HSIA
halogenated
solvents
industry
alliance, inc.

May 26, 2017

Information Quality Guidelines Processing Staff
Mail Code 2811R
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Request for Reconsideration/#16001

Dear Sirs:

On October 6, 2015, HSIA submitted a request for the correction of the TSCA Work Plan Assessment entitled Trichloroethylene: Degreasing, Spot Cleaning and Arts & Crafts Uses (June 2014) (#740-R1-4002) ("Request for Correction") under the Information Quality Act ("IQA").[1] On November 4, 2016, EPA denied this Request for Correction.[2] We respectfully request that EPA reconsider its denial of the Request for Correction.[3]

Suggested Resolution

The TCE Work Plan Assessment is now the basis for two rules banning uses of trichloroethylene (TCE) that have been proposed under § 6 of the Toxic Substances Control Act (TSCA). 81 Fed. Reg. 91592 (Dec. 16, 2016) (spot cleaning by dry cleaners and aerosol degreasing); 82 Fed. Reg. 7432 (Jan. 19, 2017); 82 Fed. Reg. 10732 (Feb. 15, 2017); 82 Fed. Reg. 20310 (May 1, 2017) (vapor degreasing). In this regard, we note that our comments on the latter state:

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[1] Section 515(a) of the Treasury and General Government Appropriations Act for Fiscal Year 2001, P.L. 106-554; 44 U.S.C. § 3516 (notes).

[2] The letter denying the Request for Correction was not received until November 30, 2016, however.

[3] This Request for Correction is separate and distinct from the request for correction filed on November 5, 2013, and denied by EPA on March 19, 2015 (#14001). That request was for correction of information disseminated in an EPA document, "Toxicological Review of Trichloroethylene (CAS No. 79-01-6) in Support of Summary Information on the Integrated Risk Information System (IRIS)" ("IRIS Assessment"). A request for reconsideration of that request was denied by EPA on February 26, 2016.

Our earlier request for correction addressed in detail the deficiencies of the IRIS Assessment. The IRIS Assessment contains a reference concentration ("RfC") of 0.0004 ppm (0.4 ppb or 2 $\mu g/m^3$) and a reference dose ("RfD") of 0.0005 mg/kg/day for TCE. These are values that are considered by EPA to be protective for all of the candidate critical effects. EPA's derivation of the RfC/RfD for TCE is based, in part, on Johnson *et al.*, Threshold of Trichloroethylene Contamination in Maternal Drinking Waters Affecting Fetal Heart Development in the Rat, Environmental Health Perspectives 111: 289-92 (2003).

> "EPA's progress in meeting the ambitious goals of the Lautenberg Act will in no way be impeded by deliberate review of the subject proposal. The situation is very different for the ten priority compounds recently designated by EPA under TSCA § 6(b)(2)(A).[4] For these ten designated pollutants, TSCA establishes deadlines for risk assessments to begin later this year and a schedule for rulemakings. TCE is one of these priority compounds.
>
> Because this is only a proposed rule, subject to no statutory mandate or deadline, its devastating impact can be easily avoided simply by EPA not taking action to adopt it and instead reviewing the vapor degreasing use as part of the upcoming assessment. This approach will allow serious data quality concerns with the June 2014 Work Plan Assessment to be addressed. Moreover, given EPA's announced intent to peer review its supplemental analysis, discussed below, it would be far more efficient to address the vapor degreasing use as part of that assessment."

A similar point was made in HSIA comments on the proposed ban on use of TCE in spot cleaning by dry cleaners and in aerosol degreasing.

Consistent with these comments, we urge EPA to grant our Request for Reconsideration and to defer consideration of the issues raised therein until EPA has prepared the risk assessment that it will be developing for TCE pursuant to TSCA § 6(b)(2)(A). In this regard, I note that HSIA has sponsored a study of TCE developmental toxicity intended to fill the remaining data gap for a guideline study by the drinking water route focused on whether TCE administration is associated with cardiac abnormalities in offspring. Regrettably, although the in-life portion of the study was conducted during October and November, 2016, the concentrations of TCE measured in the drinking water solutions were found to be below the acceptable target range of 100% ± 15%. The laboratory is conducting additional studies to identify the source of the problem, and HSIA intends to rerun the study as soon as the dosing methodological issues are resolved and scheduling permits. HSIA is confident that it will have completed and shared with EPA the results of this study by later this year, in ample time for EPA consideration as it initiates the new TCE risk assessment.

The denial of the Request for Correction consisted largely of conclusory statements with little factual or analytical support. For example, EPA asserted that the Work Plan Assessment is not a screening-level assessment, but it did not address its peer review Chair's own statement that it is a screening level assessment, not suitable for use in regulation: "the Agency acted prematurely in issuing this (screening level) assessment for public comment. . . . After listening

[4] Designation of Ten Chemical Substances for Initial Risk Evaluations, 81 Fed. Reg. 91927 (Dec. 19, 2016); Risk Evaluation Scoping Efforts under TSCA for Ten Chemical Substances, 82 Fed. Reg. 6545 (Jan. 19, 2017).

carefully to the comments and contributions from the other members of the Panel, I have concluded that there would little benefit in revising this draft screening assessment."[5]

As another example, the denial states that the peer review was consistent with EPA's peer review guidance and claims that EPA followed the peer reviewers' recommendations. Clearly EPA did not follow the Chair's recommendation that the assessment be abandoned and that it was not suitable for use as the basis of regulation, nor that of other reviewers that the non-cancer assessment not rely on a single flawed study that has so far not been able to be reproduced.[6]

More detailed responses to the letter denying the Request for Correction follow.

Detailed Comments

I. Deficiencies of Principal Non-Cancer Study

A. Not Reproducible

The Work Plan Assessment expressly relies on hazard values derived directly from a single academic study to estimate acute non-cancer risk.[7] Specifically, it states (p. 104):

> "The acute inhalation risk assessment used developmental toxicity data to evaluate the acute risks for the TSCA TCE use scenarios. As indicated previously, EPA's policy supports the use of developmental studies to evaluate the risks of acute exposures. This policy is based on the presumption that a single exposure of a chemical at a critical window of fetal development, as in the case of cardiac development, may produce adverse developmental effects (EPA, 1991).
>
> "After evaluating the developmental toxicity literature of TCE, the TCE IRIS assessment concluded that the fetal heart malformations are the most sensitive developmental toxicity endpoint associated with TCE exposure (EPA, 2011e). Thus, EPA/OPPT based its acute risk assessment on the most health protective endpoint (i.e., fetal cardiac malformations; Johnson et al., 2003) representing the most sensitive human population (i.e., adult women of childbearing age and fetus >16 yrs).
>
> "The acute risk assessment used the PBPK-derived hazard values (HEC_{50}, HEC_{95}, or HEC_{99}) from Johnson et al. (2003) developmental study for each degreaser and

[5] https://www.epa.gov/sites/production/files/2015-09/documents/tce consolidated peer review comments september 5 2013.pdf.

[6] As noted in the Request for Correction, the Work Plan Assessment goes beyond the IRIS Assessment by expressly relying on hazard values derived directly from Johnson *et al.* (2003) to estimate non-cancer risk.

[7] Johnson PD, *et al.*, Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat, Environ Health Perspect. 111:289-92 (2003).

spot cleaner use scenario. . . . These extremely low values result in margin of exposure ("MOE") values below 10 for almost all the occupational and residential exposure scenarios examined."

A single flawed study should not be the basis for the toxicological value that serves as the basis for regulation. Several other studies, including two GLP-compliant studies conducted under EPA guidelines to support pesticide registration (40 CFR § 870.3700) and Organization for Economic Coordination & Development ("OECD") guidelines (414) have been unable to reproduce the effect seen by Johnson *et al.* (2003).

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.[8] 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, along with results for two additional dose levels, 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.

B. Criticism in Literature and by Other Regulators

Johnson *et al.* (2003) has been heavily criticized in the published literature.[9] Indeed, its predecessor study was expressly rejected as the basis for MRLs by the Agency for Toxic Substances & Disease Registry (ATSDR) in its last final TCE Toxicological Profile Update.[10] Moreover, 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

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

[9] 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).

[10] 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.

and the participation of Dr. Johnson herself.[11] No increase in cardiac malformations was observed in the second guideline study,[12] 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.[13]

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 development, aside from those associated with maternal toxicity (Hardin et al., 2004)."[14]

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

[12] 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).

[13] "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).

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

C. Reservations of EPA Scientific Staff

Remarkably, an EPA staff review that was placed in the docket for the 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."[15]

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')."[16]

It is surprising that EPA would consider use of a dose-response value for regulation from a study in which seven of its own scientists expressed "low" confidence, and in which the other four could muster no more than "low to medium" confidence. 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 Reexamined

The TCE Work Plan Assessment relies heavily on its earlier IRIS Assessment, particularly the evaluation of the relationship between TCE exposure and the development of cardiac defects as described in Johnson *et al.* (2003). Ignoring for the moment the myriad of

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

[16] *Id.*

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. The importance of this activity cannot be overstated, 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."[17]

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 the resulting non-cancer toxicological value in the Work Plan Assessment that drives the proposed regulation.

E. Reliance on Johnson *et al.* (2003) Is Inconsistent with Use of Best Available Science

All acute inhalation exposures in the TCE Work Plan Assessment were measured against potential developmental toxicity endpoints based solely on EPA's IRIS evaluation of Johnson *et al.* (2003). 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.

[17] 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

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).[18] According to Makris *et al.* (2016):

> "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 constitutes a data quality concern sufficient to preclude Johnson *et al.* (2003) being used in support of regulation under TSCA § 6.

F. Failure to Conform to EPA Guidelines for Developmental Toxicity Risk Assessment

EPA's Guidelines for Developmental Toxicity Risk Assessment establish the framework for evaluation of developmental toxicity risk on a case-by-case basis.[19] Under these Guidelines, "[i]f data are considered *sufficient* for risk assessment, an oral or dermal reference dose for developmental toxicity (RfD_{DT}) or an inhalation reference concentration for developmental toxicity (RfC_{DT}) is then derived for comparison with human exposure estimates" (emphasis added).

In defining sufficiency, the Guidelines state: "In the case of animal data, agents that have been tested adequately in laboratory animals *according to current test guidelines* generally would be included in the 'Sufficient Experimental Animal Evidence/Limited Human Data' category (emphasis added)." Where, as here, the "database on a particular agent includes less than the minimum sufficient evidence (as defined in the 'Insufficient Evidence' category) necessary for a risk assessment, but some data are available, this information could be used to determine the need for additional testing. . . . In some cases, a database may contain conflicting data. In these instances, the risk assessor must consider each study's strengths and weaknesses

[18] 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

[19] 56 Fed. Reg. 63798 (December 5, 1991).

within the context of the overall database in an attempt to define the strength of evidence of the database for assessing the potential for developmental toxicity."

Given the demonstrated shortcomings of Johnson *et al.* (2003), which was not conducted to EPA test guidelines, and the availability to EPA of two guideline studies that are inconsistent with Johnson *et al.* (2003), we submit that the Guidelines for Developmental Toxicity Risk Assessment and TSCA §§ 6 and 26 require a weight of evidence evaluation of the database before EPA relies on Johnson *et al.* (2003) for regulatory purposes.

G. New Relevant Information

As noted above, HSIA sponsored a third guideline study of TCE developmental toxicity. The study was designed with a focus on cardiac abnormalities and included toxicokinetic measures to enable comparison with the earlier studies. It was intended to fill the remaining gap for a guideline study by the drinking water route, the same exposure route as Johnson *et al.* (2003). Regrettably, although the in-life portion of the study was conducted during October and November, 2016, the concentrations of TCE measured in the drinking water solutions were found to be below the acceptable target range of 100% ± 15%. The laboratory is conducting additional studies to identify the source of the problem, and HSIA intends to rerun the study as soon as the dosing methodological issues are resolved and scheduling permits. We note, however, that the difficulties achieving/maintaining target concentrations for the drinking water solutions by an experienced contract laboratory raise questions about the drinking water concentrations achieved by Johnson *et al.* (2003), particularly variability from batch to batch which was not discussed in the paper.

II. Deficiencies of Cancer Risk Assessment

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

While acute risks of developmental toxicity are characterized by EPA as of the greatest concern, the Work Plan Assessment also concludes that all but one of the degreaser exposure scenarios exceeded all the target cancer levels. The discussion of carcinogenicity in the Work Plan Assessment suffers from unquestioning reliance on EPA's earlier IRIS Assessment, which classified 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.[20] We briefly address below how the epidemiological data on TCE do not meet the threshold for classification as "Carcinogenic to Humans."

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

1. 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.[21]

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

"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

[21] 70 Fed. Reg. 17766-817 (April 7, 2005).

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.

2. Application of the Guidelines to TCE

In considering the data in the context of applying the "Carcinogenic to Humans" descriptor, one first considers the weight of the epidemiological evidence. 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.[22] 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 EPA assessment.[23] 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, 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 relative risks are small, and 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

[22] Alexander, D, *et al.*, A meta-analysis of occupational trichloroethylene exposure and multiple myeloma or leukemia, 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).

[23] 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).

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's 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.[24] 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 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

[24] 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).

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.[25] 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.

3. 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'

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 Institute of Medicine (IOM) report.[26] 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

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

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

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 Work Plan Assessment should not rely on the IRIS Assessment's classification of TCE as "Carcinogenic to Humans."

4. EPA Should Reassess Available Cancer Epidemiology Data, Given Publication of More Recent and Larger Studies on Worker Populations

The observation of an elevated but weak kidney cancer association reported by Charbotel *et al.* (2006)[27] 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.[28] 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

[27] 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).

[28] 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).

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.[29] 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 *et al.* (2003),[30] 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.[31] Finally, although a meta-analysis of 23 studies meeting criteria for study inclusion found a slightly increased simple summary association of 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.[32]

These more recent studies were not reviewed in the 2011 TCE IRIS Assessment or the 2014 TCE Work Plan Assessment.

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[29] 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).

[30] 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).

[31] 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).

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

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

The Work Plan Assessment of potential cancer risk focuses solely on inhalation exposures and relies on an inhalation unit risk (IUR) value developed in the 2011 IRIS Assessment. The IUR 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 concluded:

> "[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."[33]

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.[34] The authors' own conclusion 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 used as the basis for regulation.

As no cancer registry was available for this region to identify all relevant renal cell cancer cases from the target population, selection bias may be a concern. Case ascertainment relied on records of local urologists and regional medical centers. 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 RCC 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

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[33] 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.

[34] 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

workers not exposed to TCE. This preference in identifying cases among screw-cutting industry workers would bias findings in an upward direction.

The exposure assessment for the Charbotel study was based on questionnaires and expert judgment, not direct measures of exposure.[35] 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.[36] 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 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 cavalierly 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

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[35] 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.

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

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 have to be rigorously justified by EPA before it could be considered sufficiently robust to drive the types of decisions based on unit risk that are found in the proposed rule.

C. 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, and that IUR was used in the Work Plan Assessment without consideration of the scientific merit of such an approach. A recent study 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.

D. A Role for Glutathione conjugate-derived Metabolites Dichlorovinylglutathione (DCVG) and Dichlorovinylcysteine (DCVC) in TCE Renal 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.[37] However, Lash *et al.* (1999) relied on a colorimetric chromatographic method analysis of TCE glutathione conjugate-derived metabolites which had substantial potential for detection of non-TCE-specific endogenous substances. A recent study sponsored by HSIA (attached as Appendix 3) provides evidence that the HPLC/UV method used by Lash *et al.* (1999) may have been confounded by the potential of this method to detect non-TCE specific endogenous substances.

[37] 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 Env Hlth Part A, 56: 1-21 (1999). 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 (1999).

Since the publication of the IRIS Assessment in 2011, additional studies have evaluated the kidney concentrations of TCE oxidative and glutathione conjugate-derived metabolites in a variety of mouse strains administered 5 daily oral 600 mg/kg doses of TCE.[38] Metabolites were quantitated 2 hr after the last daily dose in that toxicokinetic evaluations had shown the approximate maximum plasma concentrations of TCA, DCA, DCVG and DCVC were observed 2 hr following oral TCE treatment.[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 oxidative metabolites quantitated in kidney. Trichloroethanol (TCOH) kidney concentrations were 2-4-fold greater than TCA, and TCA concentrations were 100-1000 greater than DCA. Importantly, DCA concentrations were 100-1000-fold greater than DCVG and DCVC, resulting in the conclusion that TCE oxidative metabolism was up to 5 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 plasma toxicokinetics TCA, DCA, DCVG and DCVC following a single 2140 mg/kg oral TCE dose found that the cumulative AUC of oxidative metabolites was 40,000-fold higher than the combined AUC of DCVG and DCVC; note that this study did not quantify TCOH, which would have further increased the disparity of glutathione conjugate-derived relative to oxidative-derived metabolites. These data 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 10-75 nM DCVC resulting from treatment of various strains of mice with a high oral TCE dose of 600 mg/kg/day for 5 days observed by Yoo *et al.* (2015). In addition, a likely NOAEL of 1 mg/kg/day was reported for kidney toxicity (no change in serum BUN, weak tubule dilation and no necrosis) in mice administered DCVC orally or intraperitoneally at 1, 10

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[38] Yoo HS, Bradford BU, Kosyk O, Uehara T, Shymonyak S, Collins LB, Bodnar WM, Ball LM, Gold A, Rusyn I, Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: kidney effects, J Toxicol Env Hlth Pt A, 78: 32-49.b (2015).

[39] Kim, S, Kim, D, Pollack, GM, Collins, LB, 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).

[40] Lash LH, Qian W, Putt DA, Hueni SE, Elfarra AA, Krause RJ, Parker JC, 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).

or 30 mg/kg/day, 1 day per week, for 13 weeks.[41] If, based on 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 6044 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.

III. Deficiencies in EPA's Exposure Assessment

The exposure assessment in the Work Plan Assessment was also flawed, because EPA failed to look at how it already regulates vapor degreasing. The second national emissions standard to be adopted by EPA under § 112 of the Clean Air Act (CAA) applied to vapor degreasing, and reduced emissions 80-90% ("Halogenated Solvent Cleaning NESHAP" or "NESHAP").[42] Then, in 2007, EPA revised the NESHAP to address residual risk, which essentially mandated a facility-wide emission limit for TCE of 14,100 kilograms per year in order to provide an "ample margin of safety to protect public health."[43] The NESHAP changed work practices, reduced in-facility exposure (occupational and bystander), and capped fenceline emissions.

A major shortcoming of the Work Plan Assessment is its failure to utilize information already submitted to EPA under the NESHAP. For example, the Work Plan Assessment relies on data collected before the 2008-2009 compliance deadlines for the NESHAP (primarily the NEI and TRI, and many assumptions (see pp. 34-37)) to estimate releases, exposures, and population exposed (pp. 114-15). This major source of uncertainty could easily have been eliminated by reference to data required to be reported under the NESHAP, which requires every facility to make an initial notification and report annually to EPA for each degreaser: type of machine and controls, location, date of installation, solvent consumption, and emissions.

More basically, to the extent the Work Plan Assessment references the NESHAP at all, it reflects a misunderstanding of it: "EPA's overall emission limit for implementing [the NESHAP] is 150 kilograms (kg) per square meter (m^2) per month (EPA, 2004a)" (p. 39). This reference is to the NESHAP for organic liquids distribution (non-gasoline), not here relevant. Moreover, the 150 kg/m^2 per month limit was an alternative standard for batch machines in the 1994 degreasing NESHAP, long since superseded. The current emissions limit – 14,100 kg/year facility-wide TCE emissions – is not reflected at all in the Assessment.

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

[42] 59 Fed. Reg. 61800 (Dec. 2, 1994). This rule established maximum achievable control technology for major and area sources.

[43] 72 Fed. Reg. 25138 (May 3, 2007); Halogenated Solvent Cleaning NESHAP, 40 C.F.R. Part 63, Subpart T.

IV. Other Flaws in Risk Assessment

A. Peer Review Ignored

The draft Work Plan Assessment was the subject of peer review by a panel selected by EPA in 2013. The peer review report highlights that it was a screening level assessment that inappropriately relied on an unreproducible study, and recommended that the assessment be abandoned.[44] One reviewer devoted six pages to a very detailed critique of Johnson *et al.* (2003) and EPA's reliance on such a deficient study.[45] Nevertheless, EPA largely ignored the peer review. Remarkably, even though the trade press article on the peer review was entitled *EPA Peer Reviewers Say Trichloroethylene Analysis Not Ready for Regulatory Use*,[46] the EPA Assistant Administrator for Chemical Safety and Pollution Prevention wrote to the EPA Inspector General that "[i]t is notable that *the external peer reviews of all the Work Plan assessments we have completed thus far supported our overall assessment methodologies and conclusions.*"[47] A more detailed description of the peer reviewers' comments is attached as Appendix 5.

B. Screening Level Assessment

As noted above and in Appendix 5, the peer review report highlights that the Work Plan Assessment was a screening level assessment. Specifically, the Chairperson of EPA's peer review panel wrote:

> "The draft document fails to articulate satisfactorily that the analysis described within should be characterized as a screening level assessment. . . . I believe that the Agency acted prematurely in issuing this (screening level) assessment for public comment. . . . After listening carefully to the comments and contributions from the other members of the Panel, I have concluded that there would little benefit in revising this draft screening assessment."

It is clear that a risk evaluation that supports a TSCA § 6 rule must be more robust than the screening level Work Plan Assessment that EPA carried out for TCE, which does not comply with Office of Management and Budget (OMB) guidelines implementing the Information

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[44] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

[45] *Id.*

[46] BNA Daily Environment Report (July 18, 2013).

[47] Response to Office of Inspector General Draft Report No. OPE-FY14-0012 "EPA's Risk Assessment Division Has Not Fully Adhered to Its Quality Management Plan," (July 30, 2014), Appendix A, p.10 (available at https://www.epa.gov/sites/production/files/2015-09/documents/20140910-14-p-0350.pdf) (emphasis added).

Quality Act.[48] First, EPA must conduct a "highly influential scientific assessment" to support TSCA § 6 rulemaking. OMB defines a scientific assessment as "highly influential" if dissemination of the assessment could have a potential impact of more than $500 million in any one year on either the public or private sector, or if the dissemination is novel, controversial, precedent-setting, or has significant interagency interest.

The Work Plan Assessment employed worst-case or default assumptions that led to overestimation of potential risks. Such assessments may be appropriate to support a decision that no further action or evaluation is necessary, because there is confidence that the potential risks are not a concern. However, they are inappropriate to support regulations intended to reduce risk because screening level assessments do not accurately estimate risk or quantify exposures. Second, OMB's guidelines also require agencies to subject highly influential scientific assessments to more rigorous peer review. For TCE, EPA selected a contractor to manage the peer review process, even though experts consider contractor-managed peer review to be the least rigorous level of peer review.

C. Summary of Concerns with Risk Assessment

In sum, the TCE Work Plan Assessment is inconsistent with the applicable requirements of revised § 6 in the following ways, among others:

- It expressly relies on hazard values derived directly from a single academic study to estimate acute non-cancer risk, even though several other studies, including two GLP-compliant studies conducted under EPA guidelines, have been unable to reproduce the effect;[49]

- The University of Arizona study upon which EPA relies has been heavily criticized in the published literature,[50] and other regulatory agencies have expressly declined to rely on the academic study citing data quality concerns;[51]

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[48] OMB, Final Information Quality Bulletin for Peer Review (Dec. 16, 2004) (available at https://www.whitehouse.gov/sites/default/files/omb/assets/omb/memoranda/fy2005/m05-03.pdf).

[49] *Compare* Johnson *et al.* (2003) to Fisher, J, *et al.*, Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: do they affect fetal rat heart development? Int. J. Toxicol. 20: 257-67 (2001) and 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).

[50] *E.g.*, "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); Watson, R., *et al.*, Trichloroethylene-contaminated drinking water and congenital heart defects: a critical analysis of the literature, Repro. Toxicol. 21: 117-47 (2006).

[51] *E.g.*, "The data from 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." California EPA Public Health Goal for Trichloroethylene in Drinking Water (July 2009), at 21.

- The authors of the Arizona study have published repeated corrections that fail to address the data quality concerns;[52] and a majority of EPA's own staff scientists expressed "low" confidence in its results.[53]

- It is a screening level assessment which does not meet OMB guidelines implementing the Information Quality Act for a "highly influential scientific assessment" to support TSCA § 6 rulemaking.

- The report of the peer review of the TCE Assessment highlights the foregoing points in the clearest possible terms, but EPA ignored it.[54] In fact, the EPA Assistant Administrator for Chemical Safety and Pollution Prevention wrote to the EPA Inspector General that "[i]t is notable that *the external peer reviews of all the Work Plan assessments we have completed thus far supported our overall assessment methodologies and conclusions*."

Following enactment of the Lautenberg Act, it should be clear that a risk evaluation that supports a TSCA § 6 rule must be more robust than the screening level Work Plan Assessment that EPA conducted for TCE. Peer review and public comments identified numerous scientific deficiencies with the draft assessment, including the inappropriate use of default assumptions; ignoring contrary evidence that affects the weight of the scientific evidence; reliance on inapposite exposure data; conclusions inconsistent with the evidence cited; and reliance on a study that is not reproducible. Important shortcomings in both the hazard and exposure assessments were noted. Whatever "best available science" may mean, it cannot include reliance on an unreproducible toxicity study or outdated exposure information.[55] And certainly EPA can no longer afford to ignore the conclusions of the peer review it initiated, as TSCA § 26(h) requires it to consider "the extent of independent verification or peer review of the information."

V. Conclusion

HSIA urges EPA to grant our Request for Reconsideration and to defer consideration of the issues raised herein for its development of the mandated risk assessment for TCE pursuant to

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[52] Johnson, PD, *et al.*, Environ Health Perspect 122: A94 (2014): erratum to Johnson, PD, *et al.*, Environ Health Perspect 113:A18 (2005), which is an erratum to Johnson *et al.* (2003).

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

[54] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

[55] *See* 162 Cong. Rec. S3522 (June 7, 2016) ("For far too long Federal agencies have manipulated science to fit predetermined political outcomes, hiding information and underlying data, rather than using open and transparent science to justify fair and objective decision making. This Act seeks to change all of that and ensure that EPA uses the best available science, bases scientific decisions on the weight of the scientific evidence rather than one or two individual cherry-picked studies, and forces a much greater level of transparency that forces EPA to show their work to Congress and the American public.)"

TSCA § 6(b)(2)(A). This will allow time to conduct and share with EPA the results of a study of TCE developmental toxicity intended to reproduce in an EPA guideline study, if possible, the effects observed by Johnson *et al.* (2003). More broadly, this approach will allow serious data quality concerns with the June 2014 Work Plan Assessment to be addressed.

Respectfully submitted,

Faye Graul /WCN

Faye Graul
Executive Director

Attachments

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. . . .

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. . . .

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

Appendix 2

EPA calculated an inhalation unit risk (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, 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 (EPA, 2011). 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} (EPA, 2011).

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, EPA, 2011). First, using relative risk (RR) estimates for kidney cancer, NHL, and liver cancer from its meta-analyses, 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, 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, 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 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) (EPA, 2011), 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) (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 EPA adjusted the IUR estimate for multiple cancer sites. 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, 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, 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.

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 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 EPA discussed. However, 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 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 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, 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, 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, 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 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.

Appendix 3

Abstract of manuscript submitted to the Journal of Chromatography B

Comparison of Liquid Chromatography-Ultraviolet and Liquid Chromatography-Positive Electrospray Tandem Mass Spectrometry Quantitative Analysis of the Major Glutathione Conjugate Biomarkers of Trichloroethylene: Dichlorovinyl Cysteine and Dichlorovinyl Glutathione

Fagen Zhang, Sue Marty, Robert Budinsky Michael Bartels, Lynn H. Pottenger, James Bus, Chris Bevan, Tim Erskine, Amy Clark, Brian Holzheuer, Dan Markham

Abstract

High-Performance Liquid Chromatography separation coupled to either ultraviolet detection (HPLC/UV) or tandem mass spectrometry (HPLC/MS/MS) detection, were compared for quantifying the major trichloroethylene (TCE) glutathione conjugates S-(1,2-dichlorovinyl)- glutathione (DCVG) and S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in rat and human tissues. DCVG and DCVC were initially derivatized with fluorodinitrobenzene (DNP) in the HPLC/UV method. The results showed that DCVC eluted at the solvent front and could not be quantified. DCVG, however, was quantified as the DNP derivative but with significant interference observed in all four control tissues (rat blood, liver, kidney; and human blood) with average spike recoveries of 222 to 22,990%. In contrast, the HPLC/MS/MS was used to directly analyze both DCVG and DCVC fortified tissues, with average spike recoveries of 82 to 127%. This significant difference between methods for both analytes was further confirmed with rat blood, liver, and kidney samples from TCE-treated rats, where DCVG levels in TCE-treated rat liver were 18,000 times higher by HPLC/UV as compared to HPLC/MS-MS. Substantial DCVG levels were observed in all control tissue samples using the HPLC/UV method, indicating a common interference across all tissues. Fraction collection of the DCVG peak from the HPLC/UV method, followed by peak identification via an HPLC/UV/QTOF/MS/MS (high resolution mass spectrometry) method, identified the DNP derivative of endogenous glutamate to be the primary endogenous substance contributing to the interference and thus the apparently increased recoveries of DCVG in the HPLC/UV method. Thus, existing data generated using HPLC/UV methods may not be reliable and it is recommended that future DCVG and DCVC quantitation following TCE exposure be performed using the HPLC/MS/MS method."

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

Lehrstuhl für Toxikologie
(Kommissarische Leitung Prof. Dr. Helga Stopper)

Institut für Toxikologie, Versbacher Str. 9, 97078 Würzburg, Germany

Prof. Dr. W. Dekant
TEL: +49-931-20148449
FAX: +49-931-20148865
E-mail: dekant@toxi.uni-wuerzburg.de

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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 then 180 manuscripts in these areas. Moreover, I am/was 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 would have benefited from 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. A detailed justification based on evaluation of the individual studies and a consideration of controversial data not supporting conclusions by EPA is often insufficiently developed. 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

The document concludes that the extent of formation of *S*-(1,2-dichlorovinyl)glutathione (DCVG) from TCE in humans is much higher as compared to rodents. Since this conclusion has a major impact on the derivation of RfCs and RfDs for TCE, it should be well justified and based on consideration of all available data. Apparently, EPA supports

this conclusion with 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. The consideration of this dataset without the wealth of other information therefore suggests that which therefore can not be a quantitative biomarker of metabolic flux through the glutathione conjugation pathway (Lash *et al.*, 2000) and that most of the DCVG may undergo bioactivation by ß-lyase. However, a number of observations do not support this conclusion:

- In the human study with TCE inhalation, high concentrations of DCVG 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 times 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 to locate 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 in subcellular fractions also gave much higher rates of formation (Lash *et al.*, 1998) as compared to 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 widely 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 rodents 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 1,000-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 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 with 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 app. 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 DCVG.
- 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 then 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 perchloroethene, which is also metabolized by glutathione *S*-conjugate formation and ß-lyase. The perchloroethylene (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 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 as compared to 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 (Bloemen *et al.*, 2001).

In summary, the assumption of a major flux through glutathione S-conjugate formation in TCE metabolism both in humans and in rodents is not well supported.

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.

Tissue	Species	TCE Conc (mM)	Rate of DCVC formation (pmol/minxmg)	Analytical method to determine DCVG	Reference
Liver cytosol	Rat	1.4 (14C)	0.54 (non-enzymatic reaction rates substracted)	HPLC with radiochemical detection, peak identity confirmed by LC/MS	(Green *et al.*, 1997b)
	Mouse	1.9 (14C)	0.35		
	Human	1.9 – 2.5 (14C)	0.012 – 0.055		
Liver microsomes	Rat	1.4 (14C)	Not different from non-enzymatic reaction		
	Mouse	1.9 (14C)	n.d.		
	Human	1.9 – 2.5 (14C)	n.d.		
Kidney cytosol	Rat	1.4 (14C)	Not different from non-enzymatic reaction		
	Mouse	n.d.			
	Human	n.d.			
Kidney microsomes	Rat	1.4 (14C)	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 (14C)	2		
Liver cytosol	Rat	2	121 (males) 81 (females)	Derivatisation with DNCB and ion exchange HPLC	(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 cytosol	Rat	2	7.5 (males) 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)		

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, 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 (Terracini and Parker, 1965) and questions an involvement of this pathway in nephrotoxicity.
- 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.
- 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. 82.4% (Nickerson *et al.*, 2008) or 71% in (Banks *et al.*, 2006), and technical problems using archived tissue samples may be the cause. Given that exon 3, which harbors the multiple mutations seen in TCE exposed patients, did not amplify in most of the controls, there is 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 and hence are no evidence for direct genotoxicity of TCE. 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 an 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 a micronucleus/comet assays 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 only 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 causing 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 may also causes tumors through nephroxicity).

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 that 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).

- With 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, chloral hydrate is a very weak mutagen. Therefore, chloral hydrate mutagenicity cannot adequately explain the formation of liver tumors by TCE in mice.
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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 orthologe 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 the human orthologe 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. 100fold lower then 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 and 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).
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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. Both kidney and liver damage are not often 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 as 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 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 directly identified in rodents.

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 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 has not.

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Appendix 5

Peer Reviewer Comments on Draft TCE Work Plan Assessment[1]

It is clear that a risk evaluation that supports a TSCA § 6 rule must be more robust than the screening level Work Plan assessment that EPA carried out for TCE. There can be no doubt that this is the proper characterization of the June 2014 assessment. The Chairperson of EPA's peer review panel wrote:

> "The draft document fails to articulate satisfactorily that the analysis described within should be characterized as a screening level assessment. . . . I believe that the Agency acted prematurely in issuing this (screening level) assessment for public comment. . . .
>
> "After listening carefully to the comments and contributions from the other members of the Panel, I have concluded that there would little benefit in revising this draft screening assessment. Rather, I would suggest that the effort be put into a higher tier, more refined assessment which would include empirical data gathered during the course of real-world uses, e.g., as OPP regularly asks be done for occupational exposures and sometimes for residential exposures, consumer use survey data, evaluation of exposure using additional modeling tools and a revisiting and reanalysis of the choices of toxicity and epidemiologic studies used to describe the health benchmark at the MEC99 level and the rationale for selecting the singular MOE of 30 to apply to the selected studies, each of which have varying degrees of credibility. This current draft screening level assessment could then be attached as an appendix to the new second-generation assessment, and described, in summary form, in the early chapter(s) of the new assessment. I would have saved the resources expended for the current external peer review and spent them on the next-generation assessment."
>
> She further stated:
>
> "By selecting the HEC99 and very conservative assumptions about exposure, one ends up with a very conservative (that is, health-protective) risk assessment, which assures only the certainty that the potential risk has not been under-estimated. It does little to resolve the uncertainty of the true estimate of risk."

The Chairperson's main point was that the information (*i.e.*, the screening level assessment) is not consistent with any intended use to support regulation. Her advice was that there would be little benefit in even revising the assessment, given its inadequacy for regulatory use. Taken together, these comments by the Chairperson of EPA's peer review panel establish quite clearly that the TCE risk evaluation does not meet the requirements of new TSCA § 26(h).

[1] https://www.epa.gov/sites/production/files/2015-09/documents/tce_consolidated_peer_review_comments_september_5_2013.pdf.

One of the peer review panelists, Calvin Willhite, raised serious concerns over the derivation of the non-cancer dose-response:

> "The non-cancer hazard index not only leads to calculation of the lowest equivalent 'safe' concentration of TCE in residential air, but those values are either less than or consistent with background TCE concentrations in United States urban or residential indoor air. As such, any domestic use of TCE in any amount for any use whatsoever will exceed the US EPA's published residential indoor air TCE level (0.21 µg/m3). As written, the previously published and current US EPA reports lead to the conclusion that current ambient TCE levels are associated with increased risk for human cardiovascular malformations - yet there are no suggestions from studies of occupational TCE exposures at concentrations 1-2 magnitude of orders greater than ambient pose excess non-cancer health risks to those workers."

With regard to uncertainty, weight of scientific evidence, quality and reproducibility, and other criteria identified in § 26(h), Dr. Willhite stated:

> "Question 5-4. Please comment on whether the document has adequately described the uncertainties and data limitations. Please comment on whether this information is presented in a transparent manner.
>
> "The general comments concerning the OPPT and IRIS conclusions on risk for cardiovascular malformations above illustrate the poor weight of evidence assessment carried out in this regard for TCE. The uncertainty attendant to the IRIS hazard identification for cardiovascular terata is so great that it leads to the present OPPT conclusion that all TCE exposures (including background concentrations in US urban ambient and indoor residential air) present increased risk for congenital malformation of the heart and great vessels.
>
> "It is not clear why OPPT relied on the results of the Johnson et al. (2003) study to the exclusion of all other inhalation and oral developmental toxicity studies in rodents and rabbits. If in fact the OPPT is reliant upon only the inhalation data, why is it the Carney et al. (2001), the Schwetz et al. (1975), the Hardin et al. (1981), the Beliles et al. (1980) or the Dorfmueller et al. (1979) study was not used? Why is there no discussion of all of the available developmental toxicity inhalation bioassays in the present analysis?
>
> "Summary
>
> "As submitted, the exposure parameters appear arbitrary (e.g., 0.5 and 1 hr/day) and may have been selected for sake of convenience. The data upon which conclusions put forward by OPPT on risk for developmental toxicity associated with arts and crafts use of TCE are not reliable. Nearly all developmental toxicity studies with TCE in rodents find no sign of teratogenicity (e.g., Beliles et al., 1980) or find only slight developmental delay (Dormueller et al., 1979). Chiu et al. (2013) cite the NRC (2006) report as verification of their risk assessment for TCE developmental toxicity, but actually the NRC (2006) concluded:

> ‘Additional studies evaluating the lowest-observed-adverse- effect-level and mode of action for TCE-induced developmental effects are needed to determine the most appropriate species for human modeling.’

“In its present assessment, the OPPT ignored the serious deficiencies already identified in conduct of the Johnson et al. (2003) rat drinking water study upon which the BMD01 was based (Kimmel et al., 2009; Watson et al., 2006) [Attachments 1 and 2]. In their weight-of-evidence assessment, Watson et al. (2006) concluded:

> ‘…application of Hill’s causality guidelines to the collective body of data revealed no indication of a causal link between gestational TCE exposure at environmentally relevant concentrations and congenital heart defects.’

“Those conclusions were consistent with Hardin et al. (2005). Perhaps most disturbing of all in US EPA’s reliance upon Johnson et al. (2003) as the key study (which for the basis for their lowest non-cancer TCE hazard index and margin of exposure) is the observation by Hardin and associates (2004):

‘Conventional developmental and reproductive toxicology assays in mice, rats and rabbits consistently fail to find adverse effects of TCE on fertility or embryonic development aside from embryo- or fetotoxicity associated with maternal toxicity. Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a ‘specific’ cardiac teratogen.’

“One of the fundamental tenants in science is the reliability and reproducibility of results of scientific investigations. In this regard, one of the most damning of the TCE developmental toxicity studies in rats is that by Fisher et al. (2005) who stated:

‘The objective of this study was to orally treat pregnant CDR(CD) Sprague-Dawley rats with large bolus doses of either TCE (500 mg/kg), TCA (300 mg/kg) or DCA (300 mg/kg) once per day on days 6 through 15 of gestation to determine the effectiveness of these materials to induce cardiac defects in the fetus. All-trans-retinoic acid (RA) dissolved in soybean oil was used as a positive control.

“The heart malformation incidence for fetuses in the TCE-, TCA- and DCA-treated dams did not differ from control values on a per fetus or per litter basis. The RA treatment group was significantly higher with 33% of the fetuses displaying heart defects.’

“Unfortunately, Johnson et al. (2005) failed to report the source or age of their animals, their husbandry or provide comprehensive historical control data for spontaneous cardiovascular malformations in their colony. The Johnson study with 55 control litters compared to 4 affected litters of 9 treated was apparently conducted over a prolonged period of time (perhaps years); it is possible this was due to the time required to dissect and inspect fresh rodent fetuses by a small

academic research group. However, rodent background rates for malformations, anomalies and variants show temporal fluctuations (WHO, 1984) and it is not clear whether the changes reported by Johnson et al. (2005) were due to those fluctuations or to other factors. Surveys of spontaneous rates of terata in rats and other laboratory animals are common particularly in pharmaceutical and contract laboratory safety assessment (e.g., Fritz et al., 1978; Grauwiler, 1969; Palmer, 1972; Perraud, 1976). The World Health Organization (1984) advised:

> 'Control values should be collected and permanently recorded. They provide qualitative assurance of the nature of spontaneous malformations that occur in control populations. Such records also monitor the ability of the investigator to detect various subtle structural changes that occur in a variety of organ systems.'

"Rates of spontaneous congenital defects in rodents can vary with temperature and housing conditions. For example, depending on the laboratory levocardia and cardiac hypertrophy occur in rats at background rates between 0.8-1.25% (Perraud, 1976). Laboratory conditions can also influence study outcome; for instance, maternal hyperthermia (as a result of ambient elevated temperature or infection) can induce congenital defects (including cardiovascular malformations) in rodents and it acts synergistically with other agents (Aoyama et al., 2002; Edwards, 1986; Zinskin and Morrissey, 2011). Thus while the anatomical observations made by Johnson et al. (2003) may be accurate, in the absence of data on maternal well-being (including body weight gain), study details (including investigator blind evaluations), laboratory conditions, positive controls and historical rates of cardiac terata in the colony it is not possible to discern the reason(s) for the unconventional protocol, the odd dose-response and marked differences between the Johnson et al. (2003) results and those of other groups.

"As noted by previous investigators, the rat fetus is "clearly at risk both to parent TCE and its TCA metabolite" given sufficiently high prenatal TCE exposures that can induce neurobehavioral deficits (Fisher et al., 1999; Taylor et al., 1985), but to focus on cardiac terata limited to studies in one laboratory that have not been reproduced in other (higher dose) studies and apply the BMD01 with additional default toxicodynamic uncertainty factors appears misleading."

Finally, Michael Jayjock, another peer review panelist, concluded: "Clearly, more work is needed on both the exposure and hazard side of this evaluation to tighten up the exposure assessment and to provide further justification or explanation of the exceedingly low HEC99 values used in the MOE analysis."

As discussed above, other panelists raised serious concerns going to the heart of the "best available science" criteria in TSCA § 26(h). Peer review and public comments identified numerous scientific deficiencies with the draft TCE assessment, including the inappropriate use of default assumptions; ignoring contrary evidence that affects the weight of the scientific evidence; reliance on inapposite exposure data; conclusions inconsistent with the evidence cited;

and, most importantly, reliance on a study that is not reproducible. Equally important deficiencies in both the hazard and exposure assessments were noted.

EPA completely disregarded the peer reviewers' advice and issued the final Work Plan assessment in June 2014 without making any substantial change to the draft. Under TSCA § 26(h), however, EPA must make its science-based decisions "in a manner consistent with the best available science" and "based on the weight of the scientific evidence." In addition, EPA can no longer afford to ignore the conclusions of the peer review it initiated, as it must consider "the extent of independent verification or peer review of the information."