

HSIA

halogenated
solvents
industry
alliance, inc.

December 10, 2010

BY HAND

Honorable Paul T. Anastas, Ph.D.
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Vanessa Vu, Ph.D.
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Re: Toxicological Review of Trichloroethylene (October 2009 Draft)

Dear Drs, Anastas and Vu:

The Halogenated Solvents Industry Alliance, Inc. (HSIA) represents producers and users of trichloroethylene (TCE), a chlorinated solvent primarily used to clean materials in applications where aqueous cleaning methods are not acceptable. These include precision parts used in medical, aerospace, defense and other important industries.

The Environmental Protection Agency (EPA) has since 1990 been engaged in an effort to update the health effects assessment for TCE that is reported on its Integrated Risk Information System (IRIS). HSIA participated in a stakeholder process that led to publication of a monograph of 16 articles comprising the "state-of-the-science" on issues relating to the health effects of TCE.¹ It was intended that "[s]taff of the National Center for Environmental Assessment at the U.S. EPA will use these articles to write the health risk assessment for TCE."² Regrettably, the 2001 draft Toxicological Review did not accurately reflect the work of many of the "state-of-the-science" authors, and was so flawed that EPA staff spent most of the past decade rewriting it, with the assistance of advice from the EPA Science Advisory Board (SAB) in 2002 and from the National Academy of Sciences in 2006 and 2009.³ The October 2009 draft

¹ Trichloroethylene Health Risks – State of the Science, Environ. Health Perspectives 108, Suppl. 2: 159-363 (May 2000).

² *Id.* at 159

³ Review of Draft Trichloroethylene Health Risk Assessment: Synthesis and Characterization: An EPA Science Advisory Board Report (EPA-SAB-EHC-03-002) (December 2002) is available at [http://yosemite.epa.gov/sab/sabproduct.nsf/D14C306CF5482E41852571CE00697543/\\$File/ehc03002.pdf](http://yosemite.epa.gov/sab/sabproduct.nsf/D14C306CF5482E41852571CE00697543/$File/ehc03002.pdf). The Academy's reviews are Assessing the Human Health Risks of Trichloroethylene (National Academies Press) (2006)

which resulted has been the subject of review over the past year by an SAB panel convened for this purpose.

The Chartered SAB will review draft advice prepared by the SAB TCE Panel on December 15, 2010. Enclosed is a written statement submitted by HSIA for consideration by the Chartered SAB. The statement describes how the epidemiological data on TCE fail to meet the threshold for classification as “Carcinogenic to Humans” under EPA’s 2005 Guidelines for Carcinogen Risk Assessment.⁴ It also shows how the draft assessment is in conflict with the Academy’s 2009 Camp Lejeune report. These are most serious defects that must be addressed before the assessment is made final.

The purpose of this letter is to bring to your attention two further issues that EPA must deal with as it moves toward a final assessment: (i) the inappropriate reliance on a tainted bioassay; and (ii) a conflict of interest that calls into question the SAB TCE Panel’s recommendations concerning developmental toxicity, an especially important non-cancer endpoint. These issues seem more appropriate for resolution, at least in the first instance, by the EPA program office.

I. The Draft TCE Assessment Places Major Reliance on a Study now thought to be Flawed

In April 2010, a team of pathologists from the National Toxicology Program (NTP) conducted a limited assessment of pathology procedures and histopathology for a carcinogenicity bioassay on methanol conducted by the European Ramazzini Foundation in 1990-1992 and published in 2002.⁵ The NTP review team found significant discrepancies in the interpretation of the reported results and concluded that an independent pathology review and quality review of the pathology data and specimens were necessary to address the serious discrepancies identified in the reported results of the methanol study.⁶ In its summary report, the NTP team stated that: “the diagnosis of leukemia or lymphoma was sometimes difficult to distinguish from the intense, marked lymphocytic infiltrates related to the chronic inflammation of the lung.” Additionally, the NTP pathologists questioned the basic research protocol utilized by Ramazzini in allowing animals to die spontaneously rather than being sacrificed after two years, as is the practice under US Good Laboratory Practices (GLP). They found that advanced autolysis in some tissues “occasionally precluded diagnosis by the NTP pathologists.”

and Contaminated Water Supplies at Camp Lejeune, Assessing Potential Health Effects (National Academies Press) (2009) (hereinafter “Camp Lejeune report”).

⁴ 70 Fed. Reg. 17766-817 (April 7, 2005).

⁵ Soffritti, M., Belpoggi, F., Cevolani, D., Guarino, M., Padovani, M. and Maltoni, C., Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats, *Ann. N.Y. Acad. Sci.* 982: 46–69 (2002).

⁶ US Department of Health and Human Services National Toxicology Program, Memo to John R. Bucher re report on visit (4/25/2010 - 4/30/2010) and assessment of the pathology procedures performed at the Ramazzini Institute, Bentivoglio, Italy (June 11, 2010).

The findings of the NTP review team have implications beyond the methanol study. As a result of the review, EPA immediately identified six assessments that rely on Ramazzini data, announced that it is placing on hold four ongoing IRIS assessments pending a full review of the underlying Ramazzini studies, and postponed a pending SAB review of one of those assessments.⁷ Regrettably, EPA did not announce similar action with regard to the TCE assessment. Yet the TCE assessment does rely substantially on Ramazzini data for its conclusion that TCE is a kidney carcinogen, the endpoint that drives the cancer risk assessment, and its derivation of the slope factor.

The Ramazzini TCE studies, referenced as Maltoni *et al.* in the draft assessment,⁸ account for the only inhalation and one of only two out of 74 total (inhalation and gavage) dose groups of TCE-treated rats reviewed by EPA to have had a statistically significant increase in kidney tumors. This effect (renal adenocarcinomas) was seen by Maltoni *et al.* at the high dose, 600 parts per million (ppm). The other dose group where an increase in renal carcinomas was observed in rats was the high dose group (1000 mg/kg) in an NTP gavage study. There was no statistically significant increase in kidney cancer in any of the 72 other dose groups. Only one statistically significant finding out of 74 is more likely due to chance than to treatment.⁹

Moreover, the draft TCE Toxicological Review recognizes serious weaknesses in the NTP study that preclude its use in the dose-response assessment:

“The NTP (1990) study of TCE exposure in male and female F344/N rats, and B6C3F1 mice (500 and 1,000 mg/kg for rats) is limited in the ability to demonstrate a dose-response for hepatocarcinogenicity. For rats, the NTP (1990) study reported no treatment-related non-neoplastic liver lesions in males and a decrease in basophilic cytological change reported from TCE-exposure in female rats. The results for detecting a carcinogenic response in rats were considered to be equivocal because both groups receiving TCE showed significantly reduced

⁷ “Out of an abundance of caution and to ensure the agency’s chemical assessments are grounded in the soundest possible science, EPA undertook a thorough review of all ongoing and previous chemical assessments to determine which, if any, relied substantially on cancer testing from the Ramazzini Institute. . . . EPA found four ongoing chemical assessments – on methanol, MTBE, ETBE and acrylonitrile – that rely significantly on cancer data from the Ramazzini Institute. EPA has placed those assessments on hold and will determine whether the questions raised by NTP will require EPA to revise the assessments or take additional action to verify the data used in these assessments. EPA also postponed an August 23, 2010 meeting of the agency’s Science Advisory Board, which had been previously scheduled to review the draft methanol assessment.” EPA Press Release at <http://yosemite.epa.gov/opa/admpress.nsf/0/B64D44F06A56D5B285257742007C5002>, posted June 15, 2010.

⁸ Maltoni, C, Lefemine, G, Cotti, G, *et al.*, Long-term carcinogenicity bioassays on trichloroethylene administered by inhalation to Sprague-Dawley rats and Swiss and B6C3F1 mice, in Maltoni, C, Selikoff, IJ, eds., *Living in a chemical world*, Ann. N.Y. Acad. Sci (1988), vol. 534; Maltoni, C, Lefemine, G, Cotti, G., *Experimental research on trichloroethylene carcinogenesis*, in Maltoni, C, Mehlman, MA, eds., *Archives of research on industrial carcinogenesis* (Princeton Scientific Publishing, 1986), vol. 5, pp. 316–342.

⁹ This information was presented to the SAB TCE Panel by Dr. Michael Dourson on June 24, 2010.

survival compared to vehicle controls and because of a high rate (e.g., 20% of the animals in the high-dose group) of death by gavage error.”¹⁰

Maltoni *et al.*, on the other hand, was judged by EPA to provide the best data set for its inhalation risk estimate:

“For the inhalation unit risk estimates, the preferred estimate from the most sensitive species and sex was the estimate of 8.3×10^{-2} per ppm for the male rat, which was based on multiple tumors observed in this sex/species but was dominated by the kidney tumor risk estimated with the dose metric for bioactivated DCVC. This estimate was the high end of the range of estimates (see Table 5-32) but was within an order of magnitude of other estimates. . . .”¹¹

Accordingly, “[f]rom the inhalation bioassays selected for analysis in Section 5.2.1.1, and using the preferred PBPK model-based dose metrics, the inhalation unit risk estimate for the most sensitive sex/species is 8×10^{-2} per ppm [2×10^{-5} per $\mu\text{g}/\text{m}^3$], based on kidney adenomas and carcinomas reported by Maltoni *et al.* (1986) for male Sprague-Dawley rats.”¹²

The draft then states that “confidence” in the proposed central estimate of 2×10^{-2} per ppm [4×10^{-6} per $\mu\text{g}/\text{m}^3$], based on human kidney cancer risks reported by Charbotel *et al.*, “is further increased by the similarity of this estimate to estimates based on multiple rodent data sets.”¹³

On its face, the draft TCE Toxicological Review “relied substantially on cancer testing from the Ramazzini Institute,” and thus would appear to fall within the EPA policy announced on June 15 whereby “EPA has placed those assessments on hold and will determine whether the questions raised by NTP will require EPA to revise the assessments or take additional action to verify the data used in these assessments.” When the Maltoni *et al.* results are excluded, there is no “nonequivocal” animal kidney cancer data supporting either the conclusion that TCE is a kidney carcinogen or the inhalation cancer slope factor.

II. The TCE Panel Review Has Been Tainted by Active Participation by a Conflicted Member

Regarding non-cancer human health effects, a significant issue for the TCE Panel has been the potential hazard of TCE for the developing fetus, in particular the role of TCE in inducing fetal cardiac defects. Indeed, the Panel’s draft advice makes specific recommendations regarding the studies to be given greatest emphasis in the calculation of the oral reference dose (RfD) and the inhalation reference concentration (RfC). The Panel believed that the non-cancer

¹⁰ EPA Toxicological Review of Trichloroethylene (October 2009 Draft), at 4-261.

¹¹ *Id.*, at 5-121.

¹² *Id.*, at 5-145.

¹³ *Id.*, at 5-146.

kidney toxicity data are not suitable for calculations of the RfC and RfD. Instead, the Panel advised EPA to give priority to three studies for deriving the RfC and RfD, most particularly Johnson *et al.* (fetal heart malformations in rats).¹⁴ It is the reliance on this and supporting studies from the same laboratory that raises concerns regarding the impartiality and dispassionate judgment of a member of the Panel.

The Overview of the SAB Panel Formation Process states: “If a conflict exists between a panel candidate’s private financial interests and activities and public responsibilities as a panel member, or even if there is the appearance of partiality, as defined by federal ethics regulations, the SAB Staff will, as a rule, seek to obtain the needed expertise from another individual.”¹⁵ Pursuant to the EPA’s Peer Review Handbook (3rd Edition), “each advisory committee member or peer reviewer should be evaluated to ensure that an appearance of lack of impartiality does not preclude their participation.”¹⁶

The draft TCE assessment clearly has been prepared under EPA’s IRIS program. Consequently, the peer review of the draft assessment is subject to EPA’s NCEA Policy and Procedures for Conducting IRIS Peer Reviews.¹⁷ Under these procedures, a recertification of a peer-review panelist may be requested to determine if there were any changes to the information they previously disclosed that could create either an actual conflict of interest or an appearance of bias or lack of impartiality during the period of performance. EPA may be informed about a potential emerging conflict of interest situation, including an appearance of bias or lack of impartiality, by a person or organization external to EPA.

Most importantly, the Office of Management and Budget (OMB) Final Information Quality Bulletin for Peer Review states that “agencies shall adopt or adapt the NAS policy for committee selection with respect to evaluating conflicts of interest” concerning non-federal employees. The National Academy of Sciences (NAS) Policy on Committee Composition and Balance and Conflicts of Interest for Committees Used in the Development of Reports states that “an individual should not serve as a member of a committee with respect to an activity in which a critical review and evaluation of the individual's own work, or that of his or her immediate

¹⁴ Johnson, P, *et al.*, Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat, *Environ. Health Perspect.*:111: 289-92 (2003).

¹⁵ EPA, Overview of the Panel Formation Process at the Environmental Protection Agency Science Advisory Board. Office of the Administrator, Washington DC (2002) (EPA SAB-EC-02-010), p. 9.

¹⁶ US Environmental Protection Agency Peer Review Handbook (3rd Edition), Science Policy Council, Washington, DC (2009) (EPA/100/B-06/002), p. 67. The Handbook suggests the following question to assess a candidate’s suitability to serve on a peer-review panel: “Do you know of any reason that you might be unable to provide impartial advice on the matter to come before the Panel or any reason that your impartiality in the matter might be questioned?”

¹⁷ EPA, NCEA Policy and Procedures for Conducting IRIS Peer Reviews, Office of Research and Development, Washington, DC (2009).

employer, is the central purpose of the activity, because that would constitute a conflict of interest, although such an individual may provide relevant information to the program activity.”¹⁸

The conduct at issue here is the active participation of Dr. Ornella Selmin in the discussion of the weight to be given a program of *in vivo* and *in vitro* experiments carried out over the past two decades at the University of Arizona on the relationship between TCE exposure and cardiac malformations. Dr. Selmin is a lead or co-author on a number of papers reporting these results,¹⁹ and has co-authored papers with Dr. Paula Johnson, lead author of the most important and highly criticized of these studies.

Johnson *et al.* reported cardiac effects in rats from research carried out at the University of Arizona and originally published ten years earlier by the same authors.²⁰ In the earlier-published study, there was no difference in the percentage of cardiac abnormalities in rats dosed during both pre-mating and pregnancy at drinking water exposures of 1100 ppm (9.2%) and 1.5 ppm (8.2%), even though there was a 733-fold difference in the concentrations. The authors reported that the effects seen at these exposures were statistically higher than the percent abnormalities in controls (3%). For animals dosed only during the pregnancy period, the abnormalities in rats dosed at 1100 ppm (10.4%) were statistically higher than at 1.5 ppm (5.5%), but those dosed at 1.5 ppm were not statistically different from the controls. Thus, no meaningful dose-response relationship was observed in either treatment group. Johnson *et al.* republished in 2003 data from the 1.5 and 1100 ppm dose groups published by Dawson *et al.* in 1993 and pooled control data from other studies, an inappropriate statistical practice, to conclude that rats exposed to levels of TCE greater than 250 ppb during pregnancy have increased incidences of cardiac malformations in their fetuses.

Johnson *et al.* has been heavily criticized in the published literature,²¹ and the earlier studies were rejected as the basis for minimal risk levels (MRLs) by the Agency for Toxic

¹⁸ Office of Management and Budget, Final Information Quality Bulletin for Peer Review, Executive Office of the President, Washington, DC (2004).

¹⁹ *E.g.*, Makawana O, *et al.*, Exposure to low-dose trichloroethylene alters shear stress gene expression and function in the developing chick heart, *Cardiovasc Toxicol.* 10(2): 100-7 (2010); Caldwell PT, *et al.*, Gene expression profiling in the fetal cardiac tissue after folate and low-dose trichloroethylene exposure, *Birth Defects Res A Clin Mol Teratol.* 88(2): 111-27(2010); Selmin O, *et al.*, Trichloroethylene and trichloroacetic acid regulate calcium signaling pathways in murine embryonal carcinoma cells p19, *Cardiovasc Toxicol.* 8(2): 47-56 (2008); Caldwell PT, *et al.*, Trichloroethylene disrupts cardiac gene expression and calcium homeostasis in rat myocytes, *Toxicol Sci.* 104(1): 135-43 (2008); Selmin O, *et al.*, Effects of trichloroethylene and its metabolite trichloroacetic acid on the expression of vimentin in the rat H9c2 cell line, *Cell Biol Toxicol.* 21(2): 83-95 (2005); Collier JM, *et al.*, Trichloroethylene effects on gene expression during cardiac development, *Birth Defects Res A Clin Mol Teratol.* 67(7): 488-95 (2003).

²⁰ Dawson, B, *et al.*, Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water, *J. Am. Coll. Cardiol.* 21: 1466-72 (1993).

²¹ 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.*: 21:117-147 (2006).

Substances and Disease Registry (ATSDR).²² Moreover, the Johnson *et al.* findings were not reproduced in a study designed to detect cardiac malformations; this despite employing an improved method for assessing cardiac defects and the participation of Johnson herself.²³ No increase in cardiac malformations was observed in a guideline, GLP-quality study,²⁴ despite high inhalation doses and techniques capable of detecting most of the malformation types reported by Johnson *et al.* The dose-response relationship reported in Johnson *et al.* 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. The draft TCE assessment discusses many of the uncertainties regarding the findings of Johnson *et al.*, but the SAB Panel recommended Johnson *et al.* as a preferred basis for the RfD/RfC calculation. It appears that this may be the direct result of strong support for using Johnson *et al.* expressed by Dr. Selmin during the Panel meetings.

As noted above, “Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a ‘specific’ cardiac teratogen,”²⁵ and Dr. Selmin has been directly involved in this research program for some time. At various stages in the SAB panel discussions, Dr. Selmin indicated her support for the Johnson *et al.* study and expressed her view that recent mechanistic studies made the Johnson *et al.* findings more robust. For example, on May 11, 2010, during the discussions on Charge Question 3, Dr. Selmin indicated her support for EPA’s description of the studies relating to cardiac malformations (and their admitted shortcomings) but then indicated that new studies on mechanism of action make the Johnson *et al.* findings more robust. This theme was then repeated during discussion of Charge Question 8 – derivation of RfC and RfD. During the summary discussions of Charge Question 3, Dr. Selmin proposed that EPA should include recent publications to support conclusions based on Johnson *et al.*: she is co-author of three of those studies.²⁶

The concern here is that the findings of Johnson *et al.* have now been elevated to a primary source for hazard assessment and derivation of the RfC and RfD largely at the insistence

²² See Letter from Christopher T. DeRosa, Director, ATSDR Division of Toxicology, to Peter E. Voytek, HSIA (February 28, 1996) (enclosed). 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.” ATSDR, Toxicological Profile for Trichloroethylene Update (September 1997), p. 88.

²³ Fisher, J, *et al.*, Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: do they affect fetal rat heart development? *Int. J. Toxicol.* 20: 257-67 (2001).

²⁴ 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).

²⁵ Hardin, B, *et al.*, *Repro. Toxicol.*: 21:117-147 (2006), citing several other studies from the University of Arizona, Tucson.

²⁶ Makawana O, *et al.*, Exposure to low-dose trichloroethylene alters shear stress gene expression and function in the developing chick heart, *Cardiovasc Toxicol.* 10(2): 100-7 (2010); Caldwell PT, *et al.*, Gene expression profiling in the fetal cardiac tissue after folate and low-dose trichloroethylene exposure, *Birth Defects Res A Clin Mol Teratol.* 88(2): 111-27(2010); Caldwell PT, *et al.*, Trichloroethylene disrupts cardiac gene expression and calcium homeostasis in rat myocytes, *Toxicol Sci.* 104(1): 135-43 (2008).

of Dr. Selmin. Despite the recent studies, Johnson *et al.* remains a poor basis for assigning hazard or calculation of exposure limits and the mechanistic studies do not provide information that bridges the gap to support directly the Johnson *et al.* conclusions. Without impugning Dr. Selmin's scientific integrity, the extent of criticisms of the work of the University of Arizona is likely to mean that Dr. Selmin will be drawn to defend the work done by her co-workers; a dispassionate, objective interpretation might not result. The appropriate action would have been for Dr Selmin to have recused herself from discussions of the interpretation of Johnson *et al.* and related studies.

Under the NAS conflicts policy cited above that is required to be adopted or adapted by EPA, "an individual should not serve as a member of a committee with respect to an activity in which a critical review and evaluation of the individual's own work, or that of his or her immediate employer, is the central purpose of the activity, because that would constitute a conflict of interest, although such an individual may provide relevant information to the program activity." Dr. Selmin's active participation in the discourse that has resulted in the SAB recommendation that her laboratory's controversial and unreproducible work be the basis for the RfD/RfC for TCE would seem to constitute a clear conflict of interest under this policy.

It may be possible to deal with this problem by empanelling a small group of perhaps three or four independent developmental toxicologists under the aegis of the SAB to review the cardiac malformation data. This would provide much-needed clarity in a controversial area and allow resolution of the issue to move forward, and could be accomplished while the issue of inconsistency with the EPA Guidelines for Carcinogen Risk Assessment and the Academy's Camp Lejeune report, now pending before the Chartered SAB, is resolved.

III. Conclusion

In sum, we urge EPA to refrain from reliance on the bioassays by Maltoni *et al.* in the TCE assessment until EPA's review of studies conducted by that laboratory is concluded, and to reconsider the evidence supporting the recommendation that Johnson *et al.* be used to establish the RfC/RfD for TCE.

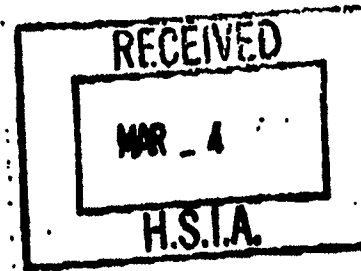
Very truly yours,


Faye Graul
Executive Director

Enclosures

5116169

February 28, 1996



Peter E. Voytek, Ph.D.
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Dear Dr. ^{Peter}Voytek:

Thank you for your recent correspondence regarding the intermediate duration oral minimal risk level for trichlorethylene chloride. Your concerns as outlined are shared by ATSDR. For this reason we have independently contacted the authors of the studies to further discuss and clarify these issues. ←

Thanks again for your input, and please be assured that it will be carefully considered as we develop the final version of the profile.

Best regards.

Sincerely,

Christopher T. DeRosa, Ph.D.
Director, Division of Toxicology