May 9, 2013

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
P.O. Box 4010, MS-19B
Sacramento, California 95812-4010

Re: Request for Relevant Information on a Chemical Being Considered for Listing by the Authoritative Bodies Mechanism: Trichloroethylene (TCE)

Dear Ms. Oshita:

On behalf of the Halogenated Solvents Industry Alliance, Inc. (HSIA), I write to provide information that is directly relevant to the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) as it considers whether to list TCE as a reproductive toxicant under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). HSIA represents producers and users of TCE, an important solvent used in vapor degreasing for precision cleaning of electronics, medical devices, aerospace equipment, and other substrates.

The request for information states:

“A chemical must be listed under Proposition 65 and its implementing regulations when two conditions are met:

1. An authoritative body formally identifies the chemical as causing reproductive toxicity (Section 25306(d)).

2. The evidence considered by the authoritative body meets the sufficiency criteria contained in the regulations (Section 25306(g)).”

In the case of TCE, neither of these conditions has been met.

First, TCE has not been “formally identified” by the US Environmental Protection Agency (EPA), in either its 2011 Toxicological Review or otherwise, as a reproductive or developmental toxin. “In spite of the preponderance of studies demonstrating effects on sperm parameters, there is an absence of overwhelming evidence in the database of adverse effects of
TCE on overall fertility in the rodent studies." 1 "In summary, an overall review of the weight of evidence in humans and experimental animals is suggestive of the potential for developmental toxicity with TCE exposure." 2 Such conclusions do not rise to the level of a "formal identification" by an authoritative body for purposes of Proposition 65. See American Chemistry Council v Office of Environmental Health Hazard Assessment, et al., Sacramento County No. 34-2013-00140720 (April 19, 2013).

Moreover, it is clear that the evidence of developmental toxicity considered by the authoritative body (EPA) is not sufficient, as a primary study relied upon by EPA has been rejected by OEHHA as deficient:

"Johnson et al. (2003) reported a dose-related increased incidence of abnormal hearts in offspring of Sprague Dawley rats treated during pregnancy with 0, 2.5 ppb, 250 ppb, 1.5 ppm, and 1,100 ppm TCE in drinking water (0, 0.00045, 0.048, 0.218, and 128.52 mg/kg-day, respectively). The NOAEL for the Johnson study was reported to be 2.5 ppb (0.00045 mg/kg-day) in this short exposure (22 days) study. The percentage of abnormal hearts in the control group was 2.2 percent, and in the treated groups was 0 percent (low dose), 4.5 percent (mid dose 1), 5.0 percent (mid dose 2), and 10.5 percent (high dose). The number of litters with fetuses with abnormal hearts was 16.4 percent, 0 percent, 44 percent, 38 percent, and 67 percent for the control, low, mid 1, mid 2, and high dose, respectively. The reported NOAEL is separated by 100-fold from the next higher dose level. The data for this study were not used to calculate a public-health protective concentration since a meaningful or interpretable dose-response relationship was not observed. These results are also not consistent with earlier developmental and reproductive toxicological studies done outside this lab in mice, rats, and rabbits: The other studies did not find adverse effects on fertility or embryonic development, aside from those associated with maternal toxicity (Hardin et al., 2004)." 3

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. 4 In the

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1 EPA TCE Toxicological Review (2011), at 4-490.
2 Id., at 4-556.
3 California EPA Public Health Goal for Trichloroethylene in Drinking Water (July 2009), at 21 (emphasis added).
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. The enclosed comment by Kimmel, Kimmel, and DeSesso, submitted to OEHHA on October 31, 2012 in connection with its announcement of an update to the Public Health Goal, addresses these and other concerns in greater detail.

Johnson et al. has been heavily criticized in the published literature, and the Arizona studies were also expressly rejected as the basis for minimal risk levels (MRLs) by the Agency for Toxic Substances & Disease Registry (ATSDR). 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. No increase in cardiac malformations was observed in a guideline, GLP-quality study, 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


6 ATSDR concluded that “[t]he study is limited in that only two widely spaced exposure concentrations were used and that a significant dose-response was not observed for several exposure scenarios.” Toxicological Profile for Trichloroethylene Update (September 1997), at 88. More recently, however, following publication by EPA in 2010 of its TCE IRIS Assessment, ATSDR issued an Addendum that bases both chronic and intermediate-duration MRLs on the EPA RfD/RfC values (0.0005 mg/kg/day /0.0004 ppm (2 ug/m³)), which in turn are based in part on Johnson et al. Addendum to Toxicological Profile for Trichloroethylene (January 2013).


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. For this reason, HSIA has proposed to ATSDR to conduct yet another developmental toxicity study, under a protocol to be established by an expert panel with input from ATSDR and EPA, to definitively confirm or reject the findings of Johnson et al.

In sum, OEHHA had very good reason to reject any reliance on Johnson et al. when the Public Health Goal for TCE was adopted. There has been absolutely no scientific evidence since 2009 to cast any doubt on OEHHA’s conclusion. We urge OEHHA to consider very carefully the scientific evidence before changing its position simply to conform to a subsequent controversial EPA review, particularly in light of the study HSIA has proposed to provide more robust information on the developmental toxicity question.

Please do not hesitate to let us know if we can provide any additional information on this important subject.

Respectfully submitted,

Faye Graul
Executive Director

Enclosure

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9 “Johnson and Dawson, with their collaborators, are alone in reporting that TCE is a ‘specific’ cardiac teratogen.” Hardin, B, et al., Trichloroethylene and cardiac malformations, Environ. Health Perspect. 112: A607-8 (2004).
Comments on the Public Review Draft of EPA’s IRIS
Toxicological Review for TCE: Developmental Effects

Carole A. Kimmel, PhD
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27 January 2010
INTRODUCTION

EPA’s assessment of TCE uses data on heart defects as a major endpoint for setting the RfD and RfC. The data on which this decision is based are weak, incomplete and flawed and do not account for more robust data that shows no increase in congenital heart defects.

- The human data are based on studies with inadequate exposure information, making it impossible to determine whether or not exposure occurred, and if it did, to what levels of TCE.
  - There are deficiencies in the human data in terms of the background rates of cardiac malformations (Bove et al, 1995), and differences in the outcome of different studies (Goldberg et al, 1990, versus the Baltimore Washington Infant Study – Wilson et al, 1998).
- The animal data reporting a link between TCE and heart defects all come from the same laboratory and were an accumulation of data over ten years (Johnson et al 2003, Dawson et al 1993). Two additional GLP- and guideline-compliant studies showing no effect on heart development were conducted by Fisher et al. (2001) and Carney et al. (2006).
  - In the Johnson and Dawson studies, there were a number of deficiencies in study design and reporting of data that make the interpretation of data tentative at best.
  - The major effect reported in the Johnson/Dawson studies was an increase in the incidence of atrial septal defects (or the foramen ovale, which closes around the time of birth) which may be related to the procedure for examining fetuses or possible delays in development, rather than actual heart defects.
- EPA uses weak human data, incomplete and flawed animal data, and in vitro/in ovo data (which are of questionable relevance to environmental exposures) to make a mechanistic argument that TCE causes heart defects.
- Although EPA notes some of the database deficiencies, they use a “strength of evidence” approach rather than a “weight of evidence” analysis by basing the RfD only on the studies reporting a positive effect and ignoring the data from subsequent well-conducted GLP studies that show no increase in heart defects associated with TCE (Fisher et al, 2001; Carney et al, 2006).

EPA Evaluation of Animal Data on Heart Defects and Comments

The EPA review of TCE (US EPA, 2009) uses the Johnson et al. (2003) and Dawson et al. (1993) data to establish reference levels for exposure - an RfC of 0.001 ppm and an RfD of 0.0004 mg/kg/day. The fetal heart malformation data reported in Johnson et al. (2003) are used to support both of these values (US EPA, 2009; see Tables 5.1.23 and 5.1.24 and the associated text). There are several limitations with this approach:

  - This was not made clear in their paper, and it required a letter to the editor (Hardin et al., 2004) to have the authors explain this situation (Johnson et al., 2004). This gives the appearance that the authors were unaware of how to design studies, or how to analyze and present developmental toxicity data.
  - There is no indication in the summary paper (Johnson et al., 2003) of which data came from Dawson et al. (1993) and which data came from later studies.
  - Dawson et al. (1993) do not mention the number of pregnant dams that were assigned to each treatment group.
The usefulness of such studies is questionable in anything more than a support role in a database of other, more well-designed studies. A study such as Johnson et al (2003) should not be used as the critical study for establishing regulatory exposure levels.

The Johnson et al. (2003) and Dawson et al. (1993) studies have significant limitations regarding the reporting of standard maternal and fetal parameters.

- Johnson et al. (2003) do not provide data on maternal and fetal parameters other than cardiac malformations, only mentioning that “maternal and fetal variables, including noncardiac congenital abnormalities, showed no significant differences between treated and control groups.”
- Dawson et al. (1993) did not provide any control data for maternal and fetal parameters, other than cardiac abnormalities. Consequently, there is no way to assess the impact of exposure on any parameter other than cardiac abnormalities, including such parameters as maternal body weight and body weight gain, fetal weight, and fetal viability.
- Johnson et al. (2004) note that “Control values were consistent throughout our studies.” However, there is no way for the reader to determine this.
- Without evaluating all of the maternal and fetal parameters, it is not possible to get a clear idea of how the animals are responding to treatment and whether the endpoint values are within historical ranges.

Studies where major components of the results are not reported or the missing data have not been evaluated by the risk assessors may be useful in supporting other, more complete, data sets, but are of questionable value as a primary study in establishing an exposure standard.

Johnson et al. (2003) indicate that their goal was to determine whether there was a threshold level of TCE in drinking water above which the incidence of congenital cardiac defects in the rodent increased significantly. Does their study design and statistical analysis permit the testing of a hypothesis derived from this goal?

- Their study pools discrete data from at least two separate studies and is an unbalanced design (55 dams in the control vs. 9-13 in the treatment groups).
- They report that their data could indicate that a threshold effect exists at a level between 1.5 and 1,100 ppm.

It would be prudent to have a qualified statistician look at this database and the statistical evaluations used to determine if the analysis can test/address the hypothesis.

The reported “threshold effect” has a range of three orders of magnitude; not very useful in establishing reference levels.

In discussing the dose-response pattern in these studies, Johnson et al. (2003) specifically comment on the response of the highest exposure (1,100,000 ppb) relative to control, but they only mention that “Intermediate exposure levels produced intermediate response rates.”

- While this is true, the intermediate levels did not produce a clear dose-response relationship. The 2.5 ppb exposure level did not show any effects, even though 16.4% of the control litters had a cardiac defect.
- Moreover, there was a reduced (or at most an equivalent) response between 250 ppb and 1500 ppb.

Although Johnson et al. (2003) provide a rationale for choosing the exposure levels that were used, the extreme range makes it difficult to examine whether a continuous response pattern exists.
To make the analysis more difficult to interpret independently, the fetus and not the dam (litter) was used as the experimental unit. EPA has noted that Johnson “has provided individual litter incidence data to the USEPA for independent statistical analysis (P. Johnson, personal communication, 2008) (see Section 6, dose-response)” (US EPA, 2009, p 857). It is unclear why EPA refers to “Section 6, dose-response” regarding this additional data, since it does not appear that anything in this section/sub-section details these data or how they were used. Hopefully, EPA has examined these data, although it is unclear if this has ever been done or how it has been incorporated into EPA’s risk assessment.

The dose-response pattern is another area where the input of a qualified statistician/modeler would be prudent.

Johnson et al. (2003) comment that TCE exposure using an in vitro chick model has been shown to have effects on several elements of epithelial–mesenchymal cell transformation at concentration ranges that correlate with their findings.

- They note a concentration range of 50-250 ppm (although it isn’t clear if this is the only concentration range used in the referenced studies). If the 50-250 ppm is correct, it does not correlate with the Johnson et al. (2003) concentration range. It is bounded by the Johnson et al. concentration range, but then, almost any range would be, given the extreme range that Johnson et al. used.

- More importantly, an application of X ppm in an in vitro chick embryo study is in no way comparable to an application of X ppm in drinking water in an in vivo rat study.

Use of in vitro/in ovo data with questionable relevance to environmental exposures as mechanistic support for heart defects reported in poorly conducted whole animal studies and weak human studies appears to build a strong case for using heart defects as the basis for risk assessment, but compounds the problem of overstating the importance of the data.

Generally, there has been too much focus on one set of studies that show a putative positive response to low-exposure levels of TCE, without considering the overall data base and the limitations of the focus studies.

- This is not a “weight of evidence” evaluation but a “strength of evidence” evaluation (NRC, 1994). All the focus is on those studies that found a compound-related effect and no attention was given to the strengths and weaknesses of those studies that found no compound-related effects. Data from GLP-compliant animal studies that were carefully designed to probe the existence of potential links between TCE or its metabolites and heart or eye defects have shown no associations at exposure levels that are several orders of magnitude higher than those that are environmentally or occupationally relevant.

- Fisher et al. (2001) specifically investigated the cardiac teratogenic potential of TCE, TCA, and DCA in groups of 19 – 20 pregnant Sprague-Dawley rats. The rats received oral bolus doses of TCE (500 mg/kg/day, in soybean oil), TCA (300 mg/kg/day, in water) or DCA (300 mg/kg/day, in water) on gestational days 6 – 15. On gestational day 21, fetuses were removed by laparohysterectomy and hearts were examined and microdissected under a stereomicroscope by an investigator experienced in the procedure (Dr. Paula Johnson, author of the earlier report that TCA caused cardiac effects at 291 mg/kg/day). The rates of cardiac malformations among treated animals did not differ from control rates.
Also, TCE caused no change in the weight of fetuses and did not inhibit maternal weight gain.

- An inhalation study of TCE in pregnant Charles River CD IGS rats (Carney et al., 2001; 2006) exposed groups of 27 animals to filtered air or to atmospheric concentrations of TCE up to and including the limit dose (600 ppm) for 6 hours/day on each of gestational days 6 – 20. Although maternal toxicity (decreased body weight gain) was elicited at the highest dose, TCE exposure caused no increase in gross, skeletal, or visceral (including heart and eye) malformations at any of the concentrations tested.

- Some early studies of TCA and DCA in pregnant Long-Evans rats (Smith et al., 1989, 1992) reported ocular malformations. In a follow-up to the Fisher et al (2001) study, Warren et al. (2006) reported that examination of the heads showed that TCE, TCA, or DCA did not elicit gross ocular malformations. Morphometric analysis of the lens area, globe area and interocular distances revealed reductions of these parameters only in the TCA- and DCA-treated fetuses, but the overall smaller sizes of the fetuses in those groups were sufficient to explain the reductions.

Weight of evidence clearly must consider all of the data, both positive and no effect data. When the majority of the positive data are derived from clearly flawed studies using methods that give results that are not replicable in other laboratories, it is difficult to understand how the Agency can justify using only these data as the basis for a regulatory assessment.

- While there were similar methods used for examining hearts in fetuses in the Dawson/Johnson laboratories and Dr. Johnson collaborated on the Fisher et al (2001) study, there were several differences among the 3 studies as noted in the EPA review (see Table 1 below). In addition, preparation of the heart for dissection also differed. Dawson et al (1993) and Johnson et al (2003) both removed the heart first, then flushed with a fixative, while Fisher et al (2001) flushed the heart in situ via the left ventricle with a staining solution for better visualization (1:3 hematoxylin-saline solution), perhaps a more physiologically normal situation, then removed the heart and immersion fixed it in 10% buffered formalin.


<table>
<thead>
<tr>
<th>Study</th>
<th>Stock of animals</th>
<th>Source of animals</th>
<th>Route of exposure</th>
<th>Dose</th>
<th>Vehicle</th>
<th>Treatment days GD</th>
<th>Day of sperm GD</th>
<th>Day of sacrifice GD</th>
<th>Heart preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dawson et al 1993</td>
<td>Sprague Dawley</td>
<td>Harlan, Indianapolis?</td>
<td>Drinking water</td>
<td>1.5 and 1100 ppm</td>
<td>Tap water</td>
<td>1-22</td>
<td>1?</td>
<td>22?</td>
<td>Flushed with 2% glutaraldehyde after heart removal, fixed for 24 hrs in the same solution, transferred to 0.1 mol/L phosphate buffer</td>
</tr>
<tr>
<td>Johnson et al 2003</td>
<td>Sprague Dawley</td>
<td>Harlan?</td>
<td>Drinking water</td>
<td>2.5 &amp; 250 ppb, 1.5 &amp; 1100 ppm</td>
<td>Distilled water</td>
<td>1-22</td>
<td>1?</td>
<td>22?</td>
<td>Flushed with 10% formalin, transferred to 10% formalin</td>
</tr>
<tr>
<td>Fisher et al 2001</td>
<td>Sprague Dawley</td>
<td>Charles River, Raleigh</td>
<td>Gavage</td>
<td>500 mg/kg</td>
<td>Soybean oil (TCE &amp; RA); IERO*</td>
<td>6-15</td>
<td>0</td>
<td>21</td>
<td>Flushed in situ via the left ventricle with staining solution for better visualization (1:3 hematoxylin-saline)</td>
</tr>
</tbody>
</table>
water (TCA, DCA), then removed and immersion fixed in 10% buffered formalin

- One major difference in the data from the Dawson/Johnson laboratory versus the Fisher laboratory appears to be the incidence of atrial septal defects (Table 2). The types of atrial septal defects reported by Dawson/Johnson et al are not detailed in any of the papers except for the statement that they are “secundum in type” (Dawson et al, 1993).
  - Since the septum primum and septum secundum both grow rapidly around the time of birth to close the foramen ovale (Momma et al, 1992), this may represent a variation in development like other structures that are developing around the time of birth in the rat, e.g., skeletal ossification of sternbrae, vertebral centra, etc., or development of the renal papillae.
  - Whether the different methods of flushing the hearts may have disturbed the position of the septum which would not be closed on the day of sacrifice is unclear.
  - Even more disturbing, however, is that neither Dawson et al (1993) nor Johnson et al (2003) provide maternal or fetal weight data, so it is impossible to know whether there were differences in fetal weight that would suggest a delay in development. Also, data on other aspects of fetal development (e.g., skeletal ossification) were not presented to give any clues about developmental stage.
  - Fisher et al (2001) report no significant difference from water controls in maternal weight, uterine weight, number of implantations or fetal weight for TCE at 500 mg/kg. In that study, the percent of fetuses with atrial septal defects was approximately the same in the two groups. Thus, there are a lot of questions about the incompleteness of the data presented in the Dawson et al (1993) and Johnson et al (2003) papers, in addition to the obvious design flaws and protracted length of time over which the studies were conducted. Without concurrent control data, it is very difficult to evaluate small changes in heart development that may or may not be related to TCE exposure.

Table 2. Comparison of Atrial Septal Defects in the Three Papers*

<table>
<thead>
<tr>
<th>Study/Data</th>
<th>Control Tap water</th>
<th>TCE – Prepreg only 1.5 ppm</th>
<th>TCE – Prepreg only 1100 ppm</th>
<th>TCE – Preg only 1.5 ppm</th>
<th>TCE – Preg only 1100 ppm</th>
<th>TCE – Preg &amp; Prepreg only 1.5 ppm</th>
<th>TCE – Preg &amp; Prepreg only 1100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dawson et al 1993</td>
<td>No. of atrial septal defects/no hearts examined (%)</td>
<td>1/232 (0.4)</td>
<td>3/130 (2.3)</td>
<td>7/147 (4.8)</td>
<td>4/181 (2.2)</td>
<td>7/105 (6.7)</td>
<td>5/256 (2.0)</td>
</tr>
<tr>
<td>Johnson et al 2003</td>
<td>No. of atrial septal defects/no hearts examined (%)</td>
<td>7/606 (1.2)</td>
<td>0/144 (0)</td>
<td>1/110 (1.0)</td>
<td>4/181 (2.2)</td>
<td>7/105 (6.7)</td>
<td>7/105 (6.7)</td>
</tr>
<tr>
<td>Fisher et al 2001</td>
<td>No. of atrial septal defects/no hearts examined (%)</td>
<td>2/273 (1.0)</td>
<td>2/269 (1.0)</td>
<td>3/298 (1.0)</td>
<td>6/367 (1.6)</td>
<td>4/290 (1.4)</td>
<td>3/155 (1.9)</td>
</tr>
</tbody>
</table>

*Data in the shaded boxes were reported in both the Dawson et al 1993 and the Johnson et al 2003 papers.
**IERO = ion exchange/reverse osmosis
Another difference is in the incidence of ventricular septal defects (VSDs). Johnson et al. (2003) reported an increase in membranous VSDs from 0.33% in controls to 1.7% at 1.5 ppm, and 2.9% at 1,100 ppm, and in muscular VSDs from 0.33% in controls to 0.55% at 1.5 ppm and 0.95% at 1,100 ppm. In the Fisher et al study, there were 2 cases of membranous VSD and one case of muscular VSD in soybean-treated controls (incidence of 0.54% and 0.27%, respectively), but there were no cases of VSD in TCE-treated fetuses.

Thus, there are significant questions about examination of the hearts in the Dawson/Johnson studies, as well as questions about whether effects on the atrial septum (the primary defect reported) are actually a reflection of developmental variation or delays, because the atrial septum is developing around the time of birth. Unfortunately, data on maternal and fetal body weight or other indicators of development (e.g., skeletal ossification) are missing from the reports by Dawson/Johnson.

**EPA Evaluation of Human Data on Heart Defects and Comments**

The existing human data are deficient for risk assessment, but even so they do not support an association between TCE exposure and cardiac defects in human infants.

- A shortcoming that is common to all of the epidemiology studies is the lack of accurate exposure information and poor control of confounding factors. In the instance of the Arizona aquifer, the authors were clear to point out that their data showed “a significant association but not a cause and effect relation between parental exposure to the contaminated water area” and cardiac defects. By this, they meant that the parents of affected children were present in the land area overlying the aquifer during early gestation – but not that they had necessarily drunk or used contaminated water. Thus, it is not clear whether exposure occurred or to how much. With respect to the Baltimore-Washington Infant Study, interviews with parents identified activities and occupations that were likely to have involved organic solvents and degreasing substances. TCE is among the substances that could have been used, but it was not singled out as a causative agent and there is no information on levels of exposure. These data sets fail to clearly identify a specific causative agent and do not quantify exposure levels, making the assessment of risk for a particular chemical (i.e., TCE) poorly founded.

- NRC (2006) cited the findings in Bove et al. (2002), a study that re-analyzed the data presented in the widely disputed Goldberg et al. (1990) study. Goldberg et al. (1990) reported an increased incidence of congenital heart defects (CHD) in Tucson, AZ, but this report was criticized for its data analysis and sampling techniques. Bove et al. (2002) reported that 10-11% of households in Tucson had at least one member that had worked or resided in the TCE contaminated area. In contrast, it was stated that 39.2% of babies born with CHD had at least one parent who had resided or worked in a contaminated area. This was based on interviews of 143 of the 365 CHD cases. Bove et al. (2002) claimed that if it was assumed that the remaining 172 cases had a similar proportion of exposed parents, then the prevalence of CHD in the exposed areas during the first trimester of pregnancy would be about 2.3 times that in the uncontaminated areas. No confidence interval for this was provided. One major problem with this evaluation is that whether the mother and/or father was exposed to the TCE was not considered, and the pathway by which paternal exposure would contribute to an increase in CHD is unclear. Additionally, because socioeconomic status and demographics were not integrated with the geographical distribution of the population, it is possible that a higher proportion of births occurred in the part of town with TCE-contaminated water. In many parts of the county, certain areas of a region are more heavily populated with households with children. The control group here is for the overall Tucson
population and not childbearing families. The absence of an appropriate control group is a potential confounding factor that was not considered. Another issue is that the control incidence of CHDs was stated to be 2.6/1,000 births, which is well below the expected U.S. background CHD rate of 8/1,000 births as reported by the American Heart Association (2005a). Therefore, it appears that the Bove et al. (2002) study suffers from many of the same problems as the original Goldberg et al. (1990) study.

- The NRC (2009) report updated the conclusions of the IOM (2003) report and concluded that "there continues to be inadequate/insufficient evidence" for a link between TCE and congenital malformations in humans.
- As discussed above, the human data cited by the assessment are inadequate for risk assessment and do not support a link between TCE and heart defects.

CONCLUSIONS

- The NRC (2006) report states that ventricular septal defects (VSDs) were the most commonly observed cardiac problems in both animal studies and the epidemiological studies. This observation is provided as support to the idea that TCE can induce heart defects. However, as indicated earlier, the Johnson et al (2003) study reported a much higher incidence of atrial septal defects than VSDs. There are serious questions about whether or not atrial septal defects are actual defects or simply delays in development. In addition, VSDs are the most common heart defect in the human population, making up anywhere from ~14-25% of CHD cases (American Heart Association, 2005b; Hoffman and Kaplan, 2002), regardless of whether or not TCE exposure is involved. TCE reportedly alters endocardial cushion proliferation at low doses when administered in ovo, but whether or not this in turn increases the incidence of CHD is unclear. An increase in cellular proliferation in the cardiac cushion and outflow tract has been noted in Drake et al., 2006a. In this study, 0.2, 4, and 200 nm/egg concentrations of TCE were injected into the yolks of eggs during cardiac cushion formation at Hamburger Hamilton (HH) stages 13, 15, 17, and 20. At the 4 nm/egg concentration and higher, an increase in cardiac cushion proliferation was observed in parallel with alterations in cardiac blood flow patterns. However, the same authors also noted in a later paper that this same increase in cellular proliferation was observed when TCE was administered at HH 18, 21, and 23, but this latter experiment the increased proliferation was not linked to any kind of functional cardiac alterations, illustrating that the two observations are not necessarily linked (Drake et al., 2006b).

REFERENCES


Hardin BD, Kelman BJ, Brent RL. Trichloroethylene and cardiac malformations, a correspondence in *Environ Health Perspect.* 2004; 112:A607-8.


