



HSIA

halogenated
solvents
industry
alliance, inc.

June 17, 2015

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

Re: Request for Reconsideration/#14001

Dear Sirs:

On November 5, 2013, HSIA submitted a request for the correction of information ("Request for Correction") under the Information Quality Act ("IQA").¹ HSIA sought correction of the reference concentration ("RfC") of 0.0004 ppm (0.4 ppb or 2 $\mu\text{g}/\text{m}^3$) and reference dose ("RfD") of 0.0005 mg/kg/day first disseminated in EPA's "Toxicological Review of Trichloroethylene (CAS No. 79-01-6) in Support of Summary Information on the Integrated Risk Information System (IRIS)."² EPA's derivation of the RfC/RfD for trichloroethylene ("TCE") was based, in part, on Johnson *et al.*, Threshold of Trichloroethylene Contamination in Maternal Drinking Waters Affecting Fetal Heart Development in the Rat, Environ. Health Perspect. 111: 289-92 (March 2003).

More recently, on July 3, 2014, HSIA supplemented its Request for Correction in light of an erratum published earlier in 2014 by Johnson *et al.*³ Thereafter, on September 8, 2014, HSIA

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 EPA/635/R-09/011F (September 2011) ("TCE IRIS Assessment").

³ Johnson *et al.*, Environ Health Perspect 122: A94 (2014): erratum to Environ Health Perspect 113:A18 (2005), which is an erratum for Johnson *et al.*, Threshold of Trichloroethylene Contamination in Maternal Drinking Waters Affecting Fetal Heart Development in the Rat, Environ Health Perspect 111:289-292 (2003). The previously published articles covered by the Johnson *et al.*, 2014 erratum are: Dawson BV, Johnson PD, Goldberg SJ, Ulreich JB, Cardiac Teratogenesis of Halogenated Hydrocarbon-contaminated Drinking Water, J Am Coll Cardiol 21(6):1466-1472 (1993); Johnson PD, Dawson BV, Goldberg SJ., Cardiac Teratogenicity of Trichloroethylene Metabolites, J Am Coll Cardiol 32(2):540-545 (1998); Johnson PD, Dawson BV, Goldberg SJ., A Review: Trichloroethylene Metabolites: Potential Cardiac Teratogens. Environ Health Perspect 106 (Suppl 4):995-999 (1998); Johnson PD, Dawson BV, Goldberg SJ, Mays MZ., Trichloroethylene: Johnson *et al.*'s Response [Letter], Environ Health Perspect 112:A608-A609 (2004).

submitted additional information in support of the Request for Correction. This additional information consisted of EPA's own assessment of the predecessor study (which reported some of the TCE data cited) to Johnson *et al.* (2003).⁴ In this EPA assessment for a different compound, vinylidene chloride (1,1-dichloroethylene),⁵ EPA rejected these data as not biologically significant and concluded that they were *not* suitable to be the basis for an RfC/RfD.

On March 19, 2015, under the signature of Acting Assistant Administrator Lek Kadeli, EPA denied the HSIA Request for Correction ("EPA Denial"). For the reasons discussed below, HSIA disagrees with this EPA decision and requests reconsideration. Specifically, HSIA recommends that the RfC/RfD for TCE be based on an endpoint other than cardiac malformations.

I. Peer Review

The EPA Denial relies heavily on the external peer review of the draft TCE IRIS Assessment by the EPA Science Advisory Board ("SAB"), noting that HSIA made presentations at five TCE meetings and made 14 presentations in all. HSIA supports independent peer review. In this case, however, there are two serious problems with EPA's reliance on the SAB review as ensuring quality assurance. First, the SAB review was influenced by the inappropriate and improper participation by a scientist with a direct interest in the outcome, indeed, a co-author of some of the research under consideration. Second, the SAB review of the TCE IRIS Assessment was only the second of three external peer reviews of the specific question of whether the Arizona studies reported by Johnson, Dawson and co-authors were of good enough quality to warrant EPA reliance: the other two peer reviews determined quite conclusively that they *were not*.

A. The SAB Review Was Tainted by the Active Participation of a Conflicted Member

The SAB panel made specific recommendations regarding the studies to be given greatest emphasis in the calculation of the RfD and the RfC. It advised EPA to give priority to three studies for deriving the RfC and RfD, most particularly Johnson *et al.* (2003) (fetal heart malformations in rats). 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.

⁴ Dawson, BV, Johnson, PD, Goldberg, SJ, *et al.*, Cardiac Teratogenesis of Halogenated Hydrocarbon-Contaminated Drinking Water, *J. Am. Coll. Cardiol.* 21:1466–1472 (1993).

⁵ Toxicological Review of 1,1-Dichloroethylene (CAS No. 75-35-4) in Support of Summary Information on the Integrated Risk Information System (IRIS) (EPA/635/R02/002) (June 2002) ("Vinylidene Chloride Assessment").

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.”⁶ 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.”⁷

The draft TCE Assessment clearly was 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.⁸ 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. HSIA did so inform EPA, by letter dated December 10, 2010 to Honorable Paul T. Anastas, Ph.D., Assistant Administrator, and Vanessa Vu, Ph.D., Director, EPA Science Advisory Board Staff.

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 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.”⁹

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 two decades at the University of Arizona on the relationship between TCE exposure and

⁶ 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.

⁷ 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?”

⁸ EPA, NCEA Policy and Procedures for Conducting IRIS Peer Reviews, Office of Research and Development, Washington, DC (2009).

⁹ Office of Management and Budget, Final Information Quality Bulletin for Peer Review, Executive Office of the President, Washington, DC (2004).

cardiac malformations. Dr. Selmin is a lead or co-author on a number of papers reporting these results,¹⁰ and has co-authored papers with Dr. Paula Johnson, lead author of the most important and highly criticized of these studies.

As noted in the Request for Correction, “Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a ‘specific’ cardiac teratogen,”¹¹ and Dr. Selmin was directly involved in this research program. At various stages in the SAB panel discussions, Dr. Selmin indicated her support for Johnson *et al.* (2003) and expressed her view that recent mechanistic studies made those 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.* (2003) findings more robust. This theme was 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.* (2003): she is co-author of three of those studies.¹²

Just as HSIA had feared, the findings of Johnson *et al.* (2003) were elevated to a primary source for hazard assessment and derivation of the RfC and RfD largely at the insistence of Dr. Selmin. Without impugning Dr. Selmin’s scientific integrity, the extent of criticisms of the work of the University of Arizona meant that Dr. Selmin would be drawn to defend the work done by her co-workers; a dispassionate, objective interpretation might not result. The appropriate action would, at the least, have been for Dr Selmin to be recused from any discussion of the interpretation of Johnson *et al.* (2003) 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

¹⁰ 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).

¹¹ Hardin, B, *et al.*, *Repro. Toxicol.* 21:117–147 (2006), citing several other studies from the University of Arizona, Tucson.

¹² 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).

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 resulted in the SAB panel’s recommendation that her laboratory’s controversial and unreproducible work be the basis for the RfD/RfC for TCE, and would seem to constitute a clear conflict of interest under this policy.

B. Other Peer Reviews Rejected Reliance on the Arizona Studies

1. Vinylidene Chloride IRIS Assessment

The EPA Denial discounts the first SAB peer review of the University of Arizona studies, in connection with the IRIS assessment of vinylidene chloride (1,1-dichloroethylene or 1,1-DCE), on the basis that “the assessment focused on a different chemical and a different set of studies” and thus is “not directly comparable.” This is disingenuous, as can be seen in the SAB panel’s advice to EPA at the time:

“General Question 3: For the RfD and the RfC, have the appropriate studies been chosen as “principal”? The principal study should present the critical effect in the clearest dose response relationship. If not, what other study (or studies) should be chosen and why?”

“The Panel unanimously agreed that Quast et al. (1983, 1986) were the appropriate studies for the RfC and RfD evaluations. The Panel also discussed the Dawson et al. (1993) developmental study, which suggested an increased incidence of cardiac malformations in neonatal rats after exposure of dams to 1,1-DCE in drinking water before mating and throughout gestation. This study was discussed both to assert why the Quast et al. (1983, 1986) studies were used and why the panel did not recommend use of the Dawson et al. (1993) developmental study as the principal study.

“Although their reasons differed, the panelists unanimously believed that the Dawson et al. (1993) developmental toxicity study should not be considered as the principal study or considered to represent a potential developmental hazard from 1,1-DCE exposure. The reasons included concerns for the high positive responses on a litter basis in the controls, the lack of increased response between the two exposures that varied by 900-fold, and quality control issues identified in a 1996 Agency for Toxic Substances and Disease Registry review of other developmental toxicity studies *with trichloroethylene (TCE)* conducted by these investigators. Quality control issues, including lack of analytical confirmation of the concentrations in the drinking water *in the TCE studies*, were brought to the attention of the Panel by one panelist on the basis of his participation in an earlier review of these studies. Finally, *other studies by Fisher et al., 2001 were cited as failing to replicate developmental cardiac changes with TCE.* [Emphasis added.]

“Before the discussion of the deficiencies in the developmental toxicity drinking water studies, no panel member felt that Dawson et al. (1993) study should be

used as the principal study. Interestingly, the panelists were against using the Dawson *et al.* (1993) study because it does not provide confidence that the effects were exposure-related and associated with DCE exposures, not because the changes were variations in cardiac morphology.”

Obviously, Dawson *et al.* (1993) reported developmental toxicity data for *both* TCE and vinylidene chloride. In fact, a single control group was used for both the TCE and vinylidene chloride treatment groups, although there appears to be some confusion as to the size of that control group (see below). The SAB reviewers’ comments are equally relevant to TCE because they address quality issues associated with a key component of Johnson *et al.* (2003) -- the evaluation of the 733-fold difference between the 1,100 and 1.5 ppm TCE exposure groups as earlier reported by Dawson *et al.* (1993).

Some of the SAB reviewers’ comments relating to study quality are particularly relevant, for example, the “concerns for the high positive responses on a litter basis in the controls.” This comment is intriguing as Dawson *et al.* (1993) do not appear to provide information on the number of control litters. Nonetheless, this issue has been raised before and suggests the existence of a colony quality concern with the animals used in the developmental toxicity studies reported by the University of Arizona researchers. Dawson *et al.* (1993) reported that three percent of fetuses in the control group had cardiac defects. For comparison, the literature includes reports of an historical spontaneously occurring cardiovascular malformation rate in Crl:CD Sprague-Dawley rat fetuses of 0.04 percent. Although the source of the Sprague-Dawley rats used in Dawson *et al.* (1993) is not identified, a fetal malformation rate two orders of magnitude higher than that seen in supplier colonies should be a major concern.

Another concern raised by the SAB reviewers was “the lack of increased response between the two exposures that varied by 900-fold.” Over that vinylidene chloride dosage range, the fetal cardiac malformation rate increased from 1.9 percent to 3.6 percent. Over a 733-fold increase in TCE exposure (*i.e.*, 1.5 ppm to 1,100 ppm) the fetal cardiac malformation rate increased from 5.5 percent to 10.4 percent in the same study, raising similar concerns for HSIA. Indeed, it is particularly interesting that the reviewers noted “[q]uality control issues, including lack of analytical confirmation of the concentrations in the drinking water in the TCE studies”. In fact, Dawson *et al.* (1993) indicates that the drinking water concentrations of TCE (and vinylidene chloride) were tested by gas chromatography at the time of preparation. In the follow-up paper, Johnson *et al.* (2003) report a 35 percent loss of TCE from drinking water solutions over a 24-hour period. It is not clear from Dawson *et al.* (1993) that TCE losses were even measured for the 1,100 and 1.5 ppm solutions.

2. TSCA Chemicals Work Plan Assessment of TCE

Even more egregiously, the EPA Denial does not even mention the third independent peer review that considered the quality of the Arizona studies – this one clearly in the context of an EPA risk assessment of TCE. The Request for Correction

quoted this review at length. As this review apparently was overlooked during the preparation of the EPA Denial, this excerpt is reproduced below:

“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?

* * * * *

“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 (Dorfmueller 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; Grauwerler, 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.”¹³

We respectfully submit that EPA should not deny our Request for Correction on the basis of prior EPA peer review and not address the most recent and directly relevant of those peer reviews.

II. EPA’s Own Review Does Not Support Use of Johnson *et al.* for Dose-Response

HSIA learned from footnote 19 of the EPA Denial that the Agency empanelled a group of 15 EPA scientists to conduct an evaluation of the potential for cardiac defects occurring as a consequence of exposures to TCE. This “TCE Developmental Cardiac Toxicity Assessment Update” was submitted to the docket (EPA-HQ-OPPT-2012-0723) for the TSCA Work Plan Chemicals Risk Assessment of TCE just two weeks before EPA’s Expert Public Workshop on Alternatives and Risk Reduction Approaches to Trichloroethylene (TCE) held on July 29-30, 2014.

Although the Update is apparently the result of extensive deliberations by a multi-disciplinary team of EPA scientists, there is no indication that it was ever subjected to any sort of external peer review. Other than through its submission to the OPPT docket, there is no indication that its existence was announced publicly. While several aspects of the Update are of interest, as discussed further below, here we focus on the very clear reservations shared by the EPA scientists on the use of Johnson *et al.* (2003) to derive an RfC/RfD, which is the heart of HSIA’s concern with the TCE IRIS Assessment:

¹³ <http://www.scgcorp.com/tcl2013/prcomments.asp>, pp. 56-73. Attachments containing more detailed critiques of Johnson *et al.* are also available via this link.

“Overall, taking into account the study’s design, its strengths and limitations, and uncertainties in the weight of evidence, 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”).* Nonetheless, the team members concluded that the point of departure derived in the 2011 TCE assessment, which used an approach consistent with standard U.S. EPA dose response practices, remained a reasonable choice.”

This statement indicates that none of the EPA scientists had more than low to medium confidence in the use of Johnson *et al.* to derive the RfC/RfD. Such concerns expressed by its own scientists raise again the question of why EPA would disregard all previous reviews of the Arizona studies, including its own, and rely on Johnson *et al.* (2003) to derive the RfC/RfD.

In addition, the summary/conclusions section of the Update supports HSIA’s position that Johnson *et al.* (2003) is scientifically unacceptable:

“The minimum evidence that would be necessary to determine whether there is or is not sufficient evidence of developmental toxicity is the existence of appropriate, well-conducted animal study(ies). The overall TCE database met this criterion, although limitations and uncertainties in the primary study used in dose response (Johnson *et al.*, 2003) are acknowledged. Those limitations and uncertainties were the basis of the only dissenting opinion (*i.e.*, of one team member) regarding whether the database supports a conclusion that TCE exposures during development are likely to cause cardiac defects.”¹⁴

“The team had a range of views as to their confidence in the conclusion regarding hazard for cardiac defects—with three out of nine scientists expressing an opinion concluding the confidence should be medium to high, and six of nine concluding confidence should be “low” or “medium.” These ratings were influenced by whether the primary focus was on the uncertainties and limitations of the Johnson *et al.* (2003) study or whether it was on the weight of evidence consideration of the entire database.”

“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”).”

¹⁴ A ‘majority’ (presumably not all) of the remaining team members disagreed with the single dissenting opinion and felt that the database supports a conclusion that TCE exposures during development are likely to cause cardiac defects. However, the statement implies that an unreported number of the remaining team members shared the concerns of the dissenter.

Given the study and data quality issues that have been identified by HSIA, the EPA scientists, and others, it is amazing that “[o]n the whole, a majority of the team members agreed that Johnson *et al.*, (2003) is suitable for use in deriving a point of departure.” We believe that it has been amply demonstrated that the study is so flawed that there can be no confidence in its reported results. Yet it remains the sole animal drinking water developmental toxicity study that demonstrates a dose-response relationship between TCE dose and cardiac defects. Without Johnson *et al.* (2003), there is at best a collection of ‘supportive’ studies with no key study to support. The Update appears to separate the quality of the study from methodologies used in the dose-response analysis and point of departure determination. This is analogous to evaluating the quality of a house construction while ignoring the fact that the foundation is inadequate.

III. The Arizona Studies Lacked Concurrent Control Groups and Had Other Quality Issues

HSIA has consistently maintained that the data presented in Johnson *et al.* (2003) and subsequently clarified in the two errata do not allow calculation of the incidence of cardiac malformations per litter that is time-matched to concurrent controls (the standard practice for evaluation of developmental toxicity studies). Accepting the authors’ claims in the 2014 erratum that exposure times cannot be confirmed for substantial amounts of either control or treatment data, it also can be presumed that it is now impossible to reconstruct a calculation of per litter incidence of cardiac malformations that is appropriately matched to concurrent controls. Thus, the data reported in Johnson *et al.* (2003), even as amended in two subsequent errata, do not allow for data analysis generally accepted as essential to interpreting outcomes of developmental toxicity study findings. The lack of data availability and clarity sufficient to construct key analyses associated with a key study should disqualify the use of that study in important agency decisions such as RfC/RfD derivation.

The EPA Denial states that “[c]ontrary to your assertions, in our review of the erratum we noted that a concurrent control group is identified for each of the TCE study groups identified. Table 1 shows that during each time period that laboratory animals were being exposed to TCE, there was a temporally overlapping control group of test animals.” We fail to understand how EPA can conclude from the available information that concurrent controls were, in fact, run.

Enclosed is a copy of a document identified in the TCE IRIS Assessment as HERO ID 783484. HSIA contacted NCEA’s Weihseh Chiu on June 6, 2013, requesting any raw data, in addition to that in HERO ID 783484, that EPA had received from the University of Arizona group that may have been used to evaluate Johnson *et al.* (2003). His response on June 13, 2013 indicated that “[w]e do not have any of the additional data you are seeking. EPA’s analyses were based on the data obtained in the posted HERO document.” Examination of the enclosure reveals data for the four treatment groups and a large control group, but there are no dates associated with any of the treated or control animals. If this does, in fact, represent the entirety of the data used by EPA in its IRIS evaluation of Johnson *et al.* (2003), HSIA must vigorously disagree with EPA’s contention that “concurrent controls were, in fact, run.” The flawed and inconsistent information provided in the 2005 and 2014 Errata do nothing to change that position.

Starting with the 2014 Erratum, in referring to the two lower doses shown in Table 1 of the 2005 Erratum, the authors claim that “although the exact dates can no longer be confirmed, the start dates for these three groups occurred in 1994, not 1995” and that “all of the animal exposure experiments were run with concurrent controls.” First, we are curious why it took the authors nine years to realize that a 21-day exposure study could not be conducted in the eight days between June 6, 1995 and June 13, 1995 (*i.e.*, as reported for the 2.5 ppb dose) or the seventeen days between July 5, 1995 and July 21, 1995 (*i.e.*, as reported for the 250 ppb dose),¹⁵ and even more curious about what prompted the authors to publish the 2014 Erratum. In referring to the “three groups,” the Erratum also implies that the start date for the concurrent control group (presumably the group listed as starting on July 18, 1995) actually began in 1994. According to the 2014 Erratum, we are left to conclude that the 21-day exposure for these two dosing groups (and presumably their concurrent control group) took place over a period exceeding one year “although the exact dates can no longer be confirmed.”

The 2014 and 2005 Errata also still fail to convince a reader that concurrent controls were run for all groups. The 2014 Erratum states that the exposure start dates were “were incorrect for the 2.5 ppb and 250 ppb TCE groups and their concurrent controls” and that the “start dates for these three groups occurred in 1994, not 1995.” However, the 2014 Erratum makes no such correction statement regarding the start times listed for the 1,100 ppm and 1.5 ppm TCE groups and claimed concurrent controls described in Table 1 of the 2005 Erratum. The latest start times were March 12, 1990 for the 1,100 ppm TCE group and December 26, 1990 for the 1.5 ppm group. In contrast the latest start times for the supposed “concurrent” controls were listed as October 10, 1992 for the 1,100 ppm TCE treatment group (meaning at least some of the 15 control “mothers” were started well after their supposed “concurrent” TCE treatment group). Even more problematic is that all the supposed “concurrent” controls for the 1.5 ppm group were started after December 11, 1992 even though the start dates for the concurrent TCE treatment groups all started at the latest on December 26, 1990. Taken at face value as described in the Table 1 of the 2005 Erratum, it is impossible for the 1.5 ppm group to have had a concurrent control, and highly unlikely for the 1,100 ppm group as well.¹⁶

In trying to resolve the issue of concurrent controls, HSIA has also discovered some troubling issues with the 2005 Erratum. Although EPA focused on Johnson *et al.* (2003) to draw conclusions about the ability of TCE to cause cardiac defects in rats, it is acknowledged that results from that paper for the two higher exposure doses (*i.e.*, 1,100 and 1.5 ppm) were actually

¹⁵ The 2014 Erratum refers to the June 6 to June 13, 1995 period as “exposure start dates,” while the 2005 Erratum calls these “dates of exposure” in the title of Table 1 and the text describes the dates as the “date ranges of experimental treatment,” also implying that this is the time period in which the animals were treated (obviously impossible as stated in the comment). The fault for the confusion is entirely that of Johnson *et al.*, who cannot seem to provide an accurate description of the experiment.

¹⁶ In addition, the earliest start dates of the controls for the 2.5 and 250 ppb treatment groups are 2-3 weeks ahead of the earliest start dates for the treatment groups, indicating that, even at best, the concurrent controls could not have been started simultaneously.

first published by Dawson *et al.* (1993). A comparison of information provided in that paper against information provided in the 2005 Erratum yields some further inconsistencies that require clarification. The Dawson *et al.* (1993) manuscript was received by the Journal of the American College of Cardiology on January 7, 1992, with a revised manuscript received on October 13, 1992. In the published paper (Table 2), the authors describe a single control group (*i.e.*, Group VII) comprising 238 fetuses (although the number of litters is not provided). An examination of the control groups in Table 1 of the 2005 Erratum reveals that there is only one group (*i.e.*, the group with the dates of June 14, 1989 to October 10, 1992)¹⁷ that could have been run concurrently with the 1,100 and 1.5 ppm treatment groups.¹⁸ Setting aside for the moment the inconsistency between the manuscript submission date (*i.e.*, January 7) and the October 10 closing date reported for the control group, an even greater inconsistency is the actual number of fetuses in that control group. Dawson *et al.* (1993) report a control group comprising 238 fetuses, whereas, Table 1 in the 2005 Erratum reports a control group comprising 135 fetuses.¹⁹ As described above, given the pertinent dates, this is the only possible concurrent control group for the 1,100 and 1.5 ppm exposure groups and the authors appear to be confused about its size.

HSIA agrees with the EPA Update that there are “study design and reporting issues” associated with Johnson *et al.* (2003) and Dawson *et al.* (1993), but we fail to see how the 2014 Erratum has done anything to adequately address those issues. The 2014 Erratum provides little of substance other than attempting to clarify an apparent reporting error in exposure start dates, while stating that “the exact dates can no longer be confirmed.” Moreover, the assertion that “all the animal exposure experiments were run with concurrent controls” does not appear to be supported by the facts.

Table 1 of the Update states that “[a]n EPA review of the available control data did not observe unusual heterogeneity in prevalence of malformations.” Examination of the control data in the enclosure (HERO ID 783484) reveals abnormality data for 55 litters and, as shown, abnormalities occurred in nine litters. However, as no dates are provided for any of the

¹⁷ As noted above, the controls with these listed start dates could not have been entirely run concurrently with the 1,100 and 1.5 ppm groups in that the last start date for either TCE group is listed as December 26, 1990, almost 2 years *before* the last start date for the controls. And *none* of the start dates for the 1.5 ppm group match up with the start dates for their supposed concurrent controls. The only way out of this confusing conundrum for Johnson *et al.* would be for them to admit (which they have not done, even in the 2014 Erratum) that the control and treated data provided on the same lines in Table 1 are not meant to infer the groups were actually associated – a very unconventional way of constructing a data table. And, even if the list of start dates for controls is not intended to be matched to the treatment groups shown on the same line, the 1.5 ppm group still has essentially no time overlap with *any* of the control group start times listed in Table 1 of the 2005 Erratum.

¹⁸ If one of the control groups had a start date of October 10, 1992, it would have been impossible to include evaluations from that group in a revision submitted on October 13, 1992 (no one can do a teratology evaluation that fast), not to mention the controls would have been held for the entire 21 days of pregnancy, putting the sacrifice of this group well beyond the October 13 date.

¹⁹ 238 fetuses were reported as controls for both 1.5 and 1,100 TCE doses. However, Table 1 of the 2005 Erratum lists $135 + 155 = 290$ control fetuses total for both groups (and possibly another 62 which are shown on the next line down in Table 1). So, the 2005 Errata serves further to illustrate the very substantial confusion on control animal numbers and associated start times.

individual control litters, there is no way of directly determining whether or not there was “unusual heterogeneity in prevalence of malformations.” From indirect evidence, however, it appears that there was indeed unusual heterogeneity. Table 3 from Dawson *et al.* (1993) indicates that there were seven abnormal hearts in the single control group associated with that study (*i.e.*, Group VII). Individual litter data from the HERO enclosure indicate that there were only 13 abnormal hearts in the entire control group comprising 606 fetuses and 55 litters. With that knowledge and the lack of dates for individual litters, HSIA does not understand how EPA concluded that there was no “unusual heterogeneity” associated with the pooled controls.

As discussed previously, it appears that the ‘concurrent’ control group for the 1,100 and 250 ppm TCE treatment groups had to be the control group described in Dawson *et al.* (1993). That study comprised six TCE-treated groups, four vinylidene chloride-treated groups, and a single control group (*i.e.*, Group VII). Although Dawson *et al.* (1993) do not provide information on the number of litters in each of the eleven treatment and control groups, the 2005 Erratum indicates that there were 15 litters in the control group and a total of 22 litters in the 1,100 and 250 ppm TCE groups treated only during pregnancy. If these three groups (out of 11 total treatment and control groups) comprised 37 litters, HSIA must question the assertion in the 2014 Erratum that rats “were ordered based on a 40-animal maximum capacity.” If that assertion is correct, there is also no way that concurrent controls were run by Dawson *et al.* (1993), despite the authors’ assertion.

The 2005 Erratum also makes clear that control groups were included in Johnson *et al.* (2003) for which there were no concurrent treatment groups. Conducting a statistical analysis of the dose-response relationship between TCE exposure and cardiac defects using a control group inflated in size through the inclusion of controls from other studies has been criticized. It remains a critical flaw both in Johnson *et al.* (2003) and in EPA’s continuing assertion that a reliable dose-response relationship exists.

HSIA agrees with the EPA Update’s understated comment that “some questions on that study [referring to Johnson *et al.* (2003)] remain unresolved.” Although most of the unresolved issues have been raised before, HSIA was unaware from reviewing the literature that the animals were “group housed” as stated in the Update. This certainly adds an additional complication to any interpretation of Dawson *et al.* (1993) and Johnson *et al.* (2003). As noted by EPA and others, there was already uncertainty associated with reported details of the TCE exposure conditions. The three groups reported in Dawson *et al.* (1993) (*i.e.*, the 1,100 and 250 ppm TCE treated groups and the concurrent control group) were exposed either to tap water, or TCE dissolved in tap water, with no chemical analysis to verify the average daily TCE concentration in the consumed water. For the two remaining TCE treatment groups and presumably for some/all of the composited control groups reported in Johnson *et al.* (2003), distilled water was used as the exposure vehicle. The use of different exposure vehicles within the same study is another experimental variable questioning the results of Johnson *et al.* (2003).

The disclosure that animals were group housed is also very troubling given EPA’s assertion of a dose-response relationship between TCE exposure and the occurrence of cardiac defects. First of all, it is not clear how many animals were housed together. Johnson *et al.* (2003) indicates that animals were housed in groups of three to four, however, once pregnant,

“the rats were randomly placed in test groups.” If, in fact, the pregnant animals were grouped, as acknowledged above by EPA, there is no way to recreate an individual TCE exposure dose based on the total amount of drinking water consumed by the group without any knowledge of water consumption rates by each individual animal within that group. Grouping of the pregnant animals would also preclude a comparison of daily water intake between control and treated rats, an important component in any dose-response analysis.

The most likely reason for the positive results reported by the Arizona researchers is that the statistics were performed differently from traditional developmental studies. Original statistics were performed on a per-fetus basis, rather than on a per-litter basis, despite the fact that per-litter analysis is the accepted method for developmental effects related to chemical exposure during pregnancy, as recommended by the EPA Office of Research and Development. Statistics should be conducted on a per-litter basis because, during gestation, the dam is the unit of treatment and exposure of the pups is dependent on her. Performing statistics on a per-fetus basis artificially inflates the significance of the findings. Had the correct statistical unit been used in these studies, a positive correlation between TCE and fetal heart malformations probably would not have been reported in the original drinking water studies.

In the later studies, the Arizona investigators re-published data from the original studies, using pooled controls from all of their studies in their statistical evaluation. Pooling of controls is not an appropriate statistical practice and is likely to have exaggerated the alleged statistical significance.²⁰ While the investigators report a significant increase in fetal heart malformations on a per-litter basis at 250 ppb TCE, there is no reported effect at 1.5 ppm, suggesting a lack of a dose-response effect. Curiously, the Arizona researchers present a dose-response curve, based on a probit analysis, at concentrations up to 4,870 ppm.²¹ The concentration of 4,870 ppm is well above water solubility for TCE, however, and the authors fail to explain how they could generate a curve using concentrations for which no data exist.

IV. Conclusion

In conclusion, HSIA has demonstrated that there are such serious quality issues associated with Johnson *et al.* (2003) that the study should not have been used by EPA as a principal study for drawing conclusions about the ability of TCE to cause cardiac defects, much less for deriving toxicological values. The RfC/RfD for TCE should be withdrawn and established without reference to the unreproducible dose-response reported by Johnson *et al.* (2003).

²⁰ Hardin, B, *et al.*, *Repro. Toxicol.* 21:117–147 (2006).

²¹ Johnson *et al.* (2003), Figure 3. The authors indicate that the data in Figure 3 were extrapolated data, not actual data from experiments, *e.g.*, probit projections of 50% and higher response rates against TCE water concentrations. However, the authors do not note in their discussion of Figure 3 that such concentrations are well above TCE water solubility limits, so Figure 3 should have terminated at 1,100 ppm (*i.e.*, projected responses of TCE in drinking water are above limits of solubility and thus are impossible based on immutable physicochemical properties of TCE).

Respectfully submitted,

A handwritten signature in dark ink that reads "Faye Graul / wcd". The signature is written in a cursive, flowing style.

Faye Graul
Executive Director

Enclosure

Maternal Statistics / Treatment Group

| | | | | | | | | | |
|--|--|-----|-------|------|------|------|-------|--------|--|
| KEY: | S#,M# Gp# = code for blind treatments | | | | | | | | |
| | TWtGn = Total Weight gain during pregnancy | | | | | | | | |
| | #Fet = number of fetuses in the litter | | | | | | | | |
| | #Res = number of resorption sites (no fetus) in the uterine horn | | | | | | | | |
| | #Imp = number of implantation sites (no fetus) in the uterine horn | | | | | | | | |
| | N Hrt = number of fetuses with Normal Hearts | | | | | | | | |
| | AbnHrt = number of fetuses with Abnormal Hearts | | | | | | | | |
| | Abnormalities = the type of cardiac defect seen | | | | | | | | |
| 1100 ppm Trichloroethylene - Pregnancy Only Drinking water | | | | | | | | | |
| S# | M# | Gp# | TWtGn | #Fet | #Res | #Imp | N Hrt | AbnHrt | Abnormalities |
| 6 | 73 | 16 | 121 | 16 | 0 | 1 | 16 | 0 | |
| 6 | 74 | 16 | 106 | 8 | 2 | 0 | 7 | 1 | 1-ASD, VSD |
| 6 | 75 | 16 | 139 | 14 | 0 | 0 | 13 | 1 | 1-ASD |
| 6 | 76 | 16 | 158 | 20 | 1 | 0 | 17 | 3 | 1-A-V canal(VSD); 2-ASD; 3-ASD |
| 6 | 77 | 16 | 120 | 10 | 2 | 1 | 10 | 0 | |
| 6 | 78 | 16 | 122 | 15 | 0 | 0 | 13 | 2 | 1-ASD; 2-AorticV-unicuspid, VSD |
| 6 | 80 | 16 | 94 | 7 | 1 | 0 | 6 | 1 | 1-AorticV-fenestrated ant. leaflet |
| 6 | 81 | 16 | 94 | 3 | 0 | 0 | 3 | 0 | |
| 6 | 82 | 16 | 134 | 12 | 5 | 0 | 9 | 3 | 1-ASD, VSD; 2-VSD-two muscular; 3-ASD |
| Stats | | 9 | 121 | 105 | 11 | 2 | 94 | 11 | |
| 1.5 ppm Trichloroethylene - Pregnancy only Drinking water | | | | | | | | | |
| S# | M# | Gp# | TWtGn | #Fet | #Res | #Imp | N Hrt | AbnHrt | Abnormalities |
| 6 | 85 | 17 | 96 | 4 | 0 | 0 | 4 | 0 | |
| 6 | 86 | 17 | 154 | 14 | 1 | 0 | 14 | 0 | |
| 6 | 88 | 17 | 109 | 13 | 1 | 0 | 13 | 0 | |
| 6 | 89 | 17 | 143 | 14 | 0 | 0 | 14 | 0 | |
| 6 | 90 | 17 | 127 | 16 | 0 | 0 | 16 | 0 | |
| 6 | 92 | 17 | 158 | 15 | 0 | 0 | 13 | 2 | 1-L transposition; 2-VSD-subaortic |
| 6 | 93 | 17 | 127 | 16 | 0 | 0 | 15 | 1 | 1-Absent RPA,PA and AO parallel,small PA |
| 6 | 94 | 17 | 136 | 14 | 0 | 0 | 14 | 0 | |
| 6 | 96 | 17 | 183 | 17 | 0 | 0 | 14 | 3 | 1-ASD; 2-ASD; 3-VSD |
| 6 | 97 | 17 | 116 | 11 | 1 | 0 | 9 | 2 | 1-VSD-apical and muscular; 2-ASD |
| 9 | 4 | 17 | 135 | 13 | 3 | 0 | 13 | 0 | |
| 9 | 11 | 17 | 134 | 17 | 0 | 0 | 17 | 0 | |
| 9 | 33 | 17 | 142 | 17 | 0 | 0 | 16 | 1 | 1-ASD |
| Stats | | 13 | 135 | 181 | 6 | 0 | 172 | 9 | |

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| 250 ppb Trichloroethylene - Drinking water, Pregnancy Only | | | | | | | | | |
|--|----|-----|--------|------|------|------|-------|--------|--|
| S# | M# | Gp# | TWtGn | #Fet | #Res | #Imp | N Hrt | AbnHrt | Abnormalities |
| 20 | 1 | 53 | 124 | 15 | 1 | 0 | 14 | 1 | 1-TriV -small (R8 and R9 twinned-N Hrts) |
| 20 | 5 | 53 | 128 | 10 | 1 | 0 | 10 | 0 | |
| 20 | 6 | 53 | 139 | 12 | 1 | 0 | 11 | 1 | 1-Huge Coronary Sinus |
| 20 | 7 | 53 | 125 | 13 | 1 | 0 | 11 | 2 | 1-AoV-dysplastic, no coronary; 2-Tri/MitrV-abn |
| 20 | 8 | 53 | 155 | 17 | 0 | 0 | 17 | 0 | |
| 20 | 11 | 53 | 166 | 14 | 6 | 0 | 14 | 0 | |
| 20 | 12 | 53 | 167 | 16 | 0 | 0 | 16 | 0 | |
| 20 | 13 | 53 | 61 | 1 | 0 | 0 | 1 | 0 | |
| 20 | 23 | 53 | | | | | | | False pregnancy |
| 20 | 19 | 53 | 116 | 12 | 1 | 0 | 11 | 1 | 1-ASD |
| Stats | | 10 | 131.22 | 110 | 11 | 0 | 105 | 5 | |
| 2.5 ppb TCE in Pregnancy Only Drinking Water | | | | | | | | | |
| S# | M# | Gp# | TWtGn | #Fet | #Res | #Imp | N Hrt | AbnHrt | Abnormalities |
| 22 | 8 | 52 | 127 | 11 | 0 | 0 | 11 | 0 | |
| 22 | 10 | 52 | 140 | 13 | 2 | 0 | 13 | 0 | |
| 22 | 12 | 52 | 137 | 13 | 1 | 0 | 13 | 0 | |
| 22 | 13 | 52 | 115 | 10 | 2 | 0 | 10 | 0 | |
| 22 | 14 | 52 | 125 | 14 | 0 | 0 | 14 | 0 | |
| 22 | 15 | 52 | 134 | 13 | 1 | 0 | 13 | 0 | |
| 22 | 17 | 52 | 120 | 14 | 1 | 0 | 14 | 0 | |
| 22 | 20 | 52 | 108 | 12 | 1 | 0 | 12 | 0 | |
| 22 | 21 | 52 | 107 | 7 | 0 | 0 | 7 | 0 | |
| 22 | 40 | 52 | 127 | 13 | 0 | 0 | 13 | 0 | |
| 22 | 43 | 52 | 118 | 12 | 2 | 0 | 12 | 0 | |
| 22 | 45 | 52 | 128 | 12 | 0 | 0 | 12 | 0 | |
| Stats | | 12 | 123.83 | 144 | 10 | 0 | 144 | 0 | |

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| Control | | | | | | | |
|---------|-----|-----|------|-------|--------|--|--|
| S# | M# | Gp# | #Fet | N Hrt | AbnHrt | Abnormalities | |
| 4 | 4 | 0.2 | 14 | 11 | 3 | 1- VSD, ECD-vent.attachmts, 2- D-loop in Rt Chest, 3- D-loop in Rt Chest | |
| 4 | 24 | 0.2 | 12 | 11 | 1 | 1- VSD | |
| 9 | 23 | 0.2 | 3 | 2 | 1 | 1- VSD-hi subAO | |
| 9 | 26 | 0.2 | 12 | 11 | 1 | 1- ASD | |
| 9 | 28 | 0.2 | 9 | 8 | 1 | 1- VSD lg muscular | |
| 17 | 16 | 0.2 | 13 | 12 | 1 | 1-ASD, Mitral V-leaflet atresia | |
| 19 | 55 | 0.2 | 17 | 15 | 2 | 1-ASD, 2-ASD | |
| 20 | 15 | 0.2 | 14 | 12 | 2 | 1-ASD, 2-ASD, large | |
| 23 | 16 | 0.2 | 13 | 12 | 1 | 1-ASD | |
| 4 | 1 | 0.2 | 11 | 11 | 0 | | |
| 4 | 10 | 0.2 | 10 | 10 | 0 | | |
| 4 | 12 | 0.2 | 15 | 15 | 0 | | |
| 4 | 14 | 0.2 | 11 | 11 | 0 | | |
| 4 | 22 | 0.2 | 19 | 19 | 0 | | |
| 5 | 5 | 0.2 | 11 | 11 | 0 | | |
| 5 | 15 | 0.2 | 11 | 11 | 0 | | |
| 5 | 21 | 0.2 | 12 | 12 | 0 | | |
| 6 | 84 | 0.2 | 4 | 4 | 0 | | |
| 6 | 109 | 0.2 | 12 | 12 | 0 | | |
| 9 | 2 | 0.2 | 5 | 5 | 0 | | |
| 9 | 3 | 0.2 | 14 | 14 | 0 | | |
| 9 | 5 | 0.2 | 14 | 14 | 0 | | |
| 9 | 12 | 0.2 | 13 | 13 | 0 | | |
| 9 | 27 | 0.2 | 17 | 17 | 0 | | |
| 12 | 8 | 0.2 | 15 | 15 | 0 | | |
| 17 | 8 | 0.2 | 16 | 16 | 0 | | |
| 17 | 19 | 0.2 | 9 | 9 | 0 | | |
| 17 | 24 | 0.2 | 12 | 12 | 0 | | |
| 19 | 10 | 0.2 | 8 | 8 | 0 | | |
| 19 | 25 | 0.2 | 7 | 7 | 0 | | |
| 19 | 35 | 0.2 | 5 | 5 | 0 | | |
| 19 | 43 | 0.2 | 9 | 9 | 0 | | |
| 19 | 57 | 0.2 | 5 | 5 | 0 | | |
| 19 | 59 | 0.2 | 12 | 12 | 0 | | |
| 20 | 18 | 0.2 | 4 | 4 | 0 | | |
| 20 | 20 | 0.2 | 8 | 8 | 0 | | |
| 20 | 22 | 0.2 | 10 | 10 | 0 | | |
| 21 | 11 | 0.2 | 13 | 13 | 0 | | |

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| | | | | | |
|--|----|-----|---|-----|----|
| 22 | 1 | 0.2 | 12 | 12 | 0 |
| 22 | 6 | 0.2 | 9 | 9 | 0 |
| 22 | 9 | 0.2 | 12 | 12 | 0 |
| 22 | 18 | 0.2 | 13 | 13 | 0 |
| 22 | 31 | 0.2 | 11 | 11 | 0 |
| 22 | 44 | 0.2 | 12 | 12 | 0 |
| 23 | 1 | 0.2 | 11 | 11 | 0 |
| 23 | 3 | 0.2 | 12 | 12 | 0 |
| 23 | 6 | 0.2 | 16 | 16 | 0 |
| 23 | 19 | 0.2 | 15 | 15 | 0 |
| 23 | 21 | 0.2 | 2 | 2 | 0 |
| 23 | 23 | 0.2 | 12 | 12 | 0 |
| 23 | 30 | 0.2 | 1 | 1 | 0 |
| 23 | 31 | 0.2 | 12 | 12 | 0 |
| 23 | 33 | 0.2 | 14 | 14 | 0 |
| 23 | 35 | 0.2 | 10 | 10 | 0 |
| 23 | 36 | 0.2 | 13 | 13 | 0 |
| 55 | | | 606 | 593 | 13 |
| 55 litters total | | | 606 Fetuses total | | |
| | | | 9 litters with fetuses with abnormal hearts out of the 55 total litters | | |
| | | | 13 Fetuses with abnormal hearts in 9 Litters | | |
| | | | 593 Normal Fetuses in 46 Litters | | |
| Control Summary (No statistical sign between groups over time, so controls were thus combined) | | | | | |
| 606 fetuses /55 maternal rats total | | | | | |
| 9 litters with fetuses with abnormal hearts out of the 55 total litters | | | | | |
| 13/606 fetuses with abnormal hearts | | | | | |