HSIA
halogenated solvents industry alliance, inc.

August 16, 2018

Toni Krasnic
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: Docket No. EPA-HQ-OPPT-2016-0737

Dear Mr. Krasnic:

The Halogenated Solvents Industry Alliance, Inc. (HSIA) represents producers and users of trichloroethylene. We offer these comments on EPA's problem formulation for the risk evaluation of trichloroethylene under the Toxic Substances Control Act (TSCA), as amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act enacted in June 2016. 83 Fed. Reg. 26998 (June 11, 2018). HSIA agrees with the condition of use proposed in the problem formulation document as being appropriate for the risk evaluation and is pleased that EPA is implementing systematic review approaches in all aspects of the risk evaluation.

HSIA further agrees with EPA that legacy sources of exposure should be excluded from the risk evaluation of trichloroethylene. Legacy sources of exposure typically refer to historical releases of a chemical to the environment associated with misuse or disposal. Although legacy environmental sources of exposure certainly exist for trichloroethylene, they have been managed for decades under various federal programs (*i.e.*, CERCLA, RCRA, CAA, etc.). Many states also have stringent programs for addressing legacy contamination from these chemicals. Management of legacy contamination through the various federal and state programs is already risk-based and adding an additional risk-management program to the existing mix would be duplicative and not needed.

I. Requirements of TSCA §§ 6 and 26

TSCA § 6(b)(4)(F), as revised by the Lautenberg Act, requires that EPA's risk evaluations must, among other things:

- "integrate and assess available information on hazards and exposures for the conditions of use of the chemical substance, including information that is relevant to specific risks of injury to health or the environment and information on potentially exposed or susceptible subpopulations identified as relevant by the Administrator;"

- "take into account, where relevant, the likely duration, intensity, frequency, and number

of exposures under the conditions of use of the chemical substance;" and

- "describe the weight of the scientific evidence for the identified hazard and exposure."

New TSCA § 26(h) requires that, in carrying out § 6, "to the extent that the Administrator makes a decision based on science, the Administrator shall use scientific information, technical procedures, measures, methods, protocols, methodologies, or models, employed in a manner consistent with the best available science, and shall consider as applicable—

> (1) the extent to which the scientific information, technical procedures, measures, methods, protocols, methodologies, or models employed to generate the information are reasonable for and consistent with the intended use of the information;
>
> (2) the extent to which the information is relevant for the Administrator's use in making a decision about a chemical substance or mixture;
>
> (3) the degree of clarity and completeness with which the data, assumptions, methods, quality assurance, and analyses employed to generate the information are documented;
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> (4) the extent to which the variability and uncertainty in the information, or in the procedures, measures, methods, protocols, methodologies, or models, are evaluated and characterized; and
>
> (5) the extent of independent verification or peer review of the information or of the procedures, measures, methods, protocols, methodologies, or models."

Further, new TSCA § 26(i) provides: "The Administrator shall make decisions under sections 4, 5, and 6 based on the weight of the scientific evidence."

The problem formulation for the risk evaluation of TCE includes degreasing and spot cleaning uses, which HSIA strongly supports. These two uses had been evaluated in 2014 in EPA's TSCA Work Plan Assessment for TCE, but the evaluation procedure was deficient as it did not comply with the "best available science" and "weight of scientific evidence" requirements under TSCA §§ 6 and 26. As the Chair noted in the peer review of the draft TSCA Work Plan Assessment:

> "The principal criterion for inclusion/exclusion [in the Work Plan assessment] would be the credibility/integrity of the study rather than simply the route of exposure. . . . If the Agency had conducted a systematic review of the literature and each study as it was developing the IRIS document, it would be a relatively easy task to identify the one best data set to represent the endpoint/duration of exposure /(sub)population to be evaluated. But there is not documentation to show that this exercise was carried out. . . . If [OPPT]

didn't do its own systematic review of those . . . studies before using them, in the screening level assessment, it should do it before keeping them in a refined assessment."

More generally, the Office of Pollution Prevention and Toxics (OPPT) has released a guidance document that describes the general systemic review principles it will use to conduct risk evaluations under the amended TSCA.[1] As noted in its risk evaluation rule, EPA has concluded that systematic review is an integral part of a weight of the scientific evidence approach and that integrating systematic review into risk evaluations is critical to meet the statutory requirements of TSCA.[2] In the systematic review, HSIA supports the use of a numerical scoring system to inform the characterization of the data information sources during the data integration phase. We also see as critical the evaluation of data quality prior to incorporation of the information into the risk evaluation. OPPT's systematic review approach should provide an objective platform upon which to address ongoing controversies regarding data quality for key endpoints.

II. Non-Cancer Assessment

A. Re-assessment of cardiac malformations from Johnson *et al.* (2003) study

EPA's derivation of the current inhalation reference concentration (RfC) and oral reference dose (RfD) for TCE in its IRIS database is based, in part, on Johnson *et al.*, Threshold of Trichloroethylene Contamination in Maternal Drinking Waters Affecting Fetal Heart Development in the Rat, Environ. Health Perspect. 111: 289-92 (2003). At least two GLP-compliant studies (Carney *et al*. 2006; Fisher *et al.* 2001) conducted under both EPA and Organization for Economic Coordination and Development (OECD) guidelines have been unable to reproduce the effect seen by Johnson *et al.* (2003).[3]

A third guideline study of TCE developmental toxicity sponsored by HSIA is underway, and the results are expected by the end of October 2018. The study is designed with a focus on cardiac abnormalities and includes toxicokinetic measures to enable comparison with the earlier studies. It is intended to fill the remaining gap for a guideline study by the drinking water route, the same exposure route as Johnson *et al.* (2003). Keeping TCE in the drinking water solutions and achieving acceptable target concentrations of TCE in the drinking water has been challenging because of the high propensity of TCE to volatilize into the air, as illustrated below

[1] EPA. Application of systematic review in TSCA risk evaluations, Office of Chemical Safety and Pollution Prevention EPA-740-P1-8001 (May 2018).

[2] Procedures for Chemical Risk Evaluation under the Amended Toxic Substances Control Act, 82 *Fed Reg* 33726, 33734 (July 20, 2017).

[3] Carney, E.W., Thorsrud, B.A., Dugard, P.H., and Zablotny, C.L., Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene. Birth Defects Res. (Part B) 77: 405-412 (2006); Fisher, J.W., Channel, S.R., Eggers, J.S., Johnson, P.D., MacMahon, K.L., Goodyear, C.D., Sudberry, G.L., Warren, D.A., Latendresse, J.R., and Graeter, L.J., Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: do they affect fetal rat heart development?, Int. J. Toxicol. 20: 257-267 (2001).

in Table 1. Table 1 lists the vapor pressure, water solubility, and Henry's Law constant for TCE and several other volatile chemicals that have been tested in drinking water toxicity studies.

Table 1: Properties of TCE and selected chemicals that determine their transfer from water to air*

Chemical	Vapor Pressure (kPa)	Water Solubility (g/L)	Henry's Law constant (Pa m^3/mol)	References
Trichloroethylene (TCE)	9.9	1.1	1,030	ECHA[4]
Chloroform	21.1	8.7	310	ECHA
Ethylene dichloride	10.2	7.9	120	ECHA; HSDB[5]
Methyl tert-butyl ether (MTBE)	33	41.9	44	ECHA
Acetone	0.24	3.3	3.5	ECHA; HSDB

*Values at 20 – 25°C.

The Henry's Law constant is the equilibrium distribution of a chemical between the concentration in air and the concentration in water; it is commonly derived simply as the ratio of vapor pressure and solubility. A comparison of the Henry's Law constants for the volatile chemicals in Table 1 shows that TCE has a far greater tendency to transfer to air than the other volatile chemicals. While there were no reported problems of volatility loss of chloroform, EDC, MTBE, or acetone from the drinking water formulations in animal toxicity studies, this was found to be problematic in the earlier drinking water study sponsored at the same laboratory by HSIA. In this study, there was a significant problem with TCE volatility loss during the preparation of the dosing formulations and in the transfer of these formulations to the drinking water bottles; it was particularly severe at the lower concentrations (0.25 and 1.5 ppm TCE). Johnson et al. (2003) reported a 34% loss of TCE from the drinking water bottles over the 24-hour period in the animal cages, but the laboratory provided almost no information on the method used to minimize TCE loss during the preparation step of the dosing formulations, the concentrations of TCE achieved in the drinking water bottles at the start of each exposure period, and the variability of these concentrations throughout the study. This lack of reporting detail and analytical chemistry testing data for dose concentrations has been identified as one of the many deficiencies of the Johnson *et al*. (2003) study (Makris *et al*., 2016; Wikoff *et al.*, 2018).[6]

[4] ECHA, European Chemical Agency (ECHA) database on chemical substances registered under REACH: https://echa.europa.eu/information-on-chemicals/registered-substances, accessed April 18, 2018.

[5] HSDB, Hazardous Substance Database, National Library of Medicine: https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm, accessed on April 18, 2018.

[6] Makris, S.L., Scott, C.S., Fox, J., Knudsen, T.B., Hotchkiss, A.K., Arzuaga, X., Euling, S.Y., Powers, C.M., Jinot, J., Hogan, K.A., Abbott, B.D., Hunter, III, E.S., and Narotsky, M.G., A systematic evaluation of the potential effects of trichloroethylene exposure on cardiac development, Reprod. Toxicol. 65: 321-358 (2016); Wikoff, D., Urban, J.D., Harvey, S., and Haws, L.C., Role of risk of bias in systematic review for chemical risk assessment: a case study in understanding the relationship between congenital heart defects and exposures to trichloroethylene, Intl. J. Toxicol. 37: 125-143 (2018).

For the re-run of the HSIA-sponsored TCE developmental toxicity study, a method has been developed by the testing facility that allows the target concentrations to be met within a reasonable range. The method involves preparing the dosing formulations on a daily basis and in a closed system; headspace is minimized. For the transfer of the dosing formulations into the water bottles, nitrogen is pumped into the inlet valve of the dosing formulation vessel, displacing the dosing formulation through the outlet value and into the drinking water bottle. A feasibility study was recently conducted to ensure that the dosing formulations could be prepared consistently on a daily basis and to quantitate how much TCE loss would occur from the drinking water bottles over the 24-hour period in the animal cages. Pregnant female SD Crl:CD(SD) rats were given in their drinking water 0.25 or 1,000 ppm TCE from gestation days (GD) 11 to 13. The dosing formulations were given to the rats at the same time of the day (within 2-3 hours) on GD 11 and 12. For the 1,000 ppm TCE dose group, the concentrations of TCE in the prepared dosing formulations for the two test days were 97% and 105% of the target concentration, and 102% and 103% after being added to the water bottles. For the 0.25 ppm TCE dose group, the concentrations of TCE in the dosing formulations for the two days were 136% and 123% of the target concentrations, and 132% and 132% after being added to the water bottles. The losses of TCE from the water bottles over the 24-hour period were 34% and 31% for the 0.25 and 1,000 ppm dosing groups, respectively. While the TCE losses from the water bottles over the 24-hour exposure period are unavoidable, these results show that the method developed by the testing facility for the HSIA-sponsored developmental study achieves minimal TCE volatility loss, resulting in consistent daily TCE drinking water concentration.

B. Critiques of Johnson *et al.* (2003) in literature and by other regulators

Johnson *et al.* (2003) reported cardiac effects in rats from research carried out at the University of Arizona and originally published ten years earlier by the same authors.[7] In the earlier-published study, there was no difference in the percentage of cardiac abnormalities in rats dosed during both pre-mating and pregnancy at drinking water exposures of 1100 ppm (9.2%) and 1.5 ppm (8.2%), even though there was a 733-fold difference in the concentrations. The authors reported that the effects seen at these exposures were statistically higher than the percent abnormalities in controls (3%). For animals dosed only during the pregnancy period, the abnormalities in rats dosed at 1100 ppm (10.4%) were statistically higher than at 1.5 ppm (5.5%), but those dosed at 1.5 ppm were not statistically different from the controls. Thus, no meaningful dose-response relationship was observed in either treatment group. Johnson *et al.* republished in 2003 data from the 1.5 and 1100 ppm dose groups published by Dawson *et al.* in 1993 and pooled control data from other studies, an inappropriate statistical practice, to conclude that rats exposed to levels of TCE greater than 250 ppb during pregnancy have increased incidences of cardiac malformations in their fetuses.

[7] Dawson, B, *et al.*, Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water, J. Am. Coll. Cardiol. 21: 1466-72 (1993).

Johnson *et al.* (2003) has been heavily criticized in the published literature.[8] Indeed, its predecessor study was expressly rejected as the basis for MRLs by the Agency for Toxic Substances & Disease Registry (ATSDR) in its last TCE Toxicological Profile Update.[9] Moreover, as noted above, the Johnson *et al.* (2003) findings were not reproduced in a study designed to detect cardiac malformations; this despite employing an improved method for assessing cardiac defects and the participation of Dr. Johnson herself.[10] No increase in cardiac malformations was observed in the second guideline study,[11] despite high inhalation doses and techniques capable of detecting most of the malformation types reported by Johnson *et al*. (2003). The dose-response relationship reported in Johnson *et al.* (2003) for doses spanning an extreme range of experimental dose levels is considered by many to be improbable, and has not been replicated by any other laboratory.[12]

Even the California Office of Environmental Health Hazard Assessment (OEHHA) rejected the study as deficient:

> "Johnson et al. (2003) reported a dose-related increased incidence of abnormal hearts in offspring of Sprague Dawley rats treated during pregnancy with 0, 2.5 ppb, 250 ppb, 1.5 ppm, and 1,100 ppm TCE in drinking water (0, 0.00045, 0.048, 0.218, and 128.52 mg/kg-day, respectively). The NOAEL for the Johnson study was reported to be 2.5 ppb (0.00045 mg/kg-day) in this short exposure (22 days) study. The percentage of abnormal hearts in the control group was 2.2 percent, and in the treated groups was 0 percent (low dose), 4.5 percent (mid dose 1), 5.0 percent (mid dose 2), and 10.5 percent (high dose). The number of litters with fetuses with abnormal hearts was 16.4 percent, 0 percent, 44 percent, 38 percent, and 67 percent for the control, low, mid 1, mid 2, and high dose, respectively. The reported NOAEL is separated by 100-fold from the next higher dose level. The data for this study were not used to calculate a public-health protective concentration since a meaningful or interpretable dose-response relationship was not observed. *These results are also not consistent with earlier developmental and reproductive toxicological studies done outside this lab in mice, rats, and rabbits: The other studies did not find adverse effects on fertility or embryonic*

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[8] Hardin, B, *et al.*, Trichloroethylene and cardiac malformations, Environ. Health Perspect. 112: A607-8 (2004); Watson, R., *et al.*, Trichloroethylene-contaminated drinking water and congenital heart defects: a critical analysis of the literature, Repro. Toxicol. 21: 117-47 (2006).

[9] ATSDR concluded that "[t]he study is limited in that only two widely spaced exposure concentrations were used and that a significant dose-response was not observed for several exposure scenarios." Toxicological Profile for Trichloroethylene Update (September 1997), at 88.

[10] Fisher, J, *et al.*, Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: do they affect fetal rat heart development?, Int. J. Toxicol. 20: 257-67 (2001).

[11] Carney, E, *et al.*, Developmental toxicity studies in Crl:Cd (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene, Birth Defects Research (Part B) 77: 405−412 (2006).

[12] "Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a 'specific' cardiac teratogen." Hardin, B, *et al.*, Trichloroethylene and cardiac malformations, Environ. Health Perspect. 112: A607-8 (2004).

development, aside from those associated with maternal toxicity (Hardin et al., 2004)."[13]

C. Reservations of EPA scientific staff

Remarkably, an EPA staff review that was placed in the docket for the earlier Work Plan Assessment reflects similar concerns. First, one staff member dissented over relying at all on the Arizona study:

> "The rodent developmental toxicology studies conducted by Dawson et al. (1993), Johnson et al. (2003), and Johnson et al. (1998) that have reported cardiac defects resulting from TCE (and metabolite) drinking water exposures have study design and reporting limitations. Additionally, two good quality (GLP) inhalation and gavage rodent studies conducted in other laboratories, Carney et al. (2006) and Fisher et al. (2001), respectively, have not detected cardiac defects. These limitations and uncertainties were the basis of the single dissenting opinion of a team member regarding whether the database supports a conclusion that TCE exposures during development are likely to cause cardiac defects."[14]

Second, even the EPA staff that agreed with use of the study had little confidence that it supported the dose-response assessment:

> "[A] majority of the team members agreed that the Johnson et al. (2003) study was suitable for use in deriving a point of departure. However, confidence of team members in the dose response evaluation of the cardiac defect data from the Johnson et al. (2003) study was characterized as between 'low' and 'medium' (with 7 of 11 team members rating confidence as 'low' and four team members rating confidence as 'low to medium')."[15]

The same report notes:

> "In conclusion, there has not been a confirmation of the results of the Johnson et al. (2003) and Dawson et al. (1993) studies by another laboratory, but there has also not been a repeat of the exact same study design that would corroborate or refute their findings."

D. EPA's dose-response analysis of Johnson *et al.* (2003) data needs to be re-examined

[13] California EPA Public Health Goal for Trichloroethylene in Drinking Water (July 2009), at 21 (emphasis added).

[14] TCE Developmental Cardiac Toxicity Assessment Update (available at http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2012-0723-0045).

[15] *Id.*

The IRIS assessment's evaluation of the relationship between TCE exposure dose and the development of cardiac defects relies heavily on Johnson *et al.* (2003). Ignoring for the moment the methodological deficiencies in the paper, a closer look at EPA's evaluation of that dose-response relationship in generating a point of departure (POD) raises several concerns. This is important, as according to a paper published by the authors of the IRIS Assessment, Johnson *et al.* (2003) represents "the only available study potentially useable for dose-response analysis of fetal cardiac defects."[16]

In discussing the dose-response evaluation, Makris *et al.* (2016) further state that "[g]iven the uncertainties in the dose-response analysis related to the nature of the data, the confidence in the POD based on Johnson *et al.* (2003) has limitations. Overall, however, the POD derived in the 2011 TCE assessment (U.S. EPA, 2011), which used an approach consistent with standard U.S. EPA dose-response practices, remains a reasonable choice." It should be noted that, in order to achieve a better model fit in its derivation of a POD, EPA dropped the highest exposure dose from Johnson *et al*. (2003). With already questionable data, and no expectation that the highest dose of TCE would result in a diminished response, that decision should be reconsidered.

Makris *et al.* (2016) describe additional dose-response analyses performed to characterize the uncertainty in the POD. In summarizing the results of this analysis, they state that "[a]lternative PODs were derived based on use of alternative models, alternative BMR levels, or alternative procedures (such as LOAEL/NOAEL approach), each with different strengths and limitations. These alternatives were within *about an order of magnitude of the POD derived in the 2011 TCE assessment*" (emphasis added). This level of uncertainty in modeling the POD when combined with the uncertainty in the PBPK modeling (discussed elsewhere) and the overall poor quality of the underlying developmental toxicity study provide little confidence in this toxicological value.

E. Reliance on Johnson *et al.* (2003) is inconsistent with use of best available science

When HSIA requested access to the data used by EPA in its evaluation of the dose-response relationship between TCE exposure and cardiac defects reported in Johnson *et al*. (2003), the Agency provided the spreadsheet, referenced as Johnson (2009) (HERO ID 783484) in the 2011 IRIS Assessment, and indicated that was the entirety of the data evaluated. Examination of that spreadsheet reveals an absence of certain critical information, including, most importantly, dates for any of the individual treatment/control animals.

Acknowledging the documented deficiencies in their paper (and the data provided to EPA), the authors published an erratum aimed at updating the public record regarding methodological issues for Johnson *et al.* (2003).[17] According to Makris *et al*. (2016):

[16] Makris SL, Scott CS, Fox J, *et al*., Systematic evaluation of the potential effects of trichloroethylene exposure on cardiac development. Repro Toxicol (2016); http://dx.doi.org/10.1016/j.reprotox.2016.08.014

[17] Johnson PD, Goldberg SJ, Mays MZ, Dawson BV, Erratum: Erratum for Johnson *et al.*, [Environ Health Perspect 113: A18 (2005)]; Environ Health Perspect 122: A94 (2014); http://dx.doi.org/10.1289/ehp.122-A94

> "some study reporting and methodological details remain unknown, *e.g.*, the precise dates that each individual control animal was on study, maternal body weight/food consumption and clinical observation data, and the detailed results of analytical chemistry testing for dose concentration. Additional possible sources of uncertainty identified for these studies include that the research was conducted over a 6-yr period, that combined control data were used for comparison to treated groups, and that exposure characterization may be imprecise because tap (rather than distilled) drinking water was used in the Dawson *et al.* (1993) study and because TCE intake values were derived from water consumption measures of group-housed animals."

HSIA submits that the information contained in the above paragraph alone should disqualify Johnson *et al.* (2003) as "best available science" as required under EPA's July 2017 procedures for chemical risk evaluation under TSCA as amended.

III. Cancer Risk Assessment

A. Deficiencies of Cancer Risk Assessment

1. Erroneous Characterization of TCE as "Carcinogenic to Humans"

The IRIS Assessment classifies TCE as "Carcinogenic to Humans." It fails to discuss (or even to recognize) that such classification is inconsistent with a definitive report by the National Academy of Sciences, discussed below.[18] First, we briefly address how the epidemiological data on TCE do not meet the threshold for classification as "Carcinogenic to Humans."

a. Guidelines for Carcinogen Risk Assessment

EPA's 2005 Guidelines for Carcinogen Risk Assessment provide the following descriptors as to the weight of evidence for carcinogenicity:

- Carcinogenic to humans,
- Likely to be carcinogenic to humans,
- Suggestive evidence of carcinogenicity,
- Inadequate information to assess carcinogenic potential, and
- Not likely to be carcinogenic to humans.[19]

According to the Guidelines, "carcinogenic to humans" means the following:

[18] National Research Council, Contaminated Water Supplies at Camp Lejeune: Assessing Potential Health Effects (2009) (hereinafter "Camp Lejeune report").

[19] 70 Fed. Reg. 17766-817 (April 7, 2005).

"This descriptor indicates strong evidence of human carcinogenicity. It covers different combinations of evidence.

- "This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

- "Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when *all* of the following conditions are met: (a) There is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, *and* (b) there is extensive evidence of carcinogenicity in animals, *and* (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, *and* (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. In this case, the narrative includes a summary of both the experimental and epidemiologic information on mode of action and also an indication of the relative weight that each source of information carries, *e.g*., based on human information, based on limited human and extensive animal experiments."

According to the Guidelines, the descriptor "likely to be carcinogenic to humans": "is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor 'Carcinogenic to Humans.' Adequate evidence consistent with this descriptor covers a broad spectrum. . . . Supporting data for this descriptor may include:

"An agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer;

- "An agent that has tested positive in animal experiments in more than one species, sex, strain, site or exposure route, with or without evidence of carcinogenicity in humans;

- "A positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy or an early age at onset;

- "A rare animal tumor response in a single experiment that is assumed to be relevant to humans; or

- "A positive tumor study that is strengthened by other lines of evidence."

According to the Guidelines, the descriptor "suggestive evidence of carcinogenicity":

"is appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. Depending on the extent of the database, additional studies may or may not provide further insights. Some examples include:

- "A small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans;'

- "A small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed;

- "Evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence; or

- "A statistically significant increase at one dose only, but no significant response at the other doses and no overall trend."

b. Application of the Guidelines to TCE

In considering the data in the context of applying the "Carcinogenic to Humans" descriptor, the weight of the epidemiological evidence must first be considered. We judge the epidemiologic evidence to be neither "convincing" nor "strong," two key terms in the Guidelines. This judgment is based on four recent reviews and meta-analyses of occupational TCE exposures and cancer as well as other reviews of this literature.[20] The recent review and meta-analysis by Kelsh *et al*. focuses on occupational TCE exposure and kidney cancer, and includes the Charbotel *et al.* study that is emphasized in the IRIS assessment.[21] Both the EPA meta-analysis and the Kelsh *et al.* meta-analysis of the TCE kidney cancer epidemiologic literature produced similar summary results. However in Kelsh *et al.* the limitations of this body of research, namely exposure assessment limitations, potential unmeasured confounding,

[20] Alexander, D, *et al.*, A meta-analysis of occupational trichloroethylene exposure and multiple myeloma or leukaemia, Occup Med (Lond) 56:485−493 (2006); Alexander, D, *et al.*, A meta-analysis of occupational trichloroethylene exposure and liver cancer, Int Arch Occup Environ Health 81(2):127−43 (2007); Mandel, J, *et al.*, Occupational trichloroethylene exposure and non-Hodgkin's lymphoma: a meta-analysis and review, Occup Environ Med 63:597−607 (2006); Kelsh, M, *et al.*, Occupational trichloroethylene exposure and kidney cancer: a meta-analysis, Epidemiology 21(1): 95-102 (January 2010).

[21] Charbotel, B, *et al.*, Case-control study on renal cell cancer and occupational exposure to trichloroethylene, Part II: Epidemiological aspects, Ann Occup Hyg 50(8):777−787 (2006).

potential selection biases, and inconsistent findings across groups of studies, did not allow for a conclusion that there is sufficient evidence of a causal association, despite a modest overall association.

There are reasonably well-designed and well-conducted epidemiologic studies that report no association between TCE and cancer, some reasonably well-designed and conducted studies that did report associations between TCE and cancer, and finally some relatively poorly designed studies reporting both positive and negative findings. Overall, the summary relative risks or odds ratios in the meta-analysis studies (EPA or published meta-analyses) generally ranged between 1.2 and 1.4. Such small odds ratios are not typically considered "convincing" or "strong." Weak or small associations may be more likely to be influenced by or be the result of confounding or bias. Smoking and body mass index are well-established risk factors for kidney cancer, and smoking and alcohol are risk factors for liver cancer, yet the potential impact of these factors on the meta-analysis associations was not fully considered. There were suggestions that these factors may have impacted findings (*e.g.*, in the large Danish cohort study of TCE exposed workers, the researchers noted that smoking was more prevalent among the TCE exposed populations, however little empirical data were provided). In addition, co-linearity of occupational exposures (*i.e.*, TCE exposure correlated with chemical and/or other exposures) may make it difficult to isolate potential effects of TCE from those of other exposures within a given study, and hinder interpretation across studies. For example, although Charbotel *et al.* reported potential exposure response trends, while controlling for many confounders of concern (which strengthens the weight of evidence), they also reported attenuated associations for cumulative TCE exposure after adjustment for exposure to cutting fluids and other petroleum oils (weakening the weight of the evidence). This study is also limited due to other potential study design considerations such as selection bias, self reporting of work histories, and residual confounding.

When examining the data for TCE and non-Hodgkin lymphoma, kidney cancer, and liver cancer, associations were inconsistent across occupational groups (summary results differed between aerospace/aircraft worker cohorts compared with workers from other industries), study design, location of the study, quality of exposure assessment (*e.g.*, evaluating studies that relied upon biomonitoring to estimate exposure *vs.* semi-quantitative estimates *vs*. self-report, etc.), and by incidence *vs*. mortality endpoints. Although EPA examined high dose categories, it did not evaluate any potential dose-response relationships across the epidemiologic studies (except for Charbotel *et al.*). Reviews of the epidemiologic data reported in various studies for different exposure levels (*e.g.*, cumulative exposure and duration of exposure metrics) did not find consistent dose-response associations between TCE and the three cancer sites under review.[22] An established dose-response trend is one of the more important factors when making assessments of causation in epidemiologic literature. Thus, based on an overall weight of evidence analysis of the epidemiologic research, these data do not support the conclusion that

[22] Mandel, J, *et al.*, Occupational trichloroethylene exposure and non-Hodgkin's lymphoma: a meta-analysis and review, Occup Environ Med 63:597−607 (2006); Alexander, D, *et al.*, A meta-analysis of occupational trichloroethylene exposure and liver cancer, Int Arch Occup Environ Health 81(2):127−43 (2007); Kelsh, M, *et al.*, Occupational trichloroethylene exposure and kidney cancer: a meta-analysis, Epidemiology 21(1): 95-102 (January 2010).

there is "strong" or "convincing" evidence of a causal association between human exposure and cancer.

EPA's Guidelines also state that a chemical may be described as "Carcinogenic to Humans" with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence, all of which must be met. One of these lines of evidence is "extensive evidence of carcinogenicity in animals." Therefore, we must briefly evaluate the animal data.

The criteria that have to be met for animal data to support a "carcinogenic to humans" classification are stated in a sequential manner with an emphasized requirement that all criteria have to be met. Since the Guidelines consider this to be an "exceptional" route to a "carcinogenic to humans" classification, we would expect rigor to have been applied in assessing animal data against the criteria. This simply was not done.

Of the four primary tissues that EPA evaluated for carcinogenicity, only one or perhaps two rise to the level of biological significance. Discussion of the remaining tumor types appears to presuppose that TCE is carcinogenic. The resulting discussion appears then to overly discount negative data, of which there are many, and to highlight marginal findings. The text does not appear to be a dispassionate rendering of the available data. Specifically, EPA's conclusion that kidney cancer is evident in rats rests on *one* statistically significant finding in over 70 dose/tumor endpoint comparisons and references to exceedances of historical control values.[23] Using a 0.05 p-value for statistical significance, a frequency of 1 or even several statistically or biologically significant events is expected in such a large number of dosed/tumor groups. EPA's overall conclusion based on these flawed studies cannot be that TCE is a known kidney tumorigen. The best that can be said is that the data are inconsistent. Certainly they do not meet the criterion of "extensive evidence of carcinogenicity in animals." Several marginal findings do not constitute "extensive evidence."

For all these reasons, EPA's classification of TCE as "Carcinogenic to Humans" is not supported by the evidence and cannot be justified under the 2005 Guidelines.

c. <u>EPA's Position that there is 'Convincing Evidence' that TCE Is Carcinogenic to Humans is Inconsistent with National Academy of Sciences Conclusion of only 'Limited or Suggestive Evidence'</u>

The IRIS Assessment states that "TCE is characterized as 'carcinogenic to humans' by all routes of exposure. This conclusion is based on convincing evidence of a causal association between TCE exposure in humans and kidney cancer."

Box 2 of the Academy's Camp Lejeune report, attached as Appendix 1, categorizes every cancer outcome reviewed in relation to exposure to TCE, the dry cleaning solvent perchloroethylene, or a mixture of the two. The categories are taken directly from a respected

[23] And that bioassay is from a laboratory whose studies EPA has reviewed and declined to rely upon in other assessments.

Institute of Medicine (IOM) report.[24] These categories are "sufficient evidence of a causal relationship," "sufficient evidence of an association," "limited or suggestive evidence of an association," "inadequate evidence to determine an association," and "limited or suggestive evidence of no association," all as defined in Box 1, also attached.

Looking at Box 2, evidence considered by EPA to be "convincing evidence of a causal association between TCE exposure in humans and kidney cancer" would seem to be considered "sufficient evidence of a causal relationship." Yet the Academy found no outcomes in that category. It would at least be "sufficient evidence of an association." Again, the Academy found no outcomes in that category. Only in the third category, "limited or suggestive evidence of an association," does one find kidney or any other cancer outcome associated with TCE.

"Limited evidence of an association" is far from "convincing evidence of causation." One would expect at the least a detailed explanation of EPA's very different conclusion. Although the 2009 Camp Lejeune study was already published, and indeed is cited in the references, there is no mention of it in the text of the IRIS Assessment, even though the previous draft had just been the subject of a multi-year review by the Academy.

The Camp Lejeune committee began with a comprehensive review of the epidemiology studies of the two solvents by the IOM for its Gulf War Report. They then identified new studies published from 2003 to 2008 and considered whether these changed the conclusions in the IOM report. In the case of TCE and kidney cancer, this was the case. The Camp Lejeune committee considered six new cohort studies and two case-control studies (including Charbotel *et al.*). They concluded that several of these studies reported an increased risk of kidney cancer, but observed that the results were often based on a relatively small number of exposed persons and varied quality of exposure data and methodology. Given these data, the committee raised the classification for TCE to match the IOM conclusion of "limited" evidence for perchloroethylene.

EPA, on the other hand, offered the summary conclusion of convincing human evidence, based on the "consistency" of increased kidney cancer across the different studies. The authors of these studies, however, do not agree with EPA's characterization of them. For example, the authors of Charbotel *et al.*, the study EPA finds most compelling, state that the "study suggests an association between exposures to high levels of TCE and increased risk of [renal cell carcinoma]. Further epidemiological studies are necessary to analyze the effect of lower levels of exposure."

Given the flaws in the IRIS Assessment, and the very different conclusion reached by the Academy in its Camp Lejeune report on the same body of data, the forthcoming evaluation under TSCA as amended should not rely on the IRIS Assessment's classification of TCE as "Carcinogenic to Humans."

2. <u>EPA Should Reassess Available Cancer Epidemiology Data, Given Publication of More Recent and Larger Studies on Worker Populations</u>

[24] Institute of Medicine, Gulf War and Health, Vol. 2, Insecticides and Solvents (National Academies Press) (2003).

The observation of an elevated but weak kidney cancer association reported by Charbotel *et al.* (2006)[25] contrasts with other occupational studies which did not find an elevation in kidney cancer in industries using TCE as a metal degreaser, *e.g.*, aircraft manufacturing, metal cleaning, etc., where exposures may be higher than for screw cutters. Lipworth and coworkers (2011) found no evidence of increased kidney cancer in a large worker cohort with multiple decades of TCE exposure and extended cancer follow-up evaluations.[26] The aircraft manufacturing study involved a total cohort of 77,943 workers, of which 5,443 were identified as exposed to TCE. The study involved evaluations from 1960 through 2008, at which time 34,248 workers had died. Approximately 30% of the workers were hired before 1960 (60% born before 1940), 52% terminated employment by 1980, and approximately a third of the workers were employed for more than 20 years. The standardized incidence ratio (SIR) for kidney cancer in the TCE-exposed workers was reported as 0.66 (CI 95%: 0.38-1.07). This value for the SIR indicates that these workers were potentially less likely to get kidney cancer than the normal population (or at least had the same rate as the normal population – SIR of 1).

More recently, two large Nordic country epidemiological studies, both of which had extensive follow-up of the cohorts, have likewise failed to find an association between TCE and kidney cancer. An SIR of 1.01 (0.70-1.42) was found by Hansen *et al*. (2013) for kidney cancer based on 32 cases out of a total of 997 cancer cases in a cohort of 5,553 workers in Finland, Sweden, and Denmark, indicating that rates were the same as the normal population.[27] TCE exposures in this cohort were directly confirmed from urinary biomonitoring of the TCE metabolite trichloroacetic acid (TCA). However, overall TCE exposures were likely low in this cohort in that most urinary TCA measurements were less than 50 mg/L, corresponding to approximately 20 ppm TCE exposure. Thus, consistent with the conclusions of Bruning and Bolt (2000),[28] this study indicates TCE is unlikely to be a low-dose kidney carcinogen.

Similarly, no evidence of kidney cancer was found by Vlaanderen *et al.* (2013) in a recent follow-up examination of the Nordic Occupational Cancer cohort (Finland, Iceland, Norway, Sweden) in which statistically non-significant risk ratios (RR) of 1.01 (0.95-1.07), 1.02 (0.97-1.08), and 1.00 (0.95-1.07) were reported for a total of 4,145 renal cancer cases approximately equally distributed across three respective TCE exposure groups (tertiles) assigned from a job exposure matrix analysis.[29] Finally, although a meta-analysis of 23 studies meeting criteria for study inclusion found a slightly increased simple summary association of

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[25] Charbotel, B, *et al.*, Case-control study on renal cell cancer and occupational exposure to trichloroethylene, Part II: Epidemiological aspects, Ann Occup Hyg 50(8):777−787 (2006).

[26] Lipworth L, Sonderman JS, Mumma MT, *et al.*, Cancer mortality among aircraft manufacturing workers: an extended follow-up, J Occup Environ Med 53(9): 992-1007 (2011).

[27] Hansen J, Sallmén M, Seldén AI, *et al.*, Risk of cancer among workers exposed to trichloroethylene: analysis of three Nordic cohort studies, J Natl Cancer Inst 105(12): 869-877 (2013).

[28] Brüning T, Pesch B, Wiesenhütter B, *et al.*, Renal cell cancer risk and occupational exposure to trichloroethylene: Results of a consecutive case-control study in Arnsberg, Germany, Am J Ind Med. 43(3): 274-285 (2003).

[29] Vlaanderen J, Straif K, Pukkala E, *et al*., Occupational exposure to trichloroethylene and perchloroethylene and the risk of lymphoma, liver, and kidney cancer in four Nordic countries, Occup Environ Med 70(6): 393-401 (2013).

TCE and kidney cancer, RR 1.42 (1.17-1.77), more detailed analyses of subgroups suggested no association, or possibly a moderate elevation in kidney cancer risk, and no evidence of increasing risk with increasing exposure.[30]

These more recent studies were not reviewed in the 2011 TCE IRIS assessment.

3. EPA's reliance on Charbotel *et al.* (2006) Resulted in an Overly Conservative Estimate of Risk

The inhalation unit risk (IUR) value developed in the 2011 IRIS assessment was based primarily on epidemiology data from the case-control study on renal cell carcinoma (RCC) by Charbotel *et al.* (2006), discussed above. Although other epidemiological studies were used to derive an adjusted IUR estimate for the combined risk of developing RCC, NHL, or liver cancer, EPA concedes a lower level of confidence in both the NHL and liver cancer databases. While the Charbotel *et al.* study suggests a relationship between cumulative TCE exposure and RCC incidence, the reliability of the exposure estimates is a major concern.

The National Academy of Sciences Committee that reviewed the draft IRIS assessment released in 2001 recommended that:

> "[t]here appear to be insufficient epidemiologic data to support quantitative dose-response modeling for trichloroethylene and cancer. The committee recommends that toxicologic data be used to fit the primary dose-response model(s) and that the available epidemiologic data be used only for validation. The committee does not believe that the available information is sufficient to determine the best dose-response model for trichloroethylene."[31]

EPA should follow the recommendation of the National Academy of Sciences, which referenced the Charbotel *et al.* (2005) final study report in its review of TCE.[32] The authors' own conclusions that the study only "suggests that there is a weak association between exposures to TRI [TCE] and increased risk of RCC" argues against the existence of the robust relationship which should be required for a dose-response assessment that may be used as the basis for regulation.[33]

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[30] Kelsh MA, Alexander DD, Mink PJ, Mandel JH, Occupational trichloroethylene exposure and kidney cancer: a meta-analysis, Epidemiology 21(1): 95-102 (2010).

[31] National Research Council, Assessing the human health risks of trichloroethylene: key scientific issues, National Academies Press, Washington, DC (2006); http://www.nap.edu/openbook.php?record_id=11707&page=R1.

[32] Charbotel B, Fevotte J, Hours M, *et al.*, Case-control study on renal cell cancer and occupational trichloroethylene exposure, in the Arve Valley (France), Lyon, France: Institut Universitaire de Médecine du Travail, UMRESTTE, Université Claude Bernard (2005); http://hal.archives-ouvertes.fr/docs/00/54/59/80/PDF/charbotel_octobre_05.pdf

[33] This concern was recognized by the European Chemicals Agency (ECHA) in its 2013 Chemical Safety Report on TCE: "[T]here are several concerns with this study that should be taken into consideration when assessing its use in risk assessment and hazard characterization. For example, potential selection bias, the quality of the exposure assessment, and the potential confounding due to other exposures in the work place. With respect to the potential

The exposure assessment for the Charbotel study was based on questionnaires and expert judgment, not direct measures of exposure.[34] Worker exposure data from deceased individuals were included in the study. In contrast to living workers, who were able to respond to the questionnaires themselves, exposure information from deceased workers (22.1% of cases and 2.2% of controls) was provided by surviving family members. The authors acknowledge that "this may have led to a misclassification for exposure to TCE due to the lower levels in the quality of information collected."

Analysis of the data revealed evidence of confounding from cutting fluid exposure. Unfortunately, TCE and cutting oil were co-exposures that could not be disaggregated and the majority of the TCE exposed population, the screw cutters, could be expected to experience similar patterns of exposure for both TCE and cutting fluids (probably in aerosol form). Thus, the apparent dose-response relationship for TCE could be wholly, or in part, the result of exposure to cutting fluids.

In their 2006 publication of the study results, the authors assigned cumulative exposures into tertiles (i.e., low, medium and high), yet the dose-response evaluation conducted as part of the IRIS assessment relied on mean cumulative exposure levels provided at a later date.[35] Although the IRIS assessment references the email submission of the data to EPA, it provides no detail on the technical basis for the table, raising serious transparency issues.

In an apparent acknowledgement of the uncertainty of the exposure information, Charbotel *et al.* (2006) included an evaluation of "the impact of including deceased patients (proxy interviews) and elderly patients (>80 years of age)" on the relationship between exposure to TCE and RCC. Interestingly, it was stated that "only job periods with a high level of confidence with respect to TCE exposure were considered" in the study, an apparent reference to the use of two different occupational questionnaires, one "devoted to the screw-cutting industry and a general one for other jobs." As the Adjusted Odds Ratio (OR) for the high cumulative

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for selection bias, no cancer registry was available for this region to identify all relevant renal cell cancer cases from the target population. Case ascertainment relied on records of local urologists and regional medical centers; therefore, selection bias may be a concern. Given the concerns of the medical community in this region regarding renal cell cancer (RCC) among screw cutting industry workers, it is likely that any cases of renal cell cancer among these workers would likely be diagnosed more accurately and earlier. It is also much more unlikely that an RCC case among these workers would be missed compared to the chance of missing an RCC case among other workers not exposed to TCE. This preference in identifying cases among screw-cutting industry workers would bias findings in an upward direction. Concerning the potential for other exposures that could have contributed to the association, screw-cutting industry workers used a variety of oils and other solvents. Charbotel *et al.* reported lower risks for TCE exposure and renal cell cancer once data were adjusted for cutting oils. In fact, they noted, 'Indeed many patients had been exposed to TCE in screw-cutting workshops, where cutting fluids are widely used, making it difficult to distinguish between cutting oil and TCE effects.' This uncertainty questions the reliability of using data from Charbotel *et al*. since one cannot be certain that the observed correlation between kidney cancer and exposure is due to trichloroethylene."

[34] Fevotte J, Charbotel B, Muller-Beauté P, *et al.*, Case-control study on renal cell cancer and occupational exposure to trichloroethylene, Part I: Exposure assessment, Ann Occup Hyg 50: 765-775 (2006); http://dx.doi.org/10.1093/annhyg/mel040.

[35] Charbotel B (2008) [Email from Barbara Charbotel, University of Lyon, to Cheryl Scott, EPA].

dose group was actually higher in the censored subgroup than in the uncensored group [3.34 (1.27-8.74) vs 2.16 (1.02-4.60)], the authors suggested that "misclassification bias may have led to an underestimation of the risk."

What the authors and EPA appear to have overlooked is that, in addressing the misclassification bias, Charbotel may also have altered the cumulative dose-response relationship. For example, in the censored subgroup there were now only 16 exposed cases (1 in the Low Group, 4 in the Medium Group and 11 in the High Group) with Adjusted ORs of 0.85, 1.03 and 3.34, respectively. If the dose-response relationship in this higher-confidence subgroup has changed, use of the lower-confidence group to calculate the IUR would require rigorous justification.

4. EPA's Adjustment of the Kidney Cancer-Based IUR Value for TCE to Account for Potential Liver Cancer and Non-Hodgkin's Lymphoma (NHL) Endpoints is Not Scientifically Defensible and Needs to be Reconsidered

In addition to our concerns about the appropriateness of basing the IUR for TCE on epidemiology data, as described above, HSIA has serious concerns about the scientific appropriateness of adjusting the IUR derived from kidney cancer data to account for non-Hodgkin's lymphoma (NHL) and liver cancer. Derivation of the modified IUR is described in Section 5.2.2.2 of the IRIS Assessment. A recent review sponsored by HSIA concludes that it was not appropriate for EPA to adjust the IUR based on kidney cancer for multiple cancer sites because the available epidemiology data are not sufficiently robust to allow such calculations and the data that are available indicate that the IUR for kidney cancer is protective for all three cancer types. See Appendix 2 (attached) for a complete discussion of this issue.

5. A Role for Glutathione Conjugate-derived Metabolites in TCE Kidney Toxicity and Cancer Risk Assessment Should be Reconsidered

The TCE IRIS Assessment relies in part on the conclusion that DCVG and DCVC, which are weakly active renal toxicants and genotoxicants, are formed in toxicologically significant concentrations following human exposures to TCE. This conclusion rests primarily on studies in which a relatively high blood DCVG concentration (100 nM) was observed in volunteers exposed for 4 hours to 50 or 100 ppm TCE.[36] However, Lash *et al.* (1999) relied on a spectrophotometric chromatographic method analysis of TCE glutathione conjugate-derived metabolites which had substantial potential for detection of non-TCE-specific endogenous substances.

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[36] Lash, L.H., Putt, D.A., Brashear, W.T., Abbas, R., Parker, J.C., and Fisher, J.W., Identification of S-(1,2-dichlorovinyl) glutathione in the blood of human volunteers exposed to trichloroethylene, J. Toxicol. Environ. Health Part A 56: 1-21 (1999a). It is also supported by *in vitro* kinetic studies that measured the glutathione conjugation of TCE in human hepatocytes and human liver and kidney subcellular fractions. Lash, L.H., Lipscomb, J.C., Putt, D.A., and Parker, J.C., Glutathione conjugation of trichloroethylene in human liver and kidney: kinetics and individual variation, Drug. Metab. Dispos. 27: 351-35 (1999b).

In a published paper sponsored by HSIA (abstract attached as Appendix 3), the HPLC/UV method used by Lash *et al*. (1999) was found to overestimate the levels of DCVG in blood, liver, and kidney compared to the more specific and reliable HPLC/MS/MS method. [37] The reason for this overestimation was an interfering peak that was primarily from endogenous glutamate. It is imperative that the analytical data used in human health risk assessments be as accurate and reliable as possible, particularly if those data are used as surrogates for exposure to estimate potential health effects in humans. Our findings suggest that DCVG formation may have been substantially overestimated based on the levels that were quantified by the HPLC/UV method. The implications of this apparent uncertainty are that the GSH pathway may play a more limited role, if any, in kidney toxicity from TCE exposure; and that the risk of kidney toxicity and carcinogenicity from TCE exposure, particularly in humans, may be overestimated and may be occurring by alterative mode(s) of action not inclusive of reactive GSH-derived metabolites.

Since the publication of the IRIS Assessment in 2011, additional studies have evaluated the kidney concentrations of the oxidative and glutathione conjugate-derived metabolites of TCE in a variety of mouse strains administered five daily oral doses of 600 mg/kg TCE.[38] Metabolites were quantitated two hours after the last daily dose; this time point was chosen because previous studies had shown that the approximate maximum plasma concentrations of TCA, DCA, DCVG and DCVC occurs two hours after an oral dose of TCE.[39] Using a structure-specific HPLC-ESI-MS/MS method, Yoo *et al.* (2015) demonstrated that DCVG and DCVC were only a very small fraction of total metabolites quantitated in kidney. Trichloroethanol (TCOH) kidney concentrations were 2- to 4-fold greater than TCA, and TCA concentrations were 100- to 1,000-fold greater than DCA. Importantly, DCA concentrations were 100- to 1,000-fold greater than either DCVG or DCVC, resulting in the conclusion that TCE oxidative metabolism was up to five orders of magnitude greater than glutathione conjugate-derived metabolism.

These findings were consistent with the earlier report from Kim *et al.* (2009), in which the time course of TCA, DCA, DCVG, and DCVC in serum was investigated following a single oral dose of 2,100 mg/kg TCE dose to male B6C3F$_1$ mice. The total area under the curve (AUC) of TCA and DCA (oxidative metabolites) was 40,000-fold higher than the total AUC of DCVG and DCVC (glutathione conjugates). It should be noted that this study did not quantify the oxidative metabolite TCOH, which would have further increased the disparity of glutathione conjugate-derived metabolites relative to the oxidative-derived metabolites. These data

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[37] Zhang, F., Marty, S., Budinsky, R., Bartels, M., Pottenger, L.H., Bus, J., Bevan, C., Erskine, T., Clark, A., Holzheuer, B., and Markham, D., Analytical methods impact estimates of trichloroethylene's glutathione conjugation and risk assessment, Toxicol. Lett. 296: 82-94 (2018); available on-line at https://doi.org/10.1016/j.toxlet.2018.07.006.

[38] Yoo, H.S., Bradford, B.U., Kosyk, O., Uehara, T., Shymonyak, S., Collins, L.B., Bodnar, W.M., Ball, L.M., Gold, A., and Rusyn, I., Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: kidney effects, J Toxicol Env Health Pt. A 78: 32-49 (2015).

[39] Kim, S., Kim, D., Pollack, G.M., Collins, L.B., and Rusyn, I., Pharmacokinetic analysis of trichloroethylene metabolism in male B6C3F1 mice: Formation and disposition of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine, Toxicol. Appl. Pharmacol. 238: 90-99 (2009).

demonstrate a dramatically lower function for glutathione-conjugate metabolism relative to oxidative metabolism in mice, despite the observation by Dekant (2010) (attached as Appendix 4) that mice generate DCVC at slightly higher rates than rats and greater than 10-fold higher than humans.

The results of studies using structure-specific analytical methods for quantitation of DCVG and DCVC directly challenge the hypothesis that glutathione conjugate-derived metabolites plausibly account for the genotoxicity, renal cytotoxicity, and ultimate carcinogenicity in rodents.[40] DCVC was only marginally cytotoxic (LDH release), if at all, when incubated at 0.2M (200,000 nM) with isolated renal cortical cells of male and female rats. This *in vitro* concentration is substantially higher than the approximate maximum kidney concentrations of 10 to 75 nM DCVC reported in various strains of mice given a high oral dose of 600 mg/kg TCE for 5 consecutive days (Yoo *et al*., 2015). A likely No-Observed-Adverse-Effect-Level (NOAEL) of 1 mg/kg-day was also reported for kidney toxicity in mice administered DCVC orally or intraperitoneally at a dose of 1, 10 or 30 mg/kg, once a week for 13 weeks, as indicated by a lack of change in serum blood urea nitrogen (BUN), weak tubule dilation, and no signs of necrosis.[41] If, based on the data from Yoo *et al.* (2015), it is assumed that the ratio of formation of oxidative metabolites to glutathione conjugate-derived metabolites is 10,000:1, an implausibly high (occupational or general population) dose of 6,044 mg/kg TCE would be required to deliver a NOAEL dose of 1 mg/kg-day DCVC (1 mmol/kg-day TCE results in 0.0001 mmol/kg/day DCVC; 1 mg/kg-day DCVC = 0.0046 mmol/kg-day). These dose-toxicity calculations suggest that it appears toxicologically implausible that real-world exposures to TCE are capable of producing doses of DCVC sufficient to cause renal toxicity and carcinogenicity in mice.

IV. Miscellaneous

A. Worker and consumer exposure assessments should utilize all industry provided and publicly available information

The problem formulation document states that EPA will evaluate worker exposures to trichloroethylene in the TSCA risk evaluation from data that are publicly available, *i.e*, monitoring data from government agencies such as OSHA and NIOSH and from the published literature. It is recognized that these data may be from limited conditions of use or from out-or-date use/exposure scenarios. Thus, HSIA is submitting worker air monitoring data from trichloroethylene manufacturing facilities (attached as Attachment 5). We encourage EPA to utilize all available industry provided and publicly available information in its analysis of the exposure assessment in the risk evaluation.

[40] Lash, L.H., Qian, W., Putt, D.A., Hueni, S.E., Elfarra, A.A., Krause, R.J., and Parker, J.C., Renal and hepatic toxicity of trichloroethylene and its glutathione-derived metabolites in rats and mice: Sex-, species-, and tissue-dependent differences, J. Pharmacol. Exp. Ther. 297: 155-164 (2001).

[41] Shirai, N., Ohtsuji, M., Hagiwara, K., Tomisara, H., Ohtsuje, N., Hirose, S., and Hagiwara, H., Nephrotoxic effect of subchronic exposures to S-(1,2-dichlorovinyl)-L-cysteine in mice, J. Toxicol. Sci. 37: 871-878 (2012).

B. Trichloroethylene is subject to transportation regulations by the Department of Transportation (DOT) and the Pipeline and Hazardous Materials Safety Administration (PHMSA)

Appendix A.1 of the problem formulation document lists the federal laws and regulations to which trichloroethylene is subject. There are also specific transportation regulatory requirements for trichloroethylene by the DOT and PHMSA; these regulations need to be added to the list of Federal Laws and Regulations in Appendix A.1. The DOT regulations provide instructions on trichloroethylene is to be transported by air, highway, rail or water. It defines the operational measures to ensure the health and safety of workers, as well as to ensure that no product is allowed to be released into the air, soil or water. PHMSA has the responsibility to maintain the hazardous material regulations.

V. Conclusion

We hope that these comments will be useful to EPA as it develops the risk evaluation for trichloroethylene.

Respectfully submitted,

Faye Graul
Executive Director

Attachments

Attachment 1

Contaminated Water Supplies at Camp Lejeune,
Assessing Potential Health Effects
National Research Council of the National Academy of Sciences (2009)

BOX 1 Five Categories Used by IOM to Classify Associations

Sufficient Evidence of a Causal Relationship

Evidence from available studies is sufficient to conclude that a causal relationship exists between exposure to a specific agent and a specific health outcome in humans, and the evidence is supported by experimental data. The evidence fulfills the guidelines for sufficient evidence of an association (below) and satisfies several of the guidelines used to assess causality: strength of association, dose-response relationship, consistency of association, biologic plausibility, and a temporal relationship.

Sufficient Evidence of an Association

Evidence from available studies is sufficient to conclude that there is a positive association. A consistent positive association has been observed between exposure to a specific agent and a specific health outcome in human studies in which chance and bias, including confounding, could be ruled out with reasonable confidence. For example, several high-quality studies report consistent positive associations, and the studies are sufficiently free of bias, including adequate control for confounding.

Limited/Suggestive Evidence of an Association

Evidence from available studies suggests an association between exposure to a specific agent and a specific health outcome in human studies, but the body of evidence is limited by the inability to rule out chance and bias, including confounding, with confidence. For example, at least one high-quality study reports a positive association that is sufficiently free of bias, including adequate control for confounding. Other corroborating studies provide support for the association, but they were not sufficiently free of bias, including confounding. Alternatively, several studies of less quality show consistent positive associations, and the results are probably not due to bias, including confounding.

Inadequate/Insufficient Evidence to Determine Whether an Association Exists

Evidence from available studies is of insufficient quantity, quality, or consistency to permit a conclusion regarding the existence of an association between exposure to a specific agent and a specific health outcome in humans.

Limited/Suggestive Evidence of No Association

Evidence from well-conducted studies is consistent in not showing a positive association between exposure to a specific agent and a specific health outcome after exposure of any magnitude. A conclusion of no association is inevitably limited to the conditions, magnitudes of exposure, and length of observation in the available studies. The possibility of a very small increase in risk after exposure studied cannot be excluded.

Source: IOM (Institute of Medicine). 2003. Gulf War and Health, Vol. 2, Insecticides and Solvents. Washington, DC: National Academies Press.

Contaminated Water Supplies at Camp Lejeune,
Assessing Potential Health Effects
National Research Council of the National Academy of Sciences (2009)

BOX 2 Categorization of Health Outcomes[a] Reviewed in Relation to TCE, PCE, or Solvent Mixtures

Sufficient Evidence of a Causal Relationship

- No outcomes

Sufficient Evidence of an Association

- No outcomes

Limited/Suggestive Evidence of an Association

- Kidney cancer
- Adult leukemia (solvent mixtures)
- Multiple myeloma (solvent mixtures)
- Myleodysplasic syndromes (solvent mixtures)
- Scleroderma (solvent mixtures)
- Neurobehavioral effects (solvent mixtures)

Inadequate/Insufficient Evidence to Determine Whether an Association Exists

- Oral/pharyngeal cancer
- Nasal cancer
- Laryngeal cancer
- Esophageal cancer (TCE)
- Stomach cancer
- Colon cancer
- Rectal cancer
- Pancreatic cancer
- Hepatobiliary cancer
- Lung cancer (TCE)
- Bone cancer
- Soft tissue sarcoma
- Melanoma
- Non-melanoma skin cancer
- Breast cancer (TCE)
- Cervical cancer
- Ovarian/uterine cancer
- Prostate cancer
- Bladder cancer (TCE)
- Cancer of the brain or central nervous system
- Non-Hodgkin lymphoma
- Hodgkin disease
- Multiple myeloma
- Adult leukemia
- Myelodysplasic syndromes
- Childhood leukemia
- Childhood neuroblastoma
- Childhood brain cancer
- Aplastic anemia
- Congenital malformations
- Male infertility
- Female infertility (after exposure cessation)
- Miscarriage, preterm birth, or fetal growth restriction (from maternal preconception exposure or paternal exposure)
- Preterm birth or fetal growth restriction (from exposure during pregnancy)
- Cardiovascular effects
- Liver function or risk of cirrhosis
- Gastrointestinal effects
- Renal toxicity
- Amyotrophic lateral sclerosis
- Parkinson disease
- Multiple sclerosis
- Alzheimer disease
- Long-term reduction in color discrimination
- Long-term hearing loss
- Long-term reduction in olfactory function

Limited/Suggestive Evidence of No Association

- No outcomes

[a]*Outcomes for TCE and PCE unless otherwise specified**

* PCE-only outcomes omitted

Attachment 2

US EPA calculated an IUR based on data reported in Charbotel *et al*. (2006), which was a hospital-based, case-control study of kidney cancer and occupational exposure to TCE conducted in France. The study investigators estimated cumulative TCE exposures based on historical measurements of TCE concentrations in the air and a job-exposure matrix (JEM) (Fevotte *et al*., 2006). Based on cases of kidney cancer and age- and sex-matched controls who were recruited from local hospitals and urologists, the study investigators reported an elevated risk for kidney cancer with increasing cumulative exposures to TCE (p for trend = 0.04), adjusting for smoking and body mass index (BMI). Based on the risk estimates (*i.e*., odds ratios [ORs]) for kidney cancer and the mean cumulative exposure estimates of various TCE exposure categories, US EPA obtained a linear regression coefficient by regressing the ORs of kidney cancer against cumulative TCE exposures and used this coefficient to calculate lifetime extra risks using the life-table analysis (US EPA, 2011). US EPA then used the 95% lower confidence limit of the effective concentration corresponding to an extra kidney cancer risk of 1% to derive an IUR of 5.49×10^{-3} (US EPA, 2011).

US EPA adjusted this IUR estimate for additional cancer sites, including NHL and liver cancer, using two approaches to assess relative contributions of multiple cancer sites to the extra cancer risk from TCE exposure (see Table 5-46 in Section 5.2.2.2, US EPA, 2011). First, using relative risk (RR) estimates for kidney cancer, NHL, and liver cancer from its meta-analyses, US EPA calculated the extra risks of these cancers and obtained a ratio of 3.28 by comparing the total extra risk of NHL and liver cancer to that of kidney cancer. In an alternative approach, US EPA relied on standardized incidence ratios (SIRs) of these three cancers, reported in Raaschou-Nielsen *et al*. (2003), to calculate extra cancer risks and obtained a ratio of 4.36 by comparing the combined extra risks of NHL and liver cancer to the extra risk of kidney cancer. Based on these two ratios, US EPA applied a factor of 4 directly to the kidney cancer IUR estimate and obtained an IUR estimate of 2.2×10^{-2} for total cancer.

Setting aside the uncertainties regarding whether the associations between TCE exposure and these cancers are causal, the adjustment for multiple cancer sites US EPA applied to the IUR is not appropriate for several reasons.

First, the RR estimates from the meta-analyses do not accurately reflect the relative contributions from different cancers. In Appendix C of the *Toxicological Review of Trichloroethylene (CAS No. 79-01-6) In Support of Summary Information on the Integrated Risk Information System (IRIS)* (US EPA, 2011), US EPA presented detailed meta-analyses of several cancer sites, including kidney cancer, NHL, and liver cancer. Below, we compare key results from these meta-analyses (Table 1). In the primary analyses with all available studies, moderate, but statistically significant, meta risk estimates were observed for all three cancer types. However, in subgroup analyses by study design, it is apparent that while an elevated risk of kidney cancer was present in case-control studies but not cohort studies, elevated risks of NHL and liver cancer were present only in cohort studies. Case-control studies of these cancers generally obtained detailed information on potential confounders, such as smoking, BMI, and socioeconomic status (SES), and thus provided more robust estimates for the cancer risk

associated with TCE exposure. In contrast, the cohort studies of cancer and TCE, often comparing occupational populations to the general population, mostly reported SIRs or standardized mortality ratios (SMRs) that were not adjusted for confounders. Therefore, risk estimates from individual cohort studies, and the meta-estimates based on these studies, likely did not properly reflect the true associations between TCE and these cancers.

Table 1: Results of Meta-analyses of Trichloroethylene and Kidney Cancer, Non-Hodgkin's Lymphoma, and Liver Cancer[a]

Analysis	Meta-RR (95% CI) from Random-effects Models		
	Kidney Cancer	NHL	Liver Cancer
All Studies	1.27 (1.13-1.43)	1.23 (1.07-1.42)	1.29 (1.07-1.56)
Cohort Studies	1.16 (0.96-1.40)	1.33 (1.13-1.58)	1.29 (1.07-1.56)
Case-control Studies	1.48 (1.15-1.91)	1.11 (0.89-1.38)	-

Note:
CI = Confidence Interval; NHL = Non-Hodgkin's Lymphoma; RR = Relative Risk.
(a) Adapted from Tables C-3, C-6, and C-12 of Appendix C of the *Toxicological Review of Trichloroethylene (CAS No. 79-01-6) In Support of Summary Information on the Integrated Risk Information System (IRIS)* (US EPA, 2011).

Similarly, the SIRs of kidney cancer, NHL, and liver cancer reported in Raaschou-Nielsen *et al*. (2003), which was a retrospective cohort study of Danish blue-collar workers, were not robust estimates for the RRs of the three cancers. Blue-collar workers who were employed at a TCE-using company for at least three months between 1968 and 1997 were included in the study, but these workers were not all exposed to TCE (Raaschou-Nielsen *et al*., 2003). Because only SIRs were assessed in this study, key confounders for liver cancer, such as smoking, heavy alcohol consumption, and chronic viral hepatitis, and kidney cancer confounders like smoking and BMI, were not adjusted for. Therefore, the SIRs from Raaschou-Nielsen *et al*. (2003) should not be used in a regulatory human health risk assessment.

In addition, there are considerable uncertainties in the quantitative analyses in which US EPA adjusted the IUR estimate for multiple cancer sites. US EPA discussed some of the unverifiable assumptions implied in its IUR adjustment but did not fully acknowledge that most of these assumptions were not reasonable or realistic and likely did not hold.

For the approach using the meta-RR estimates, US EPA discussed several additional assumptions. First, populations of the underlying studies in the meta-analyses were assumed to have similar overall TCE exposure. But this assumption was likely not true as the underlying epidemiology studies were conducted in different counties, industries, and time periods. For example, Charbotel *et al*. (2006) was conducted in the Arve Valley in France, where there was a prevalent screw-cutting industry and exposure to TCE was known to have a high frequency and intensity. In contrast, Raaschou-Nielsen *et al*. (2003) investigated workers in a number of industries with TCE use, including iron and metal, electronics, painting, printing, chemical, and dry cleaning. It is unlikely that populations from different countries, industries, and time periods had similar TCE exposures.

Second, US EPA assumed that meta-RR estimates, which are based on RR estimates for both mortality and incidence, were appropriate estimates for cancer incidences. This assumption, again, was not reasonable. Because the survival rates for cancer generally depend

on cancer site and the stage at diagnosis, mortality rates often poorly approximate incidence rates, particularly when cancers are diagnosed at an early stage. In the context of IUR adjustment, kidney cancer (excluding Stage IV) and NHL have relatively high five-year survival rates, ranging from 50% to 80%. Therefore, mortality risk estimates are not good estimates for incidences for these two cancers.

Third, it was assumed that the meta-RR for kidney cancer was a good estimate for the RR for renal cell carcinoma, and that the meta-RR pooling studies using different classification schemes of NHL was valid. Since 90% of kidney cancers are renal cell carcinomas, the outcome misclassification was probably negligible. In contrast, diagnosis and classification of NHL have changed over time (Hartge *et al*., 1994; NCI, 2015), and this likely led to errors in outcome ascertainment in epidemiology studies. It is difficult, however, to estimate the direction and extent of this bias.

US EPA argued that because the second approach using Raaschou-Nielsen *et al*. (2003) was based on a single population and precise cancer types, it offered directly comparable RR estimates. But as discussed above, there were considerable uncertainties with regard to exposure assessment and confounder adjustment in Raaschou-Nielsen *et al*. (2003), undermining the validity of the RR estimates reported in this study.

The two approaches US EPA used for estimating the relative potencies of the three cancers both assumed that the lifetime background incidence rates for each cancer site in the US general population proportionally approximate the age-specific background incidence rates in the study populations, as US EPA discussed. However, US EPA did not acknowledge that this assumption likely does not hold, because the epidemiology study populations, generally consisting of workers with occupational exposure to TCE, often differed from the US general population with regard to several lifestyle factors such as smoking, obesity, and SES. These factors could have impacted the background cancer incidence rates in worker populations, making them different from the background rates in the US general population.

As US EPA discussed, the use of an adjustment factor on the IUR based on kidney cancer involved a key assumption that the dose-response relationships for NHL and liver cancer were similar to the linear one for kidney cancer. In Table 2, we compare characteristics of US EPA's IUR estimation based on kidney cancer and its IUR adjustment for other cancers. It is clear that, while the IUR assumed a linear relationship between the cumulative TCE exposure and RR of kidney cancer, the underlying data for IUR adjustment implied a log-linear relationship between RRs and the dichotomous TCE exposure. In addition, because of the use of dichotomous exposure in the underlying data, it is not possible to know with any degree of confidence that the dose-response relationships for NHL and liver cancer are linear.

Table 2: Comparison of IUR Derivation for Kidney Cancer and Its Adjustment for Multiple Cancers

	IUR Derivation for Kidney Cancer	IUR Adjustment for Multiple Cancers
Underlying Data	Exposure category-specific ORs and mean cumulative TCE exposure reported in Charbotel *et al*. (2006)	Meta-RRs based on study-specific RRs and dichotomous TCE exposure, SIRs reported in Raaschou-Nielsen *et al*. (2003)
Confounder Adjustment	Generally robust in the underlying study	Generally poor in underlying cohort studies, moderate in underlying case-control studies
D-R Relationship	RR = 1 + b * (Cumulative TCE Exposure)	Log(RR) = b * (Dichotomous TCE Exposure)
POD	Identified from life-table analysis	Not identified, assumed to be identical to kidney cancer

Notes:
D-R = Dose-Response; IUR = Inhalation Unit Risk; OR = Odds Ratio; POD = Point of Departure; RR = Relative Risk; SIR = Standardized Incidence Ratio; TCE = Trichloroethylene.

Also, US EPA failed to acknowledge an additional assumption that the dose-response between TCE exposure and NHL and liver cancer would yield the same point of departure (POD) as that of kidney cancer. It should be noted that the POD based on a 1% extra risk of kidney cancer was estimated based on not only the dose-response curve, but also the incidence rates of kidney cancer in the general population. Even if NHL and liver cancer had identical dose-response curves as kidney cancer, which is unlikely, the PODs based on 1% extra risks of NHL or liver cancer would be different from that of kidney cancer because these cancers have different incidence rates in the general population.

Finally, and perhaps most importantly, US EPA did not demonstrate that any potential risks of kidney cancer, NHL, or liver cancer from TCE exposures are additive. Even if all three cancers were causally associated with TCE exposure, and had identical dose-response relationships, both of which are highly unlikely, an IUR based on one cancer site should also be protective against the other two cancers. To evaluate this, we used data provided by Raaschou-Nielsen *et al*. (2003). These investigators reported observed and expected numbers of cases for multiple cancers, which allowed us to calculate and compare crude SIRs for kidney cancer, NHL, liver cancer, and the three cancers combined. As shown in Table 3, the crude SIR for the three cancers combined is comparable to the crude SIRs for individual cancers, indicating that the potential risks of these cancers from TCE exposures are not additive, and that an IUR based on kidney cancer is protective for all three cancer types. Therefore, US EPA's application of a multi-cancer adjustment factor to the IUR is not supported.

Table 3: Crude Standardized Incidence Ratios for Kidney Cancer, NHL, Liver Cancer, and the Three Cancers Combined[a]

Cancer Site	Men		Women		Both Sexes		
	Observed	Expected	Observed	Expected	Observed	Expected[b]	Crude SIR[c]
Kidney	93	77.1	10	8.7	103	85.8	1.20
NHL	83	67.6	13	9.5	96	77.1	1.25
Liver	27	24	7	2.5	34	26.5	1.28
Combined	203	168.7	30	20.7	233	189.4	1.23

Notes:
NHL = Non-Hodgkin's Lymphoma; SIR = Standardized Incidence Ratio.
(a) The observed and expected cancer cases in men and women were obtained from Raaschou-Nielsen *et al*. (2003).
(b) The expected cancer cases for both sexes were the sum of the expected cases in men and in women.
(c) The crude SIR was the ratio of the observed cases and the expected cases.

In summary, it is not appropriate for US EPA to adjust the IUR based on kidney cancer for multiple cancer sites because the available epidemiology data are not sufficiently robust to allow such calculations, and the data that are available indicate that the IUR for kidney cancer is protective for all three cancer types

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Appendix 3

Zhang, F., Marty, S., Budinsky, R., Bartels, M., Pottenger, L.H., Bus, J., Bevan, C., Erskine, T., Clark, A., Holzheuer, B., and Markham, D. (2018). Analytical methods impact estimates of trichloroethylene's glutathione conjugation and risk assessment, Toxicol. Lett. 296: 82-94; available on-line at https://doi.org/10.1016/j.toxlet.2018.07.006.

Abstract

The glutathione (GSH) conjugates, S-(1,2-dichlorovinyl)-glutathione (DCVG) and S-(1,2-dichlorovinyl)-L-cysteine (DCVC), have been implicated in kidney toxicity and kidney cancer from trichloroethylene (TCE) exposure. Considerable differences in blood and tissue levels of DCVG and DCVC have been reported, depending on whether HPLC/UV (High Performance Liquid Chromatography-Ultraviolet) or HPLC/MS (HPLC-Mass Spectrometry) was used. A side-by-side comparison of analytical results with HPLC/UV and HPLC/MS/MS (High Performance Liquid Chromatography-Tandem Mass Spectrometry) detection was undertaken to quantitatively compare estimates for DCVG and DCVG using rat and human tissues. For the HPLC method, DCVG and DCVC were initially derivatized with fluorodinitrobenzene (DNP). The results from the HPLC/UV method showed that derivatized-DCVC eluted at the solvent front and could not be quantified. Derivatized-DCVG, however, was quantified but significant interference was observed in all four control tissues (rat blood, liver, kidney; and human blood), resulting in average spike recoveries of 222 to 22,990%. In contrast, direct analysis of spiked tissues by HPLC/MS/MS resulted in recoveries of 82 - 127% and 89 - 117% for DCVG and DCVC, respectively. These differences in analytical results were further confirmed in tissues from TCE-treated rats, e.g., DCVG levels in rat liver were 18,000 times higher by HPLC/UV as compared to HPLC/MS/MS. Fraction collection of the derivatized-DCVG peak (obtained with the HPLC-UV method), followed by peak identification via an HPLC/UV/Q-TOF/MS/MS method, identified DNP-derivatized endogenous glutamate as the primary interfering substance that contributed to and exaggerated recoveries of DCVG. Thus, estimates of DCVG based on the HPLC/UV methods are not reliable; they will over-estimate the formation of the GSH conjugates of TCE and will artifactually exaggerate the potential cancer risk in humans from TCE exposure. Therefore, it is recommended that any characterization of cancer risks from TCE exposure attributable to the GSH conjugates of TCE rely on results obtained with the more specific and reliable HPLC/MS/MS method.

Attachment 4

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Würzburg, January 30, 2010

Toxicological Review of Trichloroethylene
In Support of the IRIS Database (Draft of October 2009)

Comments of Prof. W. Dekant

I have been asked to comment on the IRIS Document on trichloroethylene (TCE) by the Halogenated Solvents Industry Alliance. My laboratory has published extensively on the biotransformation of TCE and was among the first to report formation of glutathione-*S*-conjugates from TCE. My area of expertise is biotransformation of xenobiotics, mechanisms of toxicity, and genotoxicity testing and I have published more than 180 manuscripts in these areas. Moreover, I am, or have been, a member of several advisory panels charged with health risk assessment of chemicals including the European Union Scientific advisory committee on Health and Environment (SCHER). As a member of this committee, I was the lead author of the review of the European Chemicals Bureau risks assessment report on TCE. I also have followed the many controversies in the risk assessment of TCE over the last 30 years.

General comments

The toxicity database on TCE is very large, with a number of controversial areas relevant to health risk assessment. EPA has generated a large document and attempted to comprehensively cover the available toxicology information on TCE and its metabolites. Most of the available studies are covered by the assessment. However, the document fails to provide a detailed evaluation of the strengths and weaknesses of the individual studies and a selection of key studies based on a weight of evidence approach. In several places in the document, study results are just reiterated and some of the conclusions relevant for deriving RfDs and RfCs have apparently been taken from reviews. EPA should develop comprehensive detailed justifications based on evaluation of the individual studies and consideration of data not supporting conclusions by EPA. Identical criteria should be applied to the level of evidence required to support or discount a mode of action (MoA).

Specific comments:

1. Extent of glutathione *S*-conjugate formation from TCE

EPA concludes that the extent of formation of *S*-(1,2-dichlorovinyl)glutathione (DCVG) from TCE in humans is much higher than in rodents. Since this conclusion has a major impact on the derivation of RfCs and RfDs for TCE, it should be fully justified and based on consideration of all available data. Apparently, EPA supports this conclusion based on high blood concentrations of DCVG reported in humans after inhalation of TCE (Lash *et al.*, 1999b). This observation is in contrast to the very low concentrations of the isomers of *N*-acetyl-*S*-(1,2-dichlorovinly)-*L*-cysteine (N-acetyl-DCVC) in urine. If the overall wealth of information is disregarded, it is possible to conclude that urinary metabolite content cannot be used as a quantitative marker for metabolic flux through the glutathione conjugation pathway (Lash *et al.*, 2000) and that most of the DCVG may undergo bioactivation by ß-lyase and the products retained in the kidney. However, a number of observations refute these conclusions:

- In the human study with TCE inhalation, high concentrations of DCVG in blood were indicated using a complex analytical procedure, often called the "Reed-Method" (Reed *et al.*, 1980). This method was developed to determine low concentrations of glutathione and glutathione disulfide and may be used to quantify DCVG formation in biological samples. The method involves reaction of the thiol with iodoacetamide and the amino group with chlorodinitrobenzene, followed by ion exchange chromatography and UV-detection of the dinitrophenyl chromophore. Due to the ion-exchange chromatography with a high salt concentration in the eluate, retention time shifts are common due to column deterioration (Lash *et al.*, 1999b). Since the method is not selective for DCVG and analysis of biological samples produces many peaks, retention time shifts may create problems for locating the DCVG peak.

A number of inconsistent datasets questions the reliability of the "Reed-method" to determine DCVG and DCVC:

- In a study assessing DCVG and DCVC formation in rodents after high oral doses of TCE, DCVG-concentrations reported in blood were high, but did not show dose or time-dependence (Lash *et al.*, 2006). In addition, the study reports high concentrations of DCVC excreted in urine. EPA calls the results of this study "aberrant", but apparently did not further assess reliability. Others have reported a very low rate of DCVC-formation in vivo (Dekant *et al.*, 1990; Kim *et al.*, 2009) and DCVC has not been reported as urinary metabolite of TCE using either mass spectrometry or HPLC which radiochemical detection after administration of ^{14}C-TCE (Dekant *et al.*, 1986a).
- The "Reed-method" has also been used to determine DCVG-formation from TCE in subcellular fractions from liver and kidney of rats, mice, and humans. Again, high rates of formation of DCVG were reported (table 1). In contrast, using ^{14}C-TCE and radioactivity detection, much lower reaction rates were observed in other studies (table 1). In addition, isolated glutathione *S*-transferases also have a very low capacity to metabolize TCE to DCVG (Hissink *et al.*, 2002) and the application of the "Reed-method" to study formation of *S*-(1,2,2-trichlorovinyl)glutathione (TCVG) from perchloroethylene (PERC) in subcellular

fractions also gave much higher rates of formation (Lash *et al.*, 1998) when compared with methods using ^{14}C-perchloroethylene and HPLC with radioactivity detection (Dekant *et al.*, 1987; Green *et al.*, 1990; Dekant *et al.*, 1998).

Therefore, DCVG concentrations determined by the "Reed-method" may be greatly overestimated. The more reliable and consistent data support a very low extent of DCVG formation in rodents:

- Very low rates of formation of DCVG in rodent liver subcellular fractions are consistent with very low blood levels of DCVG in mice (Kim *et al.*, 2009) and a very low biliary elimination of DCVG in rats after oral administration of doses > 2 000 mg TCE/kg bw (Dekant *et al.*, 1990). In mice, DCVG concentrations were several thousand-fold lower than those of the oxidative metabolite trichloroacetic acid (TCA) (Kim *et al.*, 2009). In rats, biliary elimination of DCVG within seven hours after oral administration was 2 microg and therefore accounted for << 0.01 % of administered dose (Dekant *et al.*, 1990). Due to its molecular weight (> 350 D) and the presence of effective transport systems for glutathione *S*-conjugates in the canalicular membrane, most of the DCVG formed in rat liver is expected to be excreted in bile. Therefore, the low concentrations of DCVG in blood of mice and the low recovery of DCVG in bile of rats after TCE-administration well support very low rates of DCVG formation.
- Even when considering the high rates of DCVG formation reported in subcellular fractions and the only 3-fold difference in reaction rates between mouse, rat and humans (table 1), it is difficult to explain why DCVG-blood levels in mice after a very high oral dose are orders of magnitude lower than those reported in humans after inhalation exposures giving a much lower internal TCE-dose.
- High blood concentrations of DCVG and a high flux through ß-lyase bioactivation are not consistent with the human toxicity data on TCE. Despite high occupational exposures to TCE between the 1950s and 1970s (occupational exposure limits for TCE were 200 ppm in Germany and were often exceeded for prolonged times), overt nephrotoxicity was rarely observed even after many years of exposures (MAK, 1996). Using the blood concentrations reported and extrapolating to a daily exposure to 200 ppm TCE for 8 h, daily doses of DCVC of approx. 5-7 mg/kg bw should have been received by workers. A significant flux through ß-lyase bioactivation should have resulted in renal effects considering the alleged potency of DCVC.
- Kinetic studies on acetylation, and ß-lyase-mediated metabolism of DCVC support a low flux through ß-lyase activation since the relative flux through the *N*-acetylation pathway (detoxication) is one to two orders of magnitude higher than through ß-lyase activation (Green *et al.*, 1997a). In addition, a low flux through ß-lyase is indicated by the recovery of most of a low intravenous dose of DCVC isomers in urine as mercapturic acids in rats (Birner *et al.*, 1997), the weak nephrotoxicity of DCVC (Green *et al.*, 1997a) and observations with PERC, which is also metabolized by glutathione *S*-conjugate formation and ß-lyase. The PERC metabolite *S*-(1,2,2-trichlorovinyl)-*L*-cysteine is cleaved by ß-lyase to dichloroacetic acid (DCA) which, when formed in the kidney, is excreted with urine. While DCA is a metabolite of PERC in rats, this compound is not excreted as a PERC metabolite in humans (Völkel *et al.*, 1998). In addition, dichloroacetylated proteins were detected both in rat kidney proteins and rat blood proteins after PERC inhalation. Such protein modifications were not

detected in blood proteins from humans after identical exposures (Pähler *et al.*, 1999). These observations indicate that flux through ß-lyase in humans is even lower than in rodents.

- Chloracetic acid is formed by ß-lyase from DCVC (Dekant *et al.*, 1988). In rodents, chloroacetic acid and its metabolites (Green and Hathway, 1975; Green and Hathway, 1977) are not significant metabolites of TCE (< 0.1 % of radioactivity in urine) (Dekant *et al.*, 1984; Dekant *et al.*, 1986a). If the ß-lyase pathway is more relevant, such metabolites should be present in urine in higher concentrations. Other metabolites indicative of alternative processing of DCVC have also not been detected in humans exposed to TCE (Bloemen *et al.*, 2001).

Table 1: Reported rates of formation of DCVC from Trichloroethene (TCE) in rat, mouse and human subcellular fractions. The concentration of TCE in the incubation is based on the amount added. N.d. = not determined

Tissue	Species	TCE Conc (mM)	Rate of DCVC formation (pmol/minxmg)	Analytical method to determine DCVG	Reference
Liver cytosol	Rat	1.4 (^{14}C)	0.54 (non-enzymatic reaction rates substracted)	HPLC with radiochemical detection, peak identity confirmed by LC/MS	(Green *et al.*, 1997b)
	Mouse	1.9 (^{14}C)	0.35		
	Human	1.9 – 2.5 (^{14}C)	0.012 – 0.055		
Liver microsomes	Rat	1.4 (^{14}C)	Not different from non-enzymatic reaction		
	Mouse	1.9 (^{14}C)	n.d.		
	Human	1.9 – 2.5 (^{14}C)	n.d.		
Kidney cytosol	Rat	1.4 (^{14}C)	Not different from non-enzymatic reaction		
	Mouse	n.d.			
	Human	n.d.			
Kidney microsomes	Rat	1.4 (^{14}C)	Not different from non-enzymatic reaction		
	Mouse	n.d.			
	Human	n.d.			
Liver cytosol	Rat	4 (^{14}C)	< 2	HPLC with radioactivity detection, peak identity confirmed by GC/MS after hydrolysis	(Dekant *et al.*, 1990)
Liver microsomes	Rat	4 (^{14}C)	2		
Liver cytosol	Rat	2	121 (males) 81 (females)	Derivatisation and ion exchange HPLC ("Reed-method")	(Lash *et al.*, 1999a)
	Mouse	2	408 (males) 361 (females)		
	Human	1	1 700 – 4 180		
Liver microsomes	Rat	2	171 (males) 120 (females)		
	Mouse	2	666 (males) 426 (females)		
	Human	1	495 – 3 245		
Kidney	Rat	2	7.5 (males)		

cytosol			5.3 (females)		
	Mouse	2	93 (males) 61 (females)		
	Human	na	810 (vmax)		
Kidney microsomes	Rat	2	Nd (males) 1.0 (females)		
	Mouse	2	91 (males) 278 (females)		
	Human	na	6 290 (vmax)		

In summary, the evidence does not support EPA's conclusions that DCVG is released to the blood from TCE at a high rate in rodents and humans or that the rate is greater in humans than it is in rats and mice. The evidence indicates that the glutathione conjugation pathway is less active in humans than in rodents.

2. The role of glutathione *S*-conjugates in nephrotoxicity and renal tumor formation by TCE

Since *S*-conjugates of TCE are nephrotoxic in rodents and genotoxic in vitro, it is appealing to conclude that *S*-conjugate formation is involved in nephrotoxicity of TCE and that the MoA for kidney tumor formation is genotoxicity. However, a number of contradictory findings are not adequately considered in the IRIS-document:

- Formation rates for DCVC in subcellular fractions from mice and rats are similar (or even higher in mice) suggesting similar doses of DCVC to the kidney in both species (Green *et al.*, 1997a; Kim *et al.*, 2009). Moreover, activation of TCE by the ß-lyase pathway is higher in mice (Eyre *et al.*, 1995), DCVC is more nephrotoxic in mice, and causes higher rates of cell replication and covalent binding in mice as compared to rats (Eyre *et al.*, 1995; Green *et al.*, 1997a). Yet, mice are not sensitive to TCE induced renal tumor formation.
- Based on the nephrotoxicity of DCVC and the low rates of formation of DCVC both in rats and mice in vivo, it is questionable if the very low concentrations of DCVG formed in rodents can explain nephrotoxicity and tumor formation. Extrapolating the DCVG blood concentrations observed after single doses to the doses applied in the carcinogenicity studies with TCE in rats, daily DCVC-doses in the two year studies were less than 0.03 mg/kg bw. This is orders of magnitude below the doses of DCVC required to induce nephrotoxicity during chronic administration (Terracini and Parker, 1965) and further questions an involvement of this pathway in nephrotoxicity of TCE.
- EPA concludes that trichloroethanol and formic acid formation may not be involved in the toxicity of TCE to the kidney due to differences in pathology observed between TCE and trichloroethanol treated rats. In my opinion, such comparisons are difficult since differences in the kinetic profiles of a compound formed as a metabolite or administered per se are likely

major confounders. The mode of action for TCE-induced renal tumors due to effects of increased formic acid excretion due to disturbances in intermediary metabolism by trichloroethanol is supported by renal toxicity of trichloroethanol, insufficient rates of DCVC/DCVC-formation to account for renal toxicity and the absence of genotoxic effects of TCE on rat kidney in vivo.

- EPA states that data on VHL gene mutations support a mutagenic MoA in TCE-induced kidney tumors. This is based on studies (Bruning *et al.*, 1997; Brauch *et al.*, 2004) reporting VHL mutations in renal tumors of TCE-exposed individuals. It is concluded that comparison of TCE-exposed and non-exposed patients (Brauch *et al.*, 2004) revealed clear differences with respect to (1) frequency of somatic VHL mutations, (2) incidence of C454T transition, and (3) incidence of multiple mutations. As discussed in Brauch et al. (2004), the mutation frequency in the non-exposed patients (10%) was considerably lower than that commonly observed in sporadic renal tumors, e.g. 82% (Nickerson *et al.*, 2008) or 71% (Banks *et al.*, 2006), and technical problems using archived tissue samples may be one of the causes. Given that exon 3, which harbors the multiple mutations seen in TCE exposed patients, did not amplify in most of the controls, there is only limited evidence for a difference in the incidence of multiple mutations and frequency of somatic VHL mutations, although the C454T transition appears to be characteristic of tumors in TCE exposed patients. However, the presence of mutations in human tumors does not lead to the conclusion that VHL mutations occur early during carcinogenesis. Hence, they are not evidence for a direct genotoxicity of TCE in the kidney. In contrast, experimental data in rats show that neither TCE nor its active metabolite DCVC induce VHL mutations (Mally *et al.*, 2006), suggesting that VHL mutations in humans may be acquired at later stages of tumor development. While the document argues that the VHL gene may not be a target gene in rodent models of renal carcinogenesis, only few studies have looked at VHL in rats and there is no support for the hypothesis that the role of VHL is different in rats and humans.
- The Eker rat may be a useful rodent model for renal cell carcinoma (RCC), but the molecular basis for chemically induced tumor formation in rats and RCC in humans may be widely different from spontaneous tumor formation in this rat strain, as high-grade RCCs can develop in the absence of mutations in the Tsc2 gene in rats (Toyokuni *et al.*, 1998). Development of high-grade renal cell carcinomas in rats independently of somatic mutations in the Tsc2 and VHL tumor suppressor genes (Toyokuni *et al.*, 1998) demonstrates that mutational inactivation of TSC2 or VHL is not a prerequisite for renal carcinogenesis. The similar pathway activation in Eker rat RCC as that seen in humans with VHL mutations reported (Liu *et al.*, 2003) involves deregulation of HIFalpha and VEGF expression which frequently occur in various cancers and provide little evidence to suggest that Tsc-2 inactivation in rats is “analogous” to inactivation of VHL in human RCC.
- Epidemiological data may support an association between specific VHL mutations and TCE exposure, this does not indicate an early event in RCC and – in the absence of experimental support - should not be taken as support for a mutational MoA.
- EPA uses micronucleus and comet assay data in rat kidney after TCE-administration as support for a genotoxic MoA. However, the positive micronucleus (Robbiano *et al.*, 2004) assay applied a very high dose and used an inappropriate route of administration (ip injection of ½ of the LD_{50}). Due to the high dose applied and the route of administration, the results may be confounded by inflammatory responses and should not be used for conclusions. A

comet assay in the kidney using repeated inhalation exposures to TCE was negative (Clay, 2008). The decision to not use this study in the assessment is insufficiently justified. The inhalation study used a higher number of animals (5/group) as compared to the ip study, which states n > 3 with an apparent maximum of 5. The comet assay also shows that administered DCVC is no more than weakly active in the kidney.

- EPA argues that there is no link between nephrotoxicity and renal tumor formation. However, there are a number of compounds that cause renal tumors in rats without being genotoxic. For example, cytotoxicity and regenerative cell proliferation (Swenberg and Lehman-McKeeman, 1999) is accepted as MoA for α_{2u}-globulin binding agents (TCE does not bind to α_{2u}-globulin, but is most likely to cause renal tumors through nephrotoxicity).

In summary, the data do not support a genotoxic mode of action for kidney carcinogenicity via *S*-conjugates of TCE. The decision of EPA to employ *S*-conjugate-mediated genotoxicity in support of a linear dose response relationship for renal cell carcinoma should be revised to reflect the balance of the data. A non-linear dose response relationship is well supported by the available evidence.

3. Mode of action for liver carcinogenesis

- EPA spends considerable effort to correlate liver tumor induction by TCE in mice with liver tumor induction observed after administration of the TCE metabolites TCA and DCA. Again, such comparisons are inherently complex. Both DCA and TCA were administered with drinking water and TCE studies applied gavage in oil. The different administration regimens will result in different time courses of the administered compounds or metabolites in blood and dose-dependent bioavailability may further complicate the interpretation.
- It is highly questionable whether DCA is involved in liver tumor induction by TCE since it is only formed in very low concentrations from TCE in rodents (Dekant *et al.*, 1986a; Kim *et al.*, 2009). In mice, DCA is formed in concentrations several orders of magnitude below those of TCA. Thus, DCA would be required to be a highly potent liver carcinogen, which it is not. Therefore, the potency data on DCA do not suggest that the high liver tumor incidence induced by TCE in mice is related to DCA formation. In addition, DCA is not a human urinary metabolite of TCE (Bernauer *et al.*, 1996; Bloemen *et al.*, 2001).
- For TCA, EPA derives a dose-dependence from tumor incidence data in drinking water studies. Apparently, EPA assumes a dose-independent high bioavailability of TCA. However, the oral bioavailability of TCA from drinking water is limited, concentration-dependent and significantly reduced at higher concentrations of TCA (Larson and Bull, 1992; Templin *et al.*, 1993; Sweeney *et al.*, 2009). The incidence data therefore need to be corrected to account for the limited bioavailability of TCA at higher concentrations in drinking water.
- The mostly negative data in mutagenicity testing with TCE using liver specific activation and negative in vivo gentoxicity data including a very low DNA-binding in liver of mice (Bergman, 1983; Kautiainen *et al.*, 1997) also do not support a mutagenic MoA for liver tumors. Due to intensive metabolism by oxidation and reduction, chloral hydrate

concentrations in the liver are low and chloral hydrate is a very weak mutagen. Therefore, chloral hydrate mutagenicity cannot adequately explain the formation of liver tumors by TCE in mice.

4. Mode of action for lung tumorigenesis.

EPA considers the lung tumors induced by TCE in specific strains of mice as relevant to humans and implies a genotoxic mode-of action. EPA tries to devaluate the hypothesis that chloral may reach high concentrations in mouse lung cells. However, the arguments by EPA are not convincing.
Rat and guinea pig data should not be used to conclude on biotransformation in mouse lung.

- A delivery of TCE from the systemic circulation in mice also causes lung toxicity due to the high metabolic capacity in the target cell. If TCE-metabolites formed in the liver are transported to the lung to cause toxicity there, the species-specificity is difficult to explain since the same metabolites are also present in rats, which do not show lung toxicity.
- A high rate of chloral formation from TCE and limited capacity for further metabolism of chloral (low capacity for reduction of chloral hydrate to trichloroethanol, low capacity for conjugation of trichloroethanol) will result in much higher steady state levels of chloral hydrate in mouse lung Clara cells as compared to rat or human lung (Odum *et al.*, 1992; Green *et al.*, 1997b). The high steady state levels may result in cytotoxicity.
- Cells damaged by the high chloral concentrations formed by TCE-metabolism initiate regeneration and replication to repair and replace the damaged Clara cells (Villaschi *et al.*, 1991) and repeated cycles of damage and regeneration may finally result in lung tumor formation.

Support for a cytotoxic MoA regarding the mouse lung tumors induced by TCE can also be derived from observations with other chemicals. The consequences of Clara cell specific cytotoxicity for tumor induction has been assessed with a number of other chemicals and the very high capacity of the mouse lung Clara cell for biotransformation is also the basis for the mouse-specific lung toxicity. The assessment therefore should integrate this information.

- Styrene, naphthalene, and coumarin induce lung tumors in mice and chronic damage of Clara cells including hyperplasia, often with a time- and dose-related increase in bronchiolar hyperplasia in terminal bronchioles. As with TCE, lung lesions are induced by short term administration, recess after repeated exposures and reappear after continuing exposures. None of these chemical induced lung tumors or histopathologic changes in rat lung (Cruzan *et al.*, 1998; Cruzan *et al.*, 2001).
- Major species differences in lung tumor induction and lung anatomy are one likely basis for the selective tumorigenicity of these chemicals in mice. Lung tumors occur spontaneously in several mouse strains and the incidences of benign lung tumors in control mice are often very high. In general, murine lung tumors are mostly adenomas originating from bronchiolar Clara cells. The adenomas may progress to adenocarcinomas. (Witschi, 1991).
- Clara cells are the major site of xenobiotic metabolism in the mouse lung (Chichester *et al.*, 1991; Buckpitt *et al.*, 1995). In addition to marked species differences in metabolic capacity

of Clara cells in different species, species differences in Clara cell abundance and function may contribute to selective pulmonary toxicity in mice. Clara cell number is significantly higher within the terminal bronchioles of mice relative to rats and humans (Plopper *et al.*, 1980; Lumsden *et al.*, 1984). Clara cells represent approximately 5 % of all cell types and are distributed throughout the airways in mice. In humans, only very few Clara cells are present and are localized in specific regions. Moreover, Clara cells differ morphologically among species, with human cells containing little smooth endoplasmic reticulum.

- TCE and the other chemicals inducing selective lung damage and lung tumors in mice require biotransformation by pulmonary CYP2F and CYP2E1 (Green *et al.*, 1997b; Shultz *et al.*, 1999; Shultz *et al.*, 2001; Born *et al.*, 2002; West *et al.*, 2002; Forkert *et al.*, 2005).
- In mice, both CYP2E1 and CYP2F1 are preferentially localized in Clara cells (Forkert *et al.*, 1989; Buckpitt *et al.*, 1995; Forkert, 1995; Shultz *et al.*, 2001). In rat lung, the expression of CYP2F4, an ortholog of mouse CYP2F2 (Baldwin *et al.*, 2004) is app. 30-fold lower consistent with a much lower turnover of CYP2F substrates in rat. Evidence for the presence of the human ortholog CYP2F1 in human lung is lacking. In rhesus monkeys, CYP2F1 was not detected in the respiratory tract except in the nasal epithelium (Ding and Kaminsky, 2003; Baldwin *et al.*, 2004). CYP2E1 catalytic activity is present in human lung with an activity app. 100-fold lower than in human liver (Bernauer *et al.*, 2006).

In summary, the available information on the presence and catalytic activities of CYP2E1 and CYP2F enzymes in the lung of different species suggest a much higher activity of these enzymes in the mouse, the species susceptible to the pneumotoxicity. Studies directly quantifying relevant metabolite formation from the different pneumotoxic compounds show that mice consistently have a much higher capacity for oxidation as compared to rats and humans. The available data on the mode-of-action for induction of lung tumors share many common features with regard to the induction of Clara cell lesions in the mouse and a number of observations support a non-genotoxic mode-of-action: Glutathione depletion is a major determinant of the toxic responses in the mouse Clara toxicity (West *et al.*, 2000a; West *et al.*, 2000b; Plopper *et al.*, 2001; Phimister *et al.*, 2004; Turner *et al.*, 2005). Glutathione-depletion induced cell death induced by mouse specific Clara cell toxicants initiates extensive cell replication and subsequent hyperplasia which are considered important steps in the multi-step progression to tumor development (Gadberry *et al.*, 1996; Green *et al.*, 1997b; Green *et al.*, 2001).

Additional comments

Page 2-22: Line 36, the exposures in the cardboard workers in Germany likely were much higher, with peaks well above 1,000 ppm and prolonged exposures above the former occupational standard (> 200 ppm TWA).
Page 3-6: The major toxicity of TCE after acute high dose exposure is narcosis. Kidney and liver damage are usually not observed (MAK, 1996).
Page 3-13: Table 3-6, if the data in the table are not considered reliable why are they presented?
Page 3-15: Line 27, TCA reversibly binds to proteins and the reversible protein binding is much more relevant for toxicokinetics of TCE as compared to covalent binding. It should also be noted that the ^{14}C-TCE used in many of the early studies contained a number of reactive impurities.

Page 3-23: Regarding saturation of TCE metabolism in humans, none of the human studies used dose-ranges where saturation of metabolism was seen in rats. Therefore, this conclusion should be removed.
Page 3-24: Lines 9 to 14, the text is not logical. TCE oxide may rearrange to dichloroacetyl chloride and the TCE P450 intermediate may rearrange to give chloral (Miller and Guengerich, 1982; Liebler and Guengerich, 1983; Cai and Guengerich, 2001).
Page 3-25: Lines 20 to 23, TCE oxide does not rearrange to chloral. Therefore, the text is confusing.
Page 3-27, Lines 19 to 25, chloral hydrate has been identified as a circulating TCE metabolite and is also formed as the major product in the microsomal oxidation of TCE (Byington and Leibman, 1965; Cole *et al.*, 1975).
Page 3-35: Metabolite recovery data in male and female human beings are available. In addition, metabolite excretion in humans and rats exposed to TCE by inhalation under identical conditions are available (Bernauer *et al.*, 1996).
Page 3-44: Table 3-23 should include additional data on GSH-conjugation of TCE (Dekant *et al.*, 1990; Green *et al.*, 1997a).
Page 3-46: Information on ß-lyase catalyzed metabolism of DCVC is available (Green *et al.*, 1997a).
Page 3-47: DCVC-sulfoxide; it should be mentioned that sulfoxides and down-stream metabolites have never been identified in rodents after administration of TCE (or PERC) and therefore are, at best, formed in small traces.
Page 4-34: Line 1, conclusion on bacterial mutagenicity. A more detailed weight-of-evidence evaluation of the contradictory database is needed here.
Table 4-18: Robbiano study, the study did not apply DCVG or DCVC and thus should not be included in the table.
Page 4-83: Line 28, DCVC is not a "direct-acting" mutagen since bacteria express ß-lyase (Dekant *et al.*, 1986b). Thus, this is a difference when compared to *S*-(2-chlorethyl)-L-cysteine, which does not require enzymatic transformation.
Page4-443: Lines 6 -7, the reactivity of chloral hydrate and chloroacetaldehyde are highly different and should not be compared. Chloroacetaldehyde is highly reactive with DNA-constituents (Green and Hathway, 1978), whereas chloral hydrate is not.

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Attachment 5

Exposure monitoring data of workers at trichloroethylene manufacturing facilities are presented in the tables below. Full shift data are listed in Tables I and III; task samples are listed in Tables II and IV.

Table I. Worker Exposure Data (Full Shift Samples) from a Trichloroethylene Manufacturing Facility (Company A)

Exposure Group	Approx. Frequency/ Duration	Task Description	Sample Date	Sample Duration (minutes)	Trichloroethylene (ppm)
Operator	Full-shift	General 12-hour exposure	04/17/18	480	Not detected ≤0.062
Operator	Full-shift	General 12-hour exposure	04/19/18	480	Not detected ≤0.062
Operator	Full-shift	General 12-hour exposure	04/23/18	480	Not detected ≤0.062
Operator	Full-shift	General 12-hour exposure	04/24/18	480	Not detected ≤0.062
Operator	Full-shift	General 12-hour exposure	04/25/18	480	Not detected ≤0.062
Operator	Full-shift	General 12-hour exposure	04/27/18	480	Not detected ≤0.062

Table II. Worker Exposure Data (Task Samples) from a Perchloroethylene Manufacturing Facility (Company A)

Exposure Group	Task description	Sample Date	Sample Duration (minutes)	Perchloroethylene (ppm)
Inside/outside operator	Catch samples – closed loop system	01/18/16	23	Not detected ≤ 0.5
Inside/outside operator	Catch samples – closed loop system	01/19/16	18	Not detected ≤ 1
Inside/outside operator	Catch samples – closed loop system	01/20/16	23	Not detected ≤ 0.5
Inside/outside operator	Catch samples – closed loop system	01/21/16	23	Not detected ≤ 0.5
Inside/outside operator	Catch samples – closed loop system	02/16/17	23	2.1
Inside/outside operator	Catch samples – closed loop system	02/16/17	21	Not detected ≤ 0.7
Inside/outside operator	Catch samples – closed loop system	02/23/17	21	Not detected ≤ 1.4
Site logistics distribution operator	Rail car connect/sample/purge, waste-no vent	08/23/17	20	6.9
Site logistics distribution operator	Rail car connect/sample/purge, waste-no vent	09/25/17	33	1.7
Site logistics distribution operator	Rail car connect/sample/purge, waste-no vent	09/25/17	30	Not detected ≤ 0.12
Operator	Clearing pumps and equipment for maintenance	10/17/17	20	Not detected ≤ 0.17
Operator	Clearing pumps and equipment for maintenance	10/17/17	20	Not detected ≤ 0.17
Operator	Clearing pumps and equipment for maintenance	10/18/17	30	0.15
Operator	Clearing pumps and equipment for maintenance	10/18/17	17	Not detected ≤ 022
Operator	Clearing pumps and equipment for maintenance	10/18/17	26	Not detected ≤ 0.14
Operator	Clearing pumps and equipment for maintenance	10/18/17	24	3

Table III. Worker Exposure Data (Full Shift Samples) from a Trichloroethylene Manufacturing Facility (Company B)

Exposure Group	Approximate Frequency/ Duration	Comment	Sample Date	Sample Duration (minutes)	Trichloroethylene (ppm)
Shipping Tankerman	Full-Shift	-	2016	480	0.251
Pipefitter	Full-Shift	Supplied air	2016	449	0.306
D Operator	Full-Shift	-	2016	483	1.01
D Operator	Full-Shift	-	2016	479	1.17
D Operator	Full-Shift	-	2016	489	1.22
Shipping Tankerman	Full-Shift	-	2016	480	2.54
B Operator	Full-Shift	-	2016	477	2.75
B Operator	Full-Shift	-	2016	479	BDL
C Operator	Full-Shift	-	2016	483	BDL
C Operator	Full-Shift	-	2016	504	BDL
B Operator	Full-Shift	-	2016	501	BDL
D Operator	Full-Shift	-	2016	489	BDL
B Operator	Full-Shift	-	2016	484	BDL
B Operator	Full-Shift	-	2016	478	BDL
C Operator	Full-Shift	-	2016	476	BDL
Pipefitter	Full-Shift	-	2016	452	BDL
Insulator	Full-Shift	-	2016	449	BDL
Pipefitter	Full-Shift	-	2016	447	BDL
Pipefitter	Full-Shift	-	2016	458	BDL
Welder	Full-Shift	-	2016	445	BDL

Insulator	Full-Shift	Dust Mask-N 95	2016	455	BDL
Welder	Full-Shift	-	2016	451	BDL
Machinist	Full-Shift	-	2016	472	BDL
Insulator	Full-Shift	Dust mask-Half Face	2017	434	0.155
Pipefitter	Full-Shift	-	2017	460	0.161
Pipefitter	Full-Shift	APR/SAR	2017	439	0.18
Pipefitter	Full-Shift	APR	2017	435	0.197
Welder	Full-Shift	Supplied Air	2017	450	0.206
Pipefitter	Full-Shift	Supplied Air	2017	460	0.214
B Operator	Full-Shift	-	2017	474	0.47
B Operator	Full-Shift	-	2017	472	0.555
D Operator	Full-Shift	-	2017	468	1.57
B Operator	Full-Shift	-	2017	474	BDL
C Operator	Full-Shift	-	2017	474	BDL
Welder	Full-Shift	-	2017	444	BDL

Table IV. Worker Exposure Data (Task Samples) from a Perchloroethylene Manufacturing Facility (Company B)

Exposure Group	Task description	Sample Date	Sample Duration (minutes)	Perchloroethylene (ppm)
D Operator	-	2016	29	2.08
C Operator	-	2016	22	BDL
C Operator	-	2016	23	BDL
D Operator	-	2016	26	BDL
B Operator	-	2016	22	BDL
C Operator	-	2016	28	BDL
B Operator	-	2016	21	BDL