinking water) makes it difficult to make conclusions about the relevance of this mechanism at low doses. In addition, given that humans metabolize TCE at a much slower rate than rats, suggests that this mechanism may be less important in humans than in rats. However, humans are more folate deficient compared to rats, suggesting a potential sensitivity of humans (Dow and Green, 2000). Future studies, designed by the authors of this report, aim to collect data in humans exposed to TCE to test whether humans are excreting formic acid in their urine. This data will help answer the question of whether this vitamin B12 interaction is occurring in humans.

Lung tumors in mice are not predictive of carcinogenic potential in humans

Available animal studies show that TCE-induced lung tumors have been observed only in mice, not rats, and only following inhalation exposure. Green describes the evidence that these tumors arise almost exclusively in nonciliated Clara cells as a result of toxicity induced by chloral, a metabolite of TCE (Green, 2000). The nonciliated Clara cells in mice lungs are unique in that they have a very high rate of P450 metabolism (CYP2E1) of TCE and an impaired metabolism of chloral, resulting in an accumulation of chloral in the Clara cells. The rate of TCE metabolism in mice is 23-fold higher than in rat microsomes. A metabolic rate for TCE could not be detected in human lung tissue samples (Green, 2000) and no CYP2E1 could be detected in human lung tissue samples by various techniques.

The metabolic rate differences observed between species correlate with differences in Clara cell numbers and morphology. In mice, Clara cells are numerous and spread throughout the lung. They are fewer in number in the rat lung. In human lung, Clara cells are rare and are only found in few numbers in the distal bronchioles. The morphology is also vastly different. In mice, the Clara cells are packed with endoplasmic reticulum, the membrane where the P450 enzyme complex is located (Green, 2000). Clara cells in humans are largely devoid of

endoplasmic reticulum. Thus, the large quantitative differences between the metabolic capacity of the mouse lung and the human lung, together with differences in the number and morphology of Clara cells in mouse lung and human lung, suggest that lung cancer risks to humans are minimal (Green, 2000). Consequently, the weight of the evidence indicates that the lung tumors in mice are not predictive of lung cancer risk in humans.

Liver tumors in mice are thought to be related to peroxisome proliferation

Bull has extensively reviewed the literature regarding the mode of action of liver tumor induction by TCE and its metabolites (Bull, 2000). TCE and two of its metabolites, choral hydrate (CH) and trichloroacetate (TCA), induce liver tumors in selected strains of mice, but not rats or other species. A third metabolite, dicloroacetate (DCA), induces liver tumors in $B6C3F_1$ mice and F344 rats when administered directly.

The carcinogenicity of TCE in rodents is largely accounted for by TCA and DCA, rather than by the initial metabolite, CH. CH is rapidly reduced to tricholorethanol (TCOH) or oxidized to TCA. Most humans are more proficient at glucuronidating TCOH relative to rodents, which results in the compound's secretion into the bile and from there, to the small intestines. Enterohepatic circulation transports TCOH back to the bloodstream, with some amount lost in the feces. Oxidation of CH to TCA and DCA is thus expected to be more rapid in rodents. Chloral hydrate has been administered to humans, including children, as a sedative and is not considered by NTP to be a human carcinogen.

The available data strongly indicate that TCA and DCA induce liver tumors at high doses primarily by modification of cell signaling pathways, and the two chemicals differentially affect cell replication and death processes. TCE and its metabolites are thus thought to cause tumors through non-genotoxic, indirect mechanisms, which would likely have a sublinear or threshold dose-response curves. DCA in particular has a very strongly sublinear dose response relationship for tumorigenesis. At very high doses, DCA causes a rapid increase in growth rate of tumors with a less malignant phenotype than those caused by TCA. At lower doses, but still high compared to environmental levels, TCA appears to select initiated cells that have a greater rate of replication than those selected by DCA.

Although DCA is the TCE metabolite most likely to induce liver tumors in multiple species, it appears to be tumorigenic only when administered directly at high doses. In reality, it may never reach tumorigenic doses in humans at environmentally relevant TCE exposure concentrations. At TCE doses that lead to tumors in rodents, blood DCA levels are negligible. After TCE exposure, blood TCA concentrations are higher than DCA concentrations in all species, including humans.

Another mechanism that has been associated with the tumorigenic effects of TCE in mice is peroxisome proliferation by TCA, which occurs at doses resulting in liver tumorigenesis. Peroxisome proliferation is typically correlated with carcinogenesis, but this may be a loose association, particularly in humans. Many of the traditional responses in mice that are typically associated with peroxisome proliferators are mediated through the peroxisome proliferator activated receptor alpha (PPARα) gene. If the PPARα gene is disrupted in mice, mice become insensitive to the liver cancer-inducing properties associated with peroxisome proliferators.

Humans demonstrate a lower level of expression of PPARα. Therefore, if TCE acts through a peroxisome proliferation-mediated mechanism, it would be expected to be less potent in humans in comparison with mice. This is consistent with a review by Bull (2000), stating that chemicals that induce peroxisome synthesis in rodent livers and hepatocytes fail to produce the same response in human hepatocytes. By whatever mechanism TCA induces liver tumors in mice, the specificity of these tumors to particular strains, suggests that a trans-species risk for liver tumors by TCA and TCE is unlikely. Specificity of TCE-induced liver tumors to specific mouse strains at high doses limits the predictiveness of these findings for humans at environmentally relevant doses.

Summary and Conclusions

Laboratory animals exposed at levels much higher than typical occupational or environmental levels had elevated cancer rates. However, significant uncertainties exist regarding the relevance of each of the cancer types observed in animals for the evaluation of human health

risk. Specifically, the role of α_{2u}-globulin nephropathy kidney cancer in the male rat, the role of peroxisome proliferation in mouse liver cancer, and the substantial anatomical and physiological differences between humans and mice in the lung cancer process observed following TCE exposure are all problematic. Furthermore, the dose levels required to develop cancer and the lack of evidence for mutagenicity suggest that carcinogenic potential is a nonlinear process. **Thus, the data from laboratory animals do not support the conclusion that TCE is a "known human carcinogen."**

Causal inferences from epidemiologic studies are generally based on several criteria including, 1) Strength of the association – the size of the risk ratio; 2) Consistency of the association – the effect and a similar level of risk are observed across multiple studies and among different populations; 3) Temporality of the association – exposure precedes disease; 4) Dose-response effect – the disease rate increases with increased exposure; and 5) Biological plausibility – animal or other biological research supports a causal 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 any cancer and TCE. With the exception of two poorly designed studies by Henschler et al. and Vamvakas et al., the results are essentially not significant.**

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

Authors	Study Group	Total Number Workers	TCE Exposed	Weiss Review[1]	McLaughlin Review[1]	Wartenberg Review[1] (Tier 1)
1. Antilla et al., 1995	Finnish workers monitored for TCE and other solvents	3,974	3,089	√	√	√
2. Axelson et al., 1994	Swedish workers monitored for TCE	1,670	1,670	√	√	√
3. Blair et al., 1998	Aircraft workers, Utah airforce base	14,457	7,204	√	√	√
4. Boice et al., 1999	Aircraft manufacturing workers, Burbank, CA	77,965	2,267			√
5. Garabrant et al., 1988	Aircraft manufacturing workers, San Diego CA	14,067	NA		√	
6. Henschler et al., 1995	Cardboard factory workers, Germany	169	169		√	√
7. Morgan et al., 1998	Aircraft manufacturing workers, Tucson , AZ	20, 508	4,733	√	√	√
8. Ritz, 1999	Uranium processing plant workers	3,814	3,814			√

Notes:
1. √ = included in review

Table 1 – Summary of Occupational Cohort Studies of TCE Exposed Workers (cont.)

Authors	Study Group	Kidney cancer[2]	Liver Cancer[2]	Lung Cancer[2]	Lymphatic/Hema-topoietic[2]
1. Antilla et al., 1995	Finnish workers monitored for TCE and other solvents	SIR 0.87 (0.32 – 1.89) 6 cases	SIR 2.27 (0.74 – 5.29) 5 cases	SIR 0.92 (0.59 – 1.35) 25 cases	SIR 1.63 (1.06-3.80) 25 cases
2. Axelson et al., 1994	Swedish workers monitored for TCE	SIR 1.16 (0.42 – 2.52) 6 cases	SIR 1.41 (0.38-3.60) 4 cases	SIR 0.69 (0.31- 1.30) 9 cases	SIR 1.16 7 cases[3]
3. Blair et al., 1998	Aircraft workers, Utah airforce base	SMR 1.22 (0.85 – 1.74) 30 cases	SMR 1.15 (0.55-2.42 7 cases	SMR 0.98 (0.86 – 1.12) 213 cases	SMR 1.05 (0.88 – 1.24) 134 cases
4. Boice et al., 1999	Aircraft manufacturing workers, Burbank, CA	SMR 0.99 (0.40 – 2.04) 7 cases	SMR 0.54 (0.15-1.38) 4 cases	SMR 0.78 (0.60 – 0.95) 78 cases	SMR 1.05 146 cases[4]
5. Garabrant et al., 1988	Aircraft manufacturing workers, San Diego CA	SMR = 0.93 (0.48-1.64) 12 cases	SMR 0.94 (0.40 – 1.86) 8 cases	SMR 0.80 (0.68 – 0.95) 138 cases	SMR 0.78 (0.56 – 1.08) 38 cases
6. Henschler et al., 1995	Cardboard factory workers, Germany	SIR 7.97 (2.59 – 8.59) 5 cases	NA	NA	NA
7. Morgan et al., 1998	Aircraft manufacturing workers, Tucson , AZ	SMR 1.32 (0.57 – 2.60) 8 cases	SMR 0.98 (0.36-2.13) 6 cases	SMR 1.10 (0.89-1.34) 97 cases	SMR 0.99 (0.64 – 1.47) 25 cases
8. Ritz, 1999	Uranium processing plant workers	SMR 0.65 (0.21 – 1.51) 5 cases	SMR 1.66 (0.71 – 3.26) 8 cases	SMR 1.03 (0.85 – 1.24) 112 cases	SMR 1.28 (0.90 – 1.77) 37 cases

Notes:
2. SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio. 95% confidence intervals listed in parenthesis, NA= not available
3. Leukemias not reported, combined results for Non Hodgkin's lymphoma, Hodgkins's disease, multiple myelomas and other lymphomas
4. SMR among factory workers, not statistically significant, confidence intervals not reported (Table 3 of Boice et al., 1999)