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for the TSCA SACC Peer Review of the
Draft Risk Evaluation of Trichloroethylene (TCE)

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Thank you for the opportunity to present these comments on behalf of our more than three million members and online activists, and our staff of some 700 scientists, lawyers, and policy advocates across the globe that work to ensure the rights of all people to the air, the water, and the wild. NRDC has no financial interest in TCE or any other aspect of these comments.

These comments are focused on the following industry-sponsored report: An oral (drinking water) study of the effects of trichloroethylene (TCE) on fetal heart development in Sprague Dawley rats: Laboratory Project ID 00459506 Sponsor: Halogenated Solvents Industry Alliance, Inc (HSIA).

These comments reference the EPA Risk Evaluation for Trichloroethylene. EPA Document #740R18008. February 21, 2020, herein referred to as “TCE Draft”.

DETAILED COMMENTS

The HSIA study was designed with the stated purpose of replicating the findings of Dawson et al (1993) and Johnson et al. (2003) (HSIA p. 19). The study is so important because it supports risk estimates (and regulations) based on an acute endpoint – exposures during fetal heart formation - instead of exposures being averaged over a lifetime, which would accommodate much higher peak or short-term exposures.

> "Developmental toxicity endpoints will be considered for both acute and chronic scenarios. Although developmental studies typically involve multiple exposures, they are considered relevant for evaluating single exposures because evidence indicates that certain developmental effects may result from a single exposure during a critical window of development (Davis et al., 2009; Van Raaij et al., 2003). This is consistent with EPA’s Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996) and Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), which state that repeated exposure is not a necessary prerequisite for the

manifestation of developmental toxicity. This is a health protective assumption." (TCE Draft, p. 221)

HSIA is likely also concerned because the Johnson et al study introduces developmental malformations associated with TCE and therefore also triggers potential lawsuits.

The HSIA report did not repeat the Dawson et al (1993) and Johnson et al. (2003) findings

The HSIA report claims to have conducted a "targeted" analysis, so that it doesn't look at other developmental malformations, including some that were identified in the Johnson et al study such as atrial septal defects.[1] The epidemiology also identifies other problems in addition to septal defects, including hypersensitivity, skin disorders, and hepatitis. "The consistency among the studies and the concordance between the studies in mice and humans support an etiologic role of TCE in autoimmune disease" (Cooper et al 2009).[2]

- "In short, the methodology and positive control data indicate that the [HSIA study] (2019) was primarily focused on ventricular septal defects (VSDs) and therefore did not sufficiently examine the complete range of potential cardiac defects." (TCE Draft, p. 222)
- "The Johnson study (2003) … observed both valve and atrial septal defects using their … methodology. In contrast, while the … dissection method (1984) used by the [HSIA study] should allow visualization of valves, the [HSIA study] did not report valve defects in any TCE group or the RA positive control group even though many other published reports have identified valve defects following administration of TCE or RA." (TCE Draft, p. 222)
- "Additionally, the [HSIA study] method (1984) does not include examination of the heart for atrial septal defects, and the [HSIA study] did not report any atrial septal defects in either the RA positive control group or the TCE groups." (TCE Draft, p. 222)
- "As further indication of the potentially limited sensitivity of [the HSIA study], the defects observed from exposure to the retinoic acid (RA) positive control were also somewhat limited compared to the broader RA literature (which did identify atrial septal defects)." (TCE Draft, p. 223)

Thus, the HSIA study was only a partial replication of the Johnson study because it didn't look at all effects; it was designed to be a negative study by not fully examining TCE-induced developmental malformations that are well-established in the peer-reviewed literature.

The problem with pair-wise statistics

The HSIA study primarily used pairwise statistics instead of trends analysis, which is a weaker statistical method. The EPA Guidelines say either test may be used, so EPA should use whichever shows a response to TCE exposure. In this case, the trend test is preferable. (EPA 2005, p. 46)

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[1] Johnson PD, Goldberg SJ, Mays MZ, Dawson BV. Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat. Environ Health Perspect. 2003;111(3):289-92.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241384/ See Errata. Environ Health Perspect. 2005;113(1):A18.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1253738/

[2] Cooper GS, Makris SL, Nietert PJ, Jinot J. Evidence of autoimmune-related effects of trichloroethylene exposure from studies in mice and humans. Environ Health Perspect. 2009;117(5):696-702. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2685829/

Instead, the HSIA study uses pair-wise tests, which are weak and tend to waste statistical power by making multiple comparisons to control groups. Toxicologists don't tend to use them.

Litter instead of individual pups as unit of statistical analysis

The HSIA report relies on statistical analysis of the litter instead of individual pups for the unit of analysis (HSIA p. 25). With only 20 litters, this dramatically reduces the ability to detect an effect. Typically, one would use both the litter and individuals. It would be best if both litter and pup analyses were reported.

Two-sided statistical tests are inappropriate

The HSIA report uses only two-sided statistical tests (HSIA p. 25). This type of test is meant for a treatment such as a medical drug that could be either harmful or beneficial. This toxicology study should have used one-sided tests that are more appropriate since the alternative hypothesis (to no effect) is harm, not benefit. EPA IRIS scientists normally utilize one-sided tests for this reason.

The HSIA study authors make a big deal out of this being a hypothesis-driven study and their alternative hypothesis (to no effect) is harm, not a beneficial effect, so they should have used a one-sided test, which would have doubled the statistical strength of the effect.

The HSIA study is inherently a one-sided study – the general principle is that you don't waste statistical power by making comparisons that you aren't testing. You narrow the scope of the statistical test to increase power. The opinion of statisticians is to use the most appropriate technique to give you the most power: one-sided tests

Corrected statistics

Typically, a p-value of 0.1 or less (10% or less probability of chance or error) is considered significant. If you run the VSD on an individual animal basis through the Cochran Armitage trend test, the one-sided p-value is 0.0196. The Cochran Armitage trend test is for discrete data, which these are. EPA would have to make some adjustment for the litter effect, but for 5 test groups, it is reasonable to do a trend test.

The HSIA report misuses statistics as a weapon to cut away evidence of adverse effects, rather than a tool to identify associations where they may occur. An important 2019 paper published in Nature, one of the world's most prestigious and highly ranked scientific journals, signed by over 800 supporters, argues that over-reliance on statistical significance to deny or disregard an adverse effect is a mis-use of statistics and puts the public health at risk. "*Let's be clear about what must stop: we should never conclude there is 'no difference' or 'no association' just because a P value is larger than a threshold such as 0.05 or, equivalently, because a confidence interval includes zero. Neither should we conclude that two studies conflict because one had a statistically significant result and the other did not. These errors waste research efforts and misinform policy decisions*".[3] The HSIA report is a case study of statistical errors that bias against supporting environmental health protections.

[3] Ioannidis JPA. Retiring statistical significance would give bias a free pass. Nature. 2019 Mar;567(7749):461. doi: 10.1038/d41586-019-00969-2. PubMed PMID:30903096.

In 2016, the American Statistical Association released a statement in its professional journal, The American Statistician, warning against the misuse of statistical significance and P values.[4] In particular, the public statement argues that by itself a p-value is not a reliable measure of evidence, and - importantly - full reporting and transparency of data is required for proper evaluations. Failure to report on the full data sets, including data for each individual animal, is a failure of the basic requirements for statistical reporting.

There is a saying among statisticians, that some people use statistics as a drunkard uses a lamppost – for support, rather than illumination. Reliance on the HSIA study to dismiss evidence of harm would certainly be a drunk decision.

Misuse of historical controls

The Charles River Ashland historical control data range for major heart vessel variations is 0.0 to 0.86% per litter, according to the study (p. 37). The study dismisses the major blood vessel variations by saying they are within the historical controls – in fact, they are not. The incidence at the high dose is 2X the historical control.

Further, the HSIA's use of historical control data is pieced together after-the-fact (post hoc) from old publications from labs in China in the 1960s and early 1970s. This gives a new meaning to historical controls – ancient history from the perspective of an inbred rat with a 2 to 3-year lifespan! The HSIA report also applies a bizarre cherry-picked use of historical control data for some endpoints but not others, with no real rationale provided. This is inappropriate. Unless the experimental controls are flawed for some obvious documented reason, they should always be used. Again, EPA Cancer Guidelines dictate this: "Caution should be exercised in simply looking at the ranges of historical responses, because the range ignores differences in survival of animals among studies and is related to the number of studies in the database…When historical control data are used, the discussion should address several issues that affect comparability of historical and concurrent control data, such as genetic drift in the laboratory strains, differences in pathology examination at different times and in different laboratories (e.g., in criteria for evaluating lesions; variations in the techniques for the preparation or reading of tissue samples among laboratories), and comparability of animals from different suppliers. The most relevant historical data come from the same laboratory and the same supplier and are gathered within 2 or 3 years one way or the other of the study under review; other data should be used only with extreme caution." (EPA 2005, p. 47-48). The HSIA study presents no adequate rationale for its use of historical controls.

HSIA mistaken about the magical self-healing VSD

The HSIA study argues that, based on published data, defects in the membranous septum tend to "resolve postnatally, without adverse effects on postnatal survival of the animals" and thus should not be considered adverse (p. 15, 39), referencing two studies in rats to support this claim.[5]

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[4] American Statistical Assoc statement on statistical significance and p-values: Provides Principles to Improve the Conduct and Interpretation of Quantitative Science March 7, 2016. https://www.amstat.org/asa/files/pdfs/p-valuestatement.pdf

[5] Fleeman TL, Cappon GD, Hurtt ME. Postnatal Closure of Membranous Ventricular Septal Defects in Sprague-Dawley Rat Pups after Maternal Exposure with Trimethadione. Birth Defects Research. 2004;71:185-190.

The truth is that there is some evidence that VSD during early life may increase health risks over a lifetime in people.[6] An important study in rodents, using the same strain of rats as the HSIA study, reported that even small and seemingly healed chemically-induced VSD at birth "may permanently alter the capacity of the postnatal heart to adapt to pregnancy and this may have transgenerational effects."[7] The study found that adult rodents that were born with VSD could not fully adapt to the additional stress on the heart during pregnancy. The authors raise concern about implications for humans born with VSD: "The paucity of data pertaining to the clinical outcomes after spontaneously resolved [congenital heart defects] … is worrisome because our studies in rat suggest [congenital heart defects] might predispose women to [cardiovascular] dysfunction or complications during pregnancy. In fact, it has been reported that pregnancy has unmasked previously undetected heart disease in patients." [8]

In addition to the rodent data, a study in people by Aasa et al, 2015 demonstrates that in utero chemical exposure may permanently alter the capacity of the postnatal heart to adapt to pregnancy and this may have transgenerational effects.[9]

How a disease or defect progresses is the result of many factors, including the mechanism of the initial injury, and many other factors related to developmental progression, cell-cell interactions, genetic and epigenetic factors, and a list of others too numerous to name. HSIA has no scientific basis to dismiss evidence of adverse effects in its study.

HSIA report generally supports EPA conclusions that TCE elevates risk of fetal cardiac malformations

Ironically, despite its flaws – which all bias to the null, making it harder to detect significant adverse effects - the HSIA report does provide evidence of ventricular septal defects in the developing heart of rats treated prenatally with TCE, providing additional confirmatory evidence supporting the findings of the Johnson et al study.

> "In fact, the [HSIA study] observed a similar 1303 percentage of VSDs as (Johnson et al., 2003). Considering total VSDs, 3.5% of fetuses showed a VSD in 1304 Charles River vs 3.8% in Johnson at the highest dose, with 1.5% in Charles River vs 2.2% in Johnson at 1305 1.5ppm. When

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Solomon HM, Wier PJ, Fish CJ, et al. Spontaneous and Induced Alterations in the Cardiac Membranous Ventricular Septum of Fetal, Weanling, and Adult Rats. Teratology. 1997;55:185-194.

Hoffman JEI, Kaplan S. The Incidence of Congenital Heart Disease. J Am Coll Cardio. 2002;39:1890-1900

[6] Otterstad JE, Erikssen J, Michelsen S, Nitter-Hauge S. Long-term follow-up in isolated ventricular septal defect considered too small to warrant operation. J Intern Med. 1990 Oct;228(4):305-9.

[7] Aasa KL, Maciver RD, Ramchandani S, Adams MA, Ozolinš TR. In Utero Exposure to a Cardiac Teratogen Causes Reversible Deficits in Postnatal Cardiovascular Function, But Altered Adaptation to the Burden of Pregnancy. Toxicol Sci. 2015 Nov;148(1):155-66.

[8] Aasa KL, Maciver RD, Ramchandani S, Adams MA, Ozolinš TR. In Utero Exposure to a Cardiac Teratogen Causes Reversible Deficits in Postnatal Cardiovascular Function, But Altered Adaptation to the Burden of Pregnancy. Toxicol Sci. 2015 Nov;148(1):155-66.

[9] In Utero Exposure to a Cardiac Teratogen Causes Reversible Deficits inPostnatal Cardiovascular Function, But Altered Adaptation to the Burden of Pregnancy Aasa, Kristiina L ; Maciver, Rebecca D ; Ramchandani, Shyamlal ; Adams, Michael A ; Ozolinš, Terence R. S Toxicological Sciences, 2015, Vol. 148(1), pp.155-166. https://academic.oup.com/toxsci/article/148/1/155/1659914

considering only membranous VSDs (the only type observed in the Charles River study), 1306 observed incidences were actually higher in Charles River at the highest dose (3.5% vs 2.86%). 1307 Meanwhile, a substantial percentage of the total cardiac defects observed in (Johnson et al., 2003) were 1308 valvular or atrial." (TCE Draft, p. 222)

The HSIA study results are consistent with the Johnson et al results, almost a perfect match. See HSIA p. 39 - Text Table 15: Mean Litter Proportions of Membranous Interventricular Septal Defects in the Sprague Dawley Rat

Dose	0 ppm	0.0025 ppm (2.5 ppb)	0.25 ppm (250 ppb)	1.5 ppm	500 ppm	1000 ppm
HSIA	2.4	N/A	1.4	1.5	3.8	3.7
Johnson et al (% abnormal hearts)	3.0 (2.1%)	0	0.0 (4.5%)	1.7 (5%)	N/A	3.8 (10.5%)

However, the HSIA authors dismiss these findings by proposing, despite evidence to the contrary, that these developmental defects heal over time, “without adverse effects” on the prenatally exposed rat or presumably the exposed person.

Consider an analysis of the VSD and cardiac variations together

It may be a valuable statistical exercise to group the two reported cardiac malformations – the membraneous interventricular septal defects (VSD), and the cardiac major vessels variations - since the two tissues share the same embryonic tissue origin, the truncus arteriosus.[10] Further, developmental deformities in the membraneous septum and in variations in the great vessels often present clinically together.[11] EPA could consider presenting this analysis.

Conclusions of EPA career staff are supported by the science

As EPA experts have successfully shown in a published paper (Makris et al 2017), "Associations of TCE exposures with congenital heart defects in the human population are of concern for public health... The available epidemiological studies, though insufficient to establish a causal link, provide evidence of disruptions in cardiac development. The epidemiology data comprised one component of the integrated evidence; when considered in context with findings from animal and mechanistic studies, there is an association between TCE developmental exposures and cardiac defects."[12]

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[10] The muscular part of the interventricular septum derives from the bulboventricular flange which is developed due to differential growth of primitive ventricle and bulbous cordis. Membranous part has a neural crest origin which connects the upper free margin of the bulboventricular flange and anterior and posterior endocardial cushions of atrio ventricular canal. It also gets attached to lower border of spiral septum or the aortico pulmonary septum.

[11] LeRoy S. 2012. Michigan Medicine, CS Mott Children’s Hospital, Congenital Heart Center. https://www.mottchildren.org/conditions-treatments/ped-heart/conditions/ventricular-septal-defect

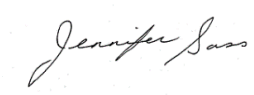
[12] Makris SL. The systematic review of TCE cardiac defects (Makris et al., 2016).Reprod Toxicol. 2017 Aug;71:124-125. doi: 10.1016/j.reprotox.2017.05.013. Epub 2017 May 30. PubMed PMID: 28571977.

Makris SL, Scott CS, Fox J, Knudsen TB, Hotchkiss AK, Arzuaga X, Euling SY, Powers CM, Jinot J, Hogan KA, Abbott BD, Hunter ES 3rd, Narotsky MG. A systematic evaluation of the potential effects of trichloroethylene exposure on

As Dr. Makris notes in her published response to John DeSesso (Exponent) and Steve Risotto (American Chemistry Council), to "dismiss or ignore the integrated evidence in this database would be a disservice to sound science" and will continue to put at risk our health and safety. [13]

Thank you for consideration of these comments.

Respectfully,



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cardiac development. Reprod Toxicol. 2016 Oct;65:321-358. doi: 10.1016/j.reprotox.2016.08.014. Epub 2016 Aug 27. Review. PubMed PMID: 27575429.

[13] Makris SL. The systematic review of TCE cardiac defects (Makris et al., 2016).Reprod Toxicol. 2017 Aug;71:124-125. doi: 10.1016/j.reprotox.2017.05.013. Epub 2017 May 30. PubMed PMID: 28571977.