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Comments on the EPA Toxicological Review of TCE: Developmental Effects

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The evidence used by the EPA to support the claim that TCE and/or its metabolites are specific cardiac teratogens comes from both human epidemiology investigations and studies in experimental animals. In both cases, the arguments are not persuasive of a causal linkage. Not only does the cited epidemiology literature fail to satisfy the Hill criteria for causation, it also fails to link the purported findings to TCE exposure. In addition, the EPA assessment is incomplete insofar as it does not consider the most recent and comprehensive animal studies (see below) regarding the effects of TCE and its major metabolites in pregnant mammals. The literature cited to support the contention that TCE and/or one of its metabolites is a cardiac teratogen is over interpreted.

The Cited Human Data Do Not Support a Causal Association between TCE Exposure and Birth Defects

The studies that allegedly support the contention that TCE exposure of pregnant women resulted in births of infants with cardiac or other developmental defects find significance by combining many types of malformations into large categories that sound related, but are not. For example, the category of "cardiac malformations" is used loosely to include alterations in the structure of the heart as well as alterations in the arrangement of the large blood vessels. Not only are the subject organs different, but also they have very dissimilar embryological development.

The human epidemiology studies used to support this contention are of two general types. The first includes investigations of births from regions that had contaminated water supplies. Goldberg et al. (1990) investigated births in a region of Arizona that had an aquifer contaminated with 6 – 239 ppb TCE. The authors reported the incidence for 33 types of malformation associated with the cardiovascular system, among which were 12 diagnoses that were predominantly malformations of the great vessels, such as transposition of the great vessels, coarctation of the aorta, interrupted aortic arch, and patent ductus arteriosus. A total of 44 non-cardiac malformation cases (18% of the total cases) was reported. What is remarkable about these studies is the low exposure concentrations of TCE. As will be discussed below, those animal studies that did report positive results had lowest observed effect levels (LOELs) that were four to six orders of magnitude higher than the highest reported contamination level in the Arizona aquifer.

The "cardiac anomalies" reported in the contaminated Arizona aquifer studies contrast with the results of other epidemiology studies. For instance, Wilson et al. (1998) examined data from the Baltimore-Washington Infant Study and concluded that "solvents/degreasing agent exposure" (inclusive of TCE and many other substances) accounts for 4.6% of the attributable risk for hypoplastic left heart syndrome, but no attributable risk for anomalies including transposition of the great vessels and coarctation of the aorta. The findings in this study contrast with those of the Goldberg study mentioned above, where the individual malformations that together comprise hypoplastic left heart syndrome (aortic valve stenosis, mitral stenosis, hypoplastic left ventricle) accounted for only 15 cases (6% of the cases reported). Thus, there is poor concordance between these two study populations (i.e., three-fold difference in rate of occurrence and different types of "cardiac anomalies").

A study of births in 75 towns of New Jersey that experienced water supply contamination by a variety of agents, including TCE at an average of 55 ppb, reported significant associations between TCE contamination and "cardiac defects" and neural tube defects (Bove et al., 1995). Once again these categories were very broad and included multiple anomalies with very different modes of formation. It is peculiar that the reference population of >55,000 births in this study was stated to have experienced no birth defects. This is an incredible statement, because the background rate of major malformation in the United States is 1 – 3% (550 – 1650 expected cases in a population of 55,000), and neural tube defects and heart defects are among the most common, having an overall expected incidence of ~100/10,000

(DeSesso et al., 1999; Hoffman and Kaplan, 2002). The authors reported as significant those effects that occurred with an odds ratio of 1.5 or greater, but they used relaxed 90% confidence intervals. In the case of the TCE-exposed population, for instance, the incidence of neural tube defects was reported as 56/81,532 or 6.9/10,000, which is well within the expected number of cases based on the national incidence rate, although it is obviously higher than the "0" seen in the reference population. Notably, hypoplastic left heart syndrome (normal rate of occurrence ~2/10,000 births) was not associated with TCE contamination (see discussion below).

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, exposure was not quantified. 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 (e.g., TCE) unfeasible.

As detailed above, the human data cited by the assessment are inadequate for risk assessment. In the absence of clear-cut human data, strong evidence from animal studies in addition to good mechanistic information can help in the assessment of risk.

Data from Early Animal Studies Have Been Used Without Critical Evaluation

Papers from the EPA laboratories in Cincinnati (Smith et al., 1989, 1992; Epstein et al., 1992) first reported cardiovascular anomalies in fetal rats whose mothers had received doses of the TCE metabolites TCA (up to 1,800 mg/kg/day) or DCA (up to 2,400 mg/kg/day) by gastric intubation during gestational days 6 – 15. The spectrum of cardiac malformations observed in these studies was unique. They included many cases of "levocardia" (displacement of the heart towards the left side of the thorax) and a defect that appears to have been very high in the membranous portion of the interventricular septum (the wall that separates the left and right ventricles and participates in the separation of the aorta from the right ventricle). As will be detailed later, other laboratories have not reproduced these malformations.

The question arises as to the cause of the observations in these first studies. It should be noted that the doses used in these studies were six to seven orders of magnitude higher than the dose expected for a 65-kg pregnant woman who drinks water containing TCE at the concentration resulting from application of the proposed reference dose (RfD)(13μg/L). Further, the pregnant rats in the groups with anomalous fetal hearts experienced severe maternal toxicity, evidenced by diminished body weights at study termination, decreased weight gains during gestation, and total litter resorptions. The offspring from the affected litters had mean fetal weights that were approximately 33% lower than control values, as well as concomitant decreases in fetal size (e.g., decreased crown-rump lengths). In rats, the last 48 hours of gestation are a period of rapid growth; not only do the fetuses gain much weight in this period, but also the thorax grows quickly to accommodate the lungs, which develop largely after birth (Rakusan, 1984; Burri et al., 1974). While it is possible to associate the cardiac effects with the aforementioned maternal and fetal toxicities, there may be another contributing factor. Some findings in developmental toxicity studies can be caused by too over-zealous dissection methods (Harris and DeSesso, 1994). Fresh dissection of rat fetuses for examination of thoracic contents and dissection of the heart to observe internal cardiac structure is a demanding procedure because of the small size of fetuses. In fetuses that are one-third smaller than normal, the effort is even more difficult. The delicate tissues of compromised

heart (especially the diaphanous tissue of the membranous interventricular septum) can be easily disrupted during the incision and opening of the heart.

In a subsequent paper that laid out a proposed general toxicity-neurotoxicity-developmental toxicity screening approach, Narotsky and Kavlock (1995) administered large doses of TCE (1,125 or 1,500 mg/kg/day) by gastric intubation to pregnant Fisher 344 rats on gestational days 6 – 19 and allowed the animals to deliver their litters. The maternal animals experienced noticeable toxicity at both doses. Pup weights were significantly decreased in both treated groups, and the pups were reported to have experienced "increased incidences of micro/anophthalmia," although the numbers associated with these lesions were not reported. In the absence of data it is not possible to independently evaluate the latter conclusion. The thoracic contents (including hearts) were not examined. One notable design characteristic is the exaggerated dose of the material relative to the expected human exposure levels, as noted for the preceding studies. This brings into question the relevance of these findings for risk assessment purposes.

In 1998, Johnson et al. studied a variety of TCE metabolites for potential effects on cardiac development in pregnant Sprague-Dawley rats by providing drinking water that contained one of the TCE metabolites (including TCA, MCA [monochloroacetic acid], DCVC, and others) from gestational day 1 throughout pregnancy. They reported an increased incidence of cardiac anomalies only in pups from the eleven rats that had received water that contained 2,730 ppm of TCA. The defects included four cases of defects in the membranous interventricular septum. These findings are provocative, given the early reports by Smith and colleagues, but are in need of verification because of the small number of maternal animals in the TCA group, the lack of a dose-response design, and the low number of cases. As discussed in the next section, a robust follow-up study has been completed and was unable to reproduce the findings.

In addition to the whole animal studies mentioned above, the EPA assessment reviews data from papers that have designs that are inappropriate for risk assessment. The papers include those of Dawson et al. (1990) wherein solutions of TCE (15 or 1,500 ppm in saline) were delivered directly to the uterine lumina of pregnant Sprague-Dawley rats by osmotic mini-pumps that had been surgically implanted in the abdominal cavities on gestational day 7. While alterations were observed in several fetuses, there were no cases of ventricular septal defects. Administration of compounds by such an irrelevant route provides little information about the potential risk due to environmental or occupational exposure to TCE. The other paper that deserves mention is that of Boyer et al. (2000) who explanted the atrioventricular canals from stage 16 chick embryos and cultured them in a collagen gel that contained 0 - 250 ppm TCE. The authors noted that mesenchymal cell formation was inhibited in cultures containing TCE. The findings of this study are not relevant to human health risk assessment for a variety of reasons. First, avian developmental models differ significantly from mammalian models due to the absence of a maternal influence and a placenta. Second, the dose at the exposed tissues in the culture system is static and is likely to be far higher than the target tissue dose in developing mammalian hearts. Third, the culture method is not widely used and there is little background data with which to compare the results of the experiments.

Review of Johnson et al. (2003) Paper (Critical Study) and Associated Studies.

The fact that Johnson et al. (2003) is actually a compilation of data from two or more studies, the first published ten years before the 2003 publication (Dawson et al., 1993), was not made clear in their paper. In fact, it appears that it took 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, analyze and present developmental toxicity data.

There are a number of concerns regarding these studies:

First, it is not clear where all of the data reported in Johnson et al. (2003) came from. Currently, we are aware of two papers: Johnson et al. (2003) and Dawson et al. (1993). These are the two papers that are referenced in their response to Hardin et al. (2004), but, 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.

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 in their editorial reply that "Control values were consistent throughout our studies," however, there is no way for the reader to determine that.

Dawson et al. (1993) do not mention the number of pregnant dams that were assigned to each treatment group. There is no way to determine how much of the data in Johnson et al. (2003) is from the Dawson et al. (1993) study.

It would be prudent to have a qualified statistician look at this data base and the statistical evaluations used. Given the pooling of discrete data and the unbalanced study design (55 dams in the control vs. 9-13 in the treatment groups), it would be interesting to know how a statistician would view the analysis. Moreover, can the analysis address the hypothesis? 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? They do report that their data could indicate that a threshold effect exists at a level between 1.5 and 1,100 ppm. That is a range of three orders of magnitude, which is not very useful in establishing reference concentrations.

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 best an equivalent) response between 250 ppb and 1500 ppb. Johnson et al. (2003) provide a rationale for choosing the exposure levels that were used, but the extreme range makes it difficult to examine whether a continuous response pattern exists. To make the analysis more difficult to interpret, the fetus and not the dam (litter) was used as the experimental unit, or at least was the unit where statistically significant responses were noted. The dose-response pattern may be 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 any concentration of TCE in an in vitro chick embryo study is in no way comparable to an application of any other concentration of TCE in drinking water in an in vivo

rat study. It is unclear why the authors even make this statement; are they suggesting that their drinking water dose range would produce similar inhibitions of the transcription factors?

Johnson et al. (2003) do not reference Fisher et al. (2001), even though Johnson was one of the authors of the latter study and part of the cardiac examination team. Fisher et al. (2001), using techniques similar[1] to those reported in Johnson et al. (2003), did not find any cardiac defects following exposure to 500 mg/kg/day TCE. They provide some possible explanations for the differences from the Dawson et al. (1993) study: TCE purity, rat strains (both used Sprague-Dawley, different sources?), and experimental design (see above footnote), and the use of a staining procedure in the Fisher study "to better visualize heart structure." This last comment is surprising, since if the hearts were better visualized, one would expect that more, not zero, affected hearts would have been found.

One additional note: In their conclusions, Fisher et al. (2001) comment:

> "The high background of fetal heart malformations on a per litter basis provides a challenge for using these data in regulatory decisions relating to risk characterization of TCE, TCA, and DCA. Also, the lack of clear dose-related effects (Dawson et al., 1993, and the present study) provide data of questionable utility for risk assessment applications."

Comments on Specific Types of Heart Defects Reported

While there were similar methods used for examining fetuses in the Dawson/Johnson laboratories involved and Dr. Johnson collaborated on the Fisher et al (2001) study, there were several differences between the 3 studies as noted in the EPA review (see table 1). 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 1. Comparison of Methods Used in the Dawson et al (1993), Johnson et al (2003), and Fisher et al (2001)

Study	Stock of animals	Source of animals	Route of exposure	Dose	Vehicle	Treatment days GD	Day of sperm GD	Day of sacrifice GD	Heart preparation
Dawson et al 1993	Sprague Dawley	Harlan, Indianapolis?	Drinking water	1.5 and 1100 ppm	Tap water	1-22	1?	22?	flushed with 2% glutaraldehyde after heart removal, fixed for 24 hrs in the same solution, transferred to 0.1 mol/L phosphate buffer

[1] Fisher et al. (2001) used soybean oil as a vehicle for TCE and retinoic acid (positive control) and treated the animals with a daily bolus gavage (GD 6-15). Johnson et al. (2003) used water as a vehicle for TCE, provided ad lib in the drinking water, which was changed daily with fresh TCE. The treatment period was over the entire 22-day pregnancy.

Johnson et al 2003	Sprague Dawley	Harlan?	Drinking water	2.5 & 250 ppb, 1.5 & 1100 ppm	Distilled water	1-22	1?	22?	flushed with 10% formalin, transferred to 10% formalin
Fisher et al 2001	Sprague Dawley	Charles River, Raleigh	Gavage	500 mg/kg	Soybean oil (TCE & RA); IERO* water (TCA, DCA)	6-15	0	21	flushed in situ via the left ventricle with staining solution for better visualization (1:3 hematoxylin-saline solution), then removed and immersion fixed in 10% buffered formalin

* IERO = ion exchange/reverse osmosis

The major difference in the data from the Dawson/Johnson laboratory vs. the Fisher laboratory appears to be the incidence of atrial septal defects (Table 2). The types of atrial septal defects 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 sternebrae, vertebrae centra, etc., and 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 many unanswered 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*

Study/Data	**Treatment Groups**						
Dawson et al 1993	**Control Tap water**	**TCE – Pre only 1.5 ppm**	**TCE – Pre only 1100 ppm**	**TCE – Preg only 1.5 ppm**	**TCE – Preg only 1100 ppm**	**TCE – Pre & Preg 1.5 ppm**	**TCE – Pre & Preg 1100 ppm**
No. of atrial septal defects/no hearts examined (%)	1/232 (0.4)	3/130 (2.3)	7/147 (4.8)	4/181 (2.2)	7/105 (6.7)	5/256 (2.0)	19/435 (4.4)

Johnson et al 2003	**Control Distilled water**	**TCE – 2.5 ppb**	**TCE – 250 ppb**	**TCE – 1.5 ppm**	**TCE – 1100 ppm**		
No. of atrial septal defects/no hearts examined (%)	7/606 (1.2)	0/144 (0)	1/110 (1.0)	4/181 (2.2)	7/105 (6.7)		
Fisher et al 2001	**Control IERO** Water**	**TCA 300 mg/kg in IERO water**	**DCA 300 mg/kg in IERO water**	**Control Soybean oil**	**TCE 500 mg/kg in soybean oil**	**Retinoic acid – 15 mg/kg in soybean oil**	
No. of atrial septal defects/no hearts examined (%)	2/273 (1.0)	2/269 (1.0)	3/298 (1.0)	6/367 (1.6)	4/290 (1.4)	3/155 (1.9)	

*Highlighted boxes are the same data reported in both papers
**IERO = ion exchange/reverse osmosis

Later, Robustly-designed Studies in Animals Fail to Confirm Earlier Findings of Malformations in Rats

A subsequent publication (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.

Some early studies of TCA and DCA in pregnant Long-Evans rats (Smith et al., 1989, 1992) reported ocular malformations. In a study that reported findings after examination of the heads of the fetuses from the Fisher et al. 2001 study described above, Warren et al. (2006) reported 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.

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.

Assessment

Early findings of potential heart defects in rat pups associated with high doses of TCE metabolites during gestation prompted a series of investigations into the issue. The currently existing human data are deficient for risk assessment, but even so they do not support an association between TCE exposure and cardiac defects in babies. 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.

The current EPA review of TCE toxicity focuses on several endpoints for establishing a reference concentration and a reference dose. These were considered the most sensitive effects in the current data base. Two of these are developmental endpoints: fetal heart malformations in rats and developmental immunotoxicity in mice. The current preliminary review focuses on the fetal heart malformations, since this appears to be an area with some controversy.

The EPA has developed 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) is used to support both of these values (US EPA, 2009; see Tables 5.1.23 and 5.1.24 and the associated text).

Studies from the Dawson/Johnson laboratory are clearly compromised by a number of design weaknesses which are stated in the EPA review, but the weight of evidence discussion in section 4.7.3.3.2.3 only considers those studies that reported cardiovascular defects and essentially ignores more carefully designed state-of-the art studies that do not report cardiovascular defects. This is not a "weight of evidence" evaluation but a "strength of evidence" evaluation. All the focus is on those studies that found an effect and none on the strengths and weaknesses of those that did not. There is nothing in the EPA weight of evidence about the studies that did not find cardiac defects but which used sound methodology, i.e., Fisher et al., 2001, and Carney et al., 2006. Weight of evidence clearly must consider all of the data, both positive and no effect data. When studies with clear flaws that use methods giving results not replicable in other laboratories constitute the majority of the positive data, it is difficult to see how the EPA can justify using these data as the basis for regulatory end-point(s).

Final Comments

The EPA Review Draft (pp 855-857) notes that potential limitations of the cardiac malformation data base have been raised. Nevertheless, EPA considers the animal data provide "strong, but not unequivocal, evidence" of TCE-induced cardiac malformations; and EPA's final evaluation is that there is sufficient concern regarding the potential for TCE to lead to cardiac defects (p 861).

EPA puts emphasis on the Johnson et al. (2003) and Dawson et al. (1993) studies and 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. Nothing in this section described 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.

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

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Review

Trichloroethylene-contaminated drinking water and congenital heart defects: A critical analysis of the literature

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Abstract

The organic solvent trichloroethylene (TCE) is a metal degreasing agent and an intermediate in the production of fluorochemicals and polyvinyl chloride. TCE is also a common, persistent drinking water contaminant. Several epidemiological studies have alleged links between TCE exposure during pregnancy and offspring health problems including congenital heart defects (CHDs); however, the results of these studies are inconsistent, difficult to interpret, and involve several confounding factors. Similarly, the results of animal studies examining the potential of TCE to elicit cardiac anomalies have been inconsistent, and they have often been performed at doses far exceeding the highest levels ever reported in the drinking water. To determine what is known about the relationship between TCE and the incidence of CHDs, a comprehensive analysis of all available epidemiological data and animal studies was performed. Additionally, in vivo and in vitro studies examining possible mechanisms of action for TCE were evaluated. The specific types of heart defects alleged to have been caused by TCE in animal and human epidemiology studies were categorized by the morphogenetic process responsible for the defect in order to determine whether TCE might disrupt any specific developmental process. This analysis revealed that no single process was clearly affected by TCE, providing support that gestational TCE exposure does not increase the prevalence of CHDs. As a final evaluation, 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 CHDs.
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Keywords: Trichloroethylene; Developmental toxicity; Developmental processes; Cardiac defects; Congenital heart defects; Epidemiology; Water contaminants

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Abbreviations: AF, attributable fraction; ASD, atrial septal defect; CHD, congenital heart defect; CI, confidence interval, within 95% range unless otherwise indicated; CWA, contaminated water area; DCA, dichloroacetic acid; DCE, dichloroethylene; GD, gestational days; MCL, maximum contaminant level; OR, odds ratio; TCA, trichloroacetic acid; TCE, trichloroethylene; TLV, threshold limit value; VSD, ventricular septal defect

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1. Introduction

Trichloroethylene (TCE) is a halogenated hydrocarbon solvent primarily used as a metal degreasing agent and produced as an intermediate in the production of fluorochemicals and polyvinyl chloride (PVC). Historically, TCE has been used as an anesthetic, an antiseptic, and a solvent for use in dry cleaning and coffee decaffeination [1]. During biotransformation in humans, TCE is converted to chloral hydrate, a substance that is frequently prescribed for insomnia in the elderly as well as to sedate children undergoing CAT scans [2]. The terminal products of oxidative biotransformation are trichloroacetic acid (TCA) and dichloroacetic acid (DCA). TCE is volatile, and therefore most TCE released into the environment evaporates. However, in certain groundwater environments, TCE has limited contact with the air, and will therefore persist for years. For this reason, it is found as a contaminant in groundwater supplies.

According to the Toxics Release Inventory compiled by the Environmental Protection Agency, 291,000 pounds of TCE were released into water and onto land between 1987 and 1993. States with the largest releases to water include Pennsylvania, Illinois, and Georgia [3]. The American Conference of Governmental Industrial Hygienists (ACGIH) has established an 8-h time-weighted average (TWA) threshold limit value (TLV) of 50 ppm TCE vapors, and a 15-min short-term exposure limit (STEL) of 100 ppm TCE vapors. The current EPA-defined maximum contaminant level (MCL) for TCE is 0.005 mg/L (5 ppb), and the maximum contaminant level goal is 0 ppb [3]. EPA established the aforementioned regulatory levels based on the rationale that long-term exposure to TCE at levels above the MCL might contribute to an increased risk of liver problems and cancer. Additionally, the limit of detection of TCE in the water was 5 ppb at the time this MCL was determined, and this conservative limit is likely to have been selected due to the dearth of robust data on TCE's effect on human health.

Due to its occurrence in the water supply, it is important to identify and characterize any risks to human health attributed to TCE. Accordingly, several epidemiological studies have been conducted, some of which have linked TCE to health problems including, but not limited to, cancer, spontaneous abortions, and congenital heart defects (CHDs). Despite the plethora of adverse effects purportedly attributed to TCE exposure, analysis of the body of studies available fails to establish a clear cause and effect relationship [4,5]. As a result, there is a great deal of confusion regarding the toxicity of TCE. In order to assess risk and to identify appropriate avenues for future research, it is necessary to evaluate the current state of the data to determine what exactly is known about each of TCE's alleged effects on human development. In the present analysis, emphasis is placed on TCE's impact on the developing heart.

2. Etiology of CHDs

Due to the potential for dire outcomes associated with CHDs, any potentially causative factor should be investigated. In some cases CHDs are minor or treatable, but CHDs can be fatal or have a marked adverse effect on one's quality of life. According to the American Heart Association [6], at least 8/1000 infants are born with a CHD. Although certain genetic conditions (e.g., down syndrome) and drugs (e.g., isotretinoin [Accutane®]) are linked to CHDs, rarely is the cause of a CHD understood. Consequently, the relative roles of genetics and environmental agents as causative factors in the development of CHDs are unknown. There are at least 35 recognized types of CHDs, the most prevalent of which are listed and briefly described in Table 1.

A CHD is the unfortunate manifestation of a disturbance in any of several developmental processes involved in the formation of the heart. The major categories of developmental processes involved in heart development as described in Clark

Table 1
Major types of congenital heart defects

Type of congenital heart defect	Description
Atrial septal defect (ASD)	An opening between the right and left atria. ASDs can result in oxygenated and non-oxygenated blood being mixing in the atria, decreasing the heart's efficiency in delivering oxygenated blood to the body
Ventricular septal defect (VSD)	An opening between the right and left ventricles. VSDs can result in oxygenated and non-oxygenated blood being mixed in the ventricles, decreasing the heart's efficiency in delivering oxygenated blood to the body. These defects are often associated with an increase in blood pressure
Complete atrioventricular canal	A combination ASD/VSD, with abnormal tricuspid and mitral valves, resulting in oxygenated blood being re-pumped to the lungs. This defect is associated with symptoms of congenital heart failure
Aortic valve stenosis and insufficiency	The thickening and narrowing of the aortic valve, leading to a potentially harmful increase in pressure within the left ventricle
Coarctation of the aorta	Narrowing of the aorta between the upper and lower body artery branches. This can lead to very high blood pressure in the upper part of the body, very low pressure in the lower part of the body, and considerable heart strain
Pulmonary valve stenosis	The pulmonary valve becomes narrowed and/or leaky resulting in reduced blood flow to the lungs
Tetralogy of Fallot	There are four key features to tetralogy of Fallot: (1) VSD, (2) pulmonary stenosis, (3) hypertrophy of the right ventricle, and (4) the aorta "overriding" the VSD. The result is decreased blood flow to the heart, and mixing of the blood between sides of the heart, decreasing the effectiveness of the heart in delivering oxygenated blood to the body and causing significant adverse health effects
Single ventricle defects	Heart defects resulting in underdevelopment of one of the ventricles, possibly leading to cyanosis and an increased risk of endocarditis
Truncus arteriosus	A large VSD over which a large, single great vessel arises, carrying blood to both the body and the lungs
Transposition of the great arteries	The aorta and pulmonary vessels are reversed, resulting in oxygenated blood being pumped back to the lungs and non-oxygenated blood being pumped to the body. This condition will typically result in infant death if not surgically corrected
Ebsteins anomaly	Abnormally formed tricuspid valve in which one of two leaflets do not move properly. This is often associated with ASD, a large right atrium, and a small right ventricle

[7,8] are presented below:

1. *Cellular migration, particularly of the neural crest cells:* Early in development, neural crest cell migration contributes cells that participate in conotruncal septation. Disturbance of this process leads to conotruncal malformations, such as subarterial ventricular septal defects (VSDs; Type I), tetralogy of Fallot, and transposition of the great vessels [7].
2. *Cardiac hemodynamics:* As blood flows through the developing heart, the differential pressure on the various areas of the chamber walls allows for changes in the chamber shape. Abnormal cardiac hemodynamics can lead to aberrant distention of the cardiac chambers and valves, which can alter their shape and function [9]. Malformations linked to abnormal cardiac hemodynamics include hypoplastic left heart syndrome, coarctation of the aorta, and perimembranous (Type II) VSD [8].
3. *Cell death:* Cell death molds the developing heart by removing tissue, an important function in the formation of cardiac valves, the trabeculae carneae of the ventricular wall, and timely development of shunts between the developing right and left hearts [10]. Excessive cell death is associated with septal perforations, and insufficient cell death is associated with Ebstein's anomaly, a condition in which the tricuspid valve fails to separate from the ventricular wall [8].
4. *Extracellular matrix function:* Cardiac jelly, an amorphous glycosaminoglycan substrate, is the extracellular cellular matrix material of the heart. Cardiac jelly forms the endocardial cushions at the atrioventricular orifice and in the outflow tract [7]. The endocardial cushions act as anchors for the valves [9]. Cardiac jelly also fills the space between the inner wall of the heart (endocardium) and the outer surface (epimyocardium). In this location, the cardiac jelly serves as a medium through which a subpopulation of endothelial cells lining the atrioventricular lumen detaches and migrates into the jelly, where they undergo mesenchymal transformation, forming myocardial cells that proliferate and give rise to the cardiac muscle cells [7]. Atrioventricular septal defects can occur if the extracellular matrix does not form fully functional cardiac cushions [8].
5. *Targeted growth:* Targeted growth processes are necessary for the proper formation of certain heart structures. For instance, the direction of pulmonary vein growth is determined by a particular growth signal from the left atrium. Abnormal targeted growth processes during development can lead to disorders, particularly those related to abnormal venous return and cor triatriatum which emanate from the faulty incorporation of the common pulmonary vein into the left atrium [8].
6. *Establishment of visceral situs and cardiac looping:* Visceral situs (establishment of right and left sides of the

body) and looping defects result in ventricular inversion and reversed right or left position of organs [8].

One can determine the likely etiology of a defect if the morphology and location are fully described. On the other hand, if the defect is not fully described (e.g., if the specific type of VSD is not indicated), the underlying developmental process likely to be disturbed can be difficult to ascertain. The present review will critically evaluate the existing literature, including epidemiological, animal, and in vitro studies, to assess whether TCE is likely to cause CHDs, and if so, whether this is due to disturbance of a particular developmental mechanism.

3. Types of epidemiology studies

Epidemiology identifies factors that differ between two populations and are sufficiently important to play a causative role in the genesis of a disease. Several epidemiological studies have been conducted to determine if there is a link between TCE exposure during pregnancy and an increased prevalence of CHDs. To accurately evaluate these studies, it is important to recognize the various types of epidemiological study designs and to what extent conclusions can be drawn from them. The five major types of epidemiological studies are (1) case-control studies, (2) cohort studies, (3) cluster analyses, (4) general observational studies, and (5) cross-sectional studies. Detailed information regarding epidemiology and the various study designs can be found at Page et al. [11] and Rothman and Greeland [12].

The two strongest types of epidemiological studies are case-control and cohort studies. Case-control studies are retrospective investigations into the histories and habits of persons who have developed a particular disease. For TCE, the exposure history of a case population (mothers of babies with a CHD) is compared to the exposure history of a control population (mothers of unaffected babies) to determine the likelihood that the cases are associated with maternal TCE exposure. An important requirement of this type of study design is that the control and case populations be matched as closely as possible with regard to age, parity, body weight, and tobacco/drug/alcohol use. This is often a difficult requirement to fulfill, because individuals residing in an area exposed to TCE might differ from people living in unexposed areas, particularly in regards to socioeconomic status and ethnicity, and such differences can confound study results. Other issues to consider when evaluating data from case-control studies include the possibility of interviewer bias (if interviews were conducted) and the accuracy of the reporting physician and/or hospital (if birth defect registries were used).

Cohort studies involve a longitudinal prospective or retrospective investigation of persons exposed to an agent. Exposed and unexposed populations are identified, and the prevalence of a disease within both populations is then assessed. Retrospective cohort studies rely on the accuracy of interviewee recall or the information included in various registries (for example, birth and employment registries). When disease occurrence is followed in a prospective manner, it is more likely that exposure is accurately quantified, because of possible confounding factors associated with inaccurate recall are reduced.

Cluster analyses, general observational studies, and cross-sectional studies are basic epidemiology studies that are less rigorous in design than case-control and cohort studies. Cluster analyses focus on episodic observations of isolated disease cases, often related to exposure to an agent. Although this type of study might provide a good amount of detail about each particular case, the major limitation with this type of study is the lack of statistical power required in order to establish that an association exists between exposure and a specific disease. General observational studies investigate how exposure to a particular substance might be related to the likelihood of a certain outcome, but the number of cases or controls, the selection criteria, and/or the type(s) of controls are not as robust as those in the case-control and cohort studies. Such studies are frequently based on birth records, employment records, and surveys of cases and controls. A cross-sectional study is a type of observational study that primarily deals with ascertaining the disease incidence at a moment in time for a given population, as opposed to dealing with individuals and their histories. Thus, one looks at disease rates rather than cases.

The statistic generally calculated in epidemiological studies is the odds ratio (OR). In case-control studies, the OR compares the number of cases associated with the factor of concern to the number of cases observed in an appropriately matched control population. In cohort studies, the OR compares the number of exposed individuals with a particular disease of condition to the number of unexposed individuals with the same ailment. Characteristics of the OR provided in observational studies varies according to the design of that particular study. One must consider that the OR is based on data from sample populations and may not reflect the true OR value. Thus, a 95% confidence interval (CI) of the OR is typically provided. Throughout this manuscript, the CIs presented are 95% ranges unless otherwise indicated. If the 95% CI of an OR includes 1.0, then the presence of the factor of concern is not associated with an increase in the rate at which an adverse effect occurs; if it exceeds 1.0, then the exposed population has significantly more cases than the control population ($p < 0.05$). Sometimes, an attributable fraction (AF) is also calculated, representing the proportion of cases that would not have occurred had the potentially causal factor not been present.

An ideal epidemiology study would identify a sufficiently large cohort of pregnant women who were exposed to known concentrations of TCE throughout their pregnancies (or at least throughout the first trimester) as well as a large group of unexposed controls. Examinations of infants at birth and during the first 3 years of life would be performed by obstetricians and pediatricians who pay particular attention to the

cardiovascular system. Such a study has not been performed to date, and given the alacrity with which the American populace acts to reduce volatile organic compound contamination from drinking water aquifers, it is unlikely that a sufficiently robust study of exposed pregnant women will be mounted in the U.S.

4. Epidemiological studies of TCE exposure during pregnancy

Epidemiological information is available for several locations where pregnant women were likely to have been exposed to TCE or related substances, predominantly through groundwater contamination. These locations include Tucson, AZ; Northern NJ; Woburn, MA; Milwaukee, WI; Santa Clara, CA; San Francisco, CA; Baltimore, MD/Washington, DC area as well as regions of Finland and France. A total of 16 studies were reviewed, 5 of which addressed the potential cardiac toxicity of TCE alone, and 11 of which examined the effects of organic solvents and/or degreasing agents that might, or might not, include TCE. Of the five studies specifically examining TCE exposure, two are observational studies, one is a cross-sectional study, one is a case-control study, and one is a cohort study. Of the 11 studies examining organic solvents or degreasing agents, eight are case-control studies, two are cohort studies, and one is a survey. Data from these studies are presented in Table 2 and are discussed below.

4.1. Tucson Valley, AZ

Concerns about TCE in Tucson Valley, Arizona, were triggered by observations by local pediatricians that the parents of babies with CHDs tended to reside in the southwest region of the valley, an area in which the drinking water was contaminated with TCE. The contamination is thought to have begun in the 1950s, and in 1981, TCE levels were measured at 6–239 ppb [13] in the region considered to be the contaminated water area (CWA). To address concerns of a possible link between TCE exposure and CHDs, a birth registry-based observational study was performed by Goldberg et al. [13]. The authors contend that this is a case-control study; however, the proper control groups for such a study (i.e., a control population of parents of children without a CHD in which the prevalence of TCE exposure was assessed) were not included. Information was obtained through a registry of CHDs. Follow-up interviews were conducted with 707 parents of afflicted children, 246 of whom resided in the contaminated water area (CWA) during the first trimester of pregnancy. Controls for the percentage of the Tucson population exposed to the CWA were obtained by random telephone interviews of households in Tucson in which interviewees were asked if at least one person had regular contact with the CWA. These interviews were not limited to households with recent births. This study reports that 6.8/1000 live births of mothers residing in the CWA had CHDs, compared to 2.6/1000 births of mothers residing in uncontaminated areas. Following closure of the contaminated wells, the authors report that 4.6/1000 live births had CHDs. This study also reports that although only 10.8% of households in Tucson had at least one person with regular exposure to the CWA, 35% of infants with CHD were born to mothers residing in the CWA. The prevalence of particular types of CHD was not significantly different in exposed versus non-exposed mothers of afflicted infants, indicating that TCE lacked a specific effect on the heart.

Several limitations make the data from this study difficult to interpret. First, the authors assume that the percentage of childbearing families in each area of Tucson is equivalent. They ignore the equally likely possibility that families with young children might be clustered in particular areas due to socioeconomics or because of better schools and/or more family oriented housing. Second, the precise geographic area of water contamination in excess of the MCL could not be accurately identified because different wells were contaminated in different time periods. Water available to any given household within the CWA was not always contaminated, and when it was, the amount of TCE is likely to have varied considerably within the 6–239 ppb range. Furthermore, although the prevalence of CHDs in exposed versus non-exposed areas appears to be statistically different, all values fall below the expected 8/1000 U.S. background rate of CHDs [6].

4.2. Northern NJ

A cross-sectional study examined the incidence of birth defects in 75 towns in New Jersey that reported an average of 55 ppb TCE in the water supply between 1985 and 1988 [14]. Birth records of 80,938 live births and 594 fetal deaths in affected towns during this time period were reviewed. From this population, 346 infants (including live births and stillborns) had cardiac defects, and 52,334 infants with valid gestational dates had no birth defects and were not low birth weight, small for gestational age, or preterm. The former group served as cases, and the latter group served as controls. Maternal risk factors other than TCE exposure were evaluated in each group. Additionally, the amount of maternal TCE exposure was assumed based on regular tap water sample data for the area.

The authors reported weak associations between TCE exposure and CHDs [14]. Water levels exceeding 10 ppb TCE were alleged to be associated with CHDs (OR = 1.24, 50% CI = 0.75–1.94), and levels exceeding 5 ppb were alleged to be associated with an increased risk of VSDs (OR = 1.3; 50% CI = 0.88–1.87). For both conditions, the 50% CI levels (which are far less stringent than the normally reported, broad 95% CI) include 1.0. Thus, the statistical analysis of these data does not actually support the claim that TCE is likely to contribute to CHDs. Additionally, it is notable that, of this large study population, only 346 cardiac defects (~4/1000) were reported, which is lower than the U.S. background incidence of approximately 8/1000 [6]. This study also assumes

Table 2
Summary of human epidemiological data available for trichloroethylene

Reference	Location/date of exposure	Type of study	Concentration of TCE[e] or related substance	Route of exposure	Study Subjects/source(s) of data	Findings	Comments
Goldberg et al. [13]	Tucson Valley, AZ; 1969–1987	Observational study based on birth registry	6–239 ppb TCE	Purported maternal exposure to drinking water throughout pregnancy	• Parents of 707 children with a CHD[f] born 1969–1987 were interviewed. -Case group: 246 CHD infants born in TCE contaminated area -Case comparison group: 461 CHD infants born outside TCE contaminated area	• 35% of the families with a CHD child resided in TCE CWA[a], while only 10.8% of households in Tuscon, AZ resided or worked in the CWA. • Incidence of CHDs in CWA was 6.8/1000, and the incidence in non-CWA was 2.6/1000. The difference was stated to be significant; $p<0.001$; CI[b] = 1.14–4.14. OR[c] not reported.	• Exposure concentrations could not be determined. • Precise geographic area was not defined. • Controls to gauge population with contact with CWA are inappropriate. • Possible interview bias. • Authors incorrectly identify study as a case-control study. • Incidence of CHD in exposed group is well within expected background CHD rate (8/1000).
Bove et al. [14]	75 towns in Northern NJ 1985–1988	Cross-sectional study	Average of 55 ppb TCE	Maternal exposure to drinking water throughout pregnancy	• Birth records between 1985 and 1988 were evaluated. -Study population: 80,938 live births and 594 fetal deaths -Case group: 346 infants with CHD -Case comparison group: 52,334 full-term live births with no birth defects and of normal size/body weight	• TCE exposure was claimed to be associated with the following outcomes; -An increase in major CHDs at >10 ppb TCE; OR = 1.24; 50% CI = 0.75–1.94 -An increase in ventricular septal defects at >5 ppb TCE; OR = 1.3, 50% CI = 0.88–1.87.	• OR CI ranges span 1.0, indicating a lack of an association. • The incidence of CHDs in the study population is 346/80,938 (4/1000), a lower incidence than the U.S. background rate of CHDs. • Mother's residence during pregnancy assumed to be the same as that during birth.
Lagakos et al. [17]	Woburn, MA; 1970–1982	Observational study based on telephone survey	267 ppb TCE, with lesser amounts of other contaminants	Maternal exposure to drinking water throughout pregnancy	• Surveys of 3809 parents of live infants born between 1970 and 1982, 43 of which had a CHD.	• No correlation between TCE and CHDs was made. • An increase in leukemia, eye/ear and CNS/chromosomal/oral cleft malformations due to TCE exposure reported.	• Likely interviewer bias. • Authors indicate that there are too few cases to assure that Woburn's increased leukemia rate is due to TCE contamination.

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ATSDR [19]	U.S. Marine Corps base at Camp Lejeune, Onslow County, NC; 1968–1985	Retrospective cohort	20–1400 ppb TCE	Maternal exposure to contaminated drinking water throughout pregnancy	• Birth certificates were examined for infants of exposed and unexposed women. -Cohort group 1: 141 infants of mothers with short-term TCE exposure -Cohort group 2: 31 infants of mothers with long-term TCE exposure -Control group: 5681 unexposed women	• An association was reported for long-term exposure to TCE and an increased risk of low birth weight, seen only in the males, OR = 3.9; 90% CI = 1.1–11.8. • No link between TCE exposure and CHD reported.	• The link between TCE exposure and low birth weights in the males is based on a very small number of infants born to mothers with long-term TCE exposure. • The 95% CI of the OR associated with an increased risk of low birth weight is likely to include 1.0, negating the claim of a significant association. • Exposure could not be quantified.
Yauck et al. [35]	Milwaukee, WI; 1997–1999	Case-control	No specific concentration of TCE indicated	Maternal exposure route not specified; inferred by residence within 1.32 miles of any of 21 TCE-emitting facilities.	• Birth records between 1997 and 1999 were evaluated. -Case group: 245 infants with a CHD -Control group: 3780 infants without a CHD	• Of 245 CHD cases, 8 (3.3%) were born to older mothers (≥38 years old). Of the 3780 control cases (who bore infants without a CHD), only 19 (0.5%) were born to older exposed mothers. The authors concluded that TCE exposure makes older women more likely to give birth to a baby with a CHD compared to non-exposed older women (OR = 6.2, CI = 2.6–14.5). • Younger exposed mothers were not at a higher risk of giving birth to a child with CHD.	• The amount of TCE exposure was not quantified. • Older mothers are more likely to bear CHD infants, regardless of TCE exposure. • The number of births to older, exposed women is very small, making it difficult to determine the increase attributable to age vs. TCE.
Deane et al. [23]	Santa Clara, CA; 1/1980–12/1981	Retrospective cohort	Up to 8800 ppb of trichloroethane at the well head (public exposure thought to have been at much lower concentrations)	Maternal exposure to contaminated water during pregnancy	• Households with women pregnant in exposed and non-exposed regions between 1980 and 1981 were interviewed by telephone. -191 pregnancies in the contaminated area. -210 pregnancies in the non-contaminated area met the study inclusion criteria.	• After correcting for other maternal risk factors, an increased proportion of spontaneous abortions were reported in the contaminated area compared to the non-contaminated area (21.5% vs. 6.9%). OR = 2.3; CI = 1.3–4.2. • An increase proportion of live births with congenital malformations was seen in infants born in the exposed area compared to the non-exposed area (6.9% vs. 2.2%). OR = 3.1; CI = 1.1–10.4.	• Due to the small number of congenital malformations, the authors did not look at specific types. • Possible interview bias. • Authors state that results of their study cannot be used to support or refute a causal inference based on lack of information on timing and extent of contamination. Results not specific for TCE

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Table 2 (*Continued*)

Reference	Location/date of exposure	Type of study	Concentration of TCE[e] or related substance	Route of exposure	Study Subjects/source(s) of data	Findings	Comments
Hertz-Picciotto et al. [25]	Santa Clara, CA; 9/1981–6/1982	Case-control	Up to 8800 ppb of trichloroethane at the well head (public exposure thought to have been at much lower concentrations)	Maternal exposure to drinking water during the first trimester of pregnancy	• Of 1933 pregnancies identified in medical records that could have been exposed to contaminated tap water, parents of 1697 responded to a questionnaire asking about tap and bottled water consumption. • Of the 829 (48%) responding by mail: -Case group: 210 spontaneous abortions -Control group: 619 live births • Of the 868 (52%) responding by telephone: -Case group: 260 spontaneous abortions -Control group: 608 live births	• Of the telephone respondents, consumption of mainly tap water was associated with an OR of 2.2 (CI = 1.4–3.6). • Of the mail respondents, consumption of mainly bottled water was associated with an OR of 1.3 (0.8–2.0).	• There was only a statistically significant increase in CHDs due to tap water consumption when respondents were interviewed over the phone, indicating that either the phone or mail surveys were likely to be biased.
Wrensch et al. [24]	Santa Clara, CA	Retrospective cohort	Up to 8800 ppb of trichloroethane at the well head (public exposure thought to have been at much lower concentrations)	Maternal exposure to drinking water during the first trimester of pregnancy	• Data obtained from interviews of women who had been pregnant in 1980–1985 (1980–1981 is considered the exposure period). • Previously examined contaminated area: -Cohort: 266 pregnancies in CWA -Control: 293 in non-CWA • New contaminated area: -Cohort: 299 pregnancies in CWA -Control: 180 in non-CWA	• Increase in spontaneous abortion (OR = 3.5; CI = 1.2=10.3) and congenital malformations (OR = 4.3; CI = 1.2–4.7) seen in previously examined CWA in the children of mothers pregnant form 1980–1981. • Contamination was not associated with spontaneous abortion (OR = 0.3; CI = 0.1–1.1) or congenital defects (OR = 0.9; CI = 0.1–6.6) in children of mothers who resided in the new CWA from 1980 to 1981. • No increase in adverse pregnancy outcomes seen in post-contamination period in either CWA (1982–1985).	• This study examines the previously studied area described in Deane et al. [23] as well as a new contaminated area. • Maternal risk factors of maternal age, previous spontaneous abortion, mother's ethnicity, alcohol and cigarette smoking did not significantly alter these findings. • No increase in adverse pregnancy outcomes seen in post-contamination period (1982–1985).

Swan et al. [22]	Santa Clara, CA; 1981–1983	Case-control	Up to 8800 ppb of trichloroethane at the well head (public exposure thought to have been at much lower concentrations)	Maternal exposure to drinking water during the first trimester of pregnancy	• Medical records of children born in the area between 1981 and 1983 in contaminated and non-contaminated areas. • Exposed period births (1/1/81–8/31/82): -2151 in CWA -36,592 in non-CWA • Non-exposed period births (9/1/1982–12/31/1983): -1744 in CWA -28,217 in non-CWA	• During the exposed period, 5.6/1000 (12/2151) children in the CWA had CHDs compared to 2.6/1000 (94/36,592) in the non-CWA. OR for exposure and CHD was 2.2 (CI = 1.2–4.0). However, the authors concluded that temporal association of TCE exposure is inconsistent with a link between TCE and CHD. • Residence in the exposed area during the the non-exposed period not linked with CHD.	• Authors note that closer evaluation of temporal and spatial distribution of the cases is inconsistent with a link between TCE and CHD. • Interview bias • Not specific for TCE. • Exposure not quantified.
Shaw et al. [26]	Santa Clara, CA; 1981–1983	Case-control	Up to 8800 ppb of trichloroethane at the well head (public exposure thought to have been at much lower concentrations)	Maternal exposure to drinking water during the first trimester of pregnancy	• Mothers of cases and controls were interviewed. -Case group: 145 infants with CHD -Control group: 176 infants without a CHD	• Increased prevalence of CHD in the infants of women who drank tap water vs. bottled water was reported, but no dose–response relationship was observed except for births in 1981.	• Temporal distribution of births with CHD inconsistent with TCE being a causative factor.
Shaw et al. [27]	San Francisco, CA Bay Area; 1983–1985	Case-control	Specific concentrations not indicated; study examined the effect of hydrocarbon solvents	No specific route indicated; maternal exposure throughout pregnancy	San Francisco vital statistics files were studied. Of a database of 214,499 births, there were: -5046 CHD cases -20,882 randomly selected non-CHD controls	• The OR for CHDs in group possibly exposed to hydrocarbon solvents is 1.5 (CI = 1.1–2.0). • The OR for CHDs involving the conotruncus = 2.2 (CI = 0.93–5.2).	• Weak association for CHD; CI includes 1.0. • Results not specific for TCE. • Several possible confounding factors Assumed residence at time of delivery was residence throughout pregnancy.
Wilson et al. [20]	Baltimore, MD/Washington DC area; 1981–1989	Case-control with AF analysis	No specific concentrations indicated; study examines effect of solvents and/or degreasing agents	No specific route indicated; maternal exposure during pregnancy	• At least one parent of each infant was interviewed. -Case group: 4296 infants with a CHD -Control group: 3572 infants without a CHD	• AF[d]-linked solvent and/or degreasing agents with three heart/great vessel defects: -Hypoplastic left heart AF = 4.6%, $p \leq 0.01$ CI = 3.2–6.0 -Transposition of great arteries AF = 4.8%, $p \leq 0.01$ CI = 3.0–6.6 -Coarctation of aorta AF = 3.0%, $p \leq 0.05$ CI = 1.6–4.5	• Results not specific for TCE. • Possible interview bias

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Table 2 (*Continued*)

Reference	Location/date of exposure	Type of study	Concentration of TCE[e] or related substance	Route of exposure	Study Subjects/source(s) of data	Findings	Comments
Tikkanen et al. [28]	Finland; 1980–1981	Case-control	No specific concentrations or compounds indicated; study examines effect of hydrocarbon solvents	No specific route indicated Maternal exposure at home and work during pregnancy first trimester only	• Mothers of cases and controls were interviewed. -Case group: 160 infants with CHDs -Control group: 160 infants without CHDs	• No significant association was found between organic solvent exposure during pregnancy and the likelihood of bearing a child with a CHD.	• Results not specific for TCE. • Mothers were interviewed 3 months after birth, minimizing some recall errors (this was in follow-on study as well).
Tikkanen and Heinonen [29]	Finland; 1982–1984	Case-control	No specific concentrations or compounds indicated; study examines effect of hydrocarbon solvents.	No specific route indicated; maternal exposure during the first trimester of pregnancy at work and at home	• Mothers of cases and controls were interviewed. -Case group: 569 infants with CHD -Control group: 1052 infants without CHD control parents	• Exposure to organic solvents at work during the first month of pregnancy was associated with a slight increase in CHDs compared to non-exposed mothers (10.4 vs. 7.8%). • An OR = 1.5; CI = 1.0–3.7 for exposure to TCE and ventricular septal defect OR = 1.3; CI = 0.8–1.3 for CHDs in general.	• Results not specific to TCE. • Possible sources of bias in interview. • Results are not statistically significant; CIs of ORs include 1.0.
Taskinen et al. [30]	Finland; 1973–1982	Case-control	No specific concentrations or compounds indicated; study examines effect of organic solvents.	No specific route indicated; paternal exposure	• Surveys of men and/or their wives exposed to organic solvents -Case group: 25 infants with CHD -Referents: 96 infants without CHD	• No significant association between paternal exposure and congenital malformations. • Paternal exposure to organic solvents present in 72% of cases, and 73% referents.	Not specific to TCE.
Cordier et al. [31]	France	Survey	No specific concentrations or compounds indicated; study examines effect of organic solvents.	No specific route indicated; maternal workplace exposure during pregnancy	Surveys of mothers of 325 cases of major malformations and 325 normal births from 15 maternity hospitals in France	No significant correlation between exposure to organic solvents and heart defects. OR for CHD was reported to be 1.3 with a large 90% CI of 0.3–6.2.	Industrial hygienist assessed the presence of chemical exposure and the probability of exposure.

[a] CWA: contaminated water area.
[b] CI: confidence interval (95% unless otherwise indicated).
[c] OR: odds ratio.
[d] AF: attributable fraction.
[e] TCE: trichloroethylene.
[f] CHD: congenital heart defect.

that the mother's town of residence at the time of birth was her town of residence during her entire pregnancy. Approximately, 20% of women change residences during pregnancy [15].

4.3. Woburn, MA

In Woburn, MA, two municipal wells were contaminated with several chlorinated organics, including, but not limited to, TCE. In 1979, the average level of TCE in these wells was 267 ppb [16,17]. An observational study based on interviews of the parents of 3809 children born between 1970 and 1982 was performed [17]. This study was partly to address the issue of the high rates of leukemia in Woburn compared to the national rates. The authors sought to determine if exposure to contaminated wells was associated with an increased risk of leukemia or any types of congenital malformations, including CHDs. There was no statistically significant association between access to water from the contaminated wells in the year of pregnancy and the incidence of CHDs, though increases in eye/ear and CNS/chromosomal/oral cleft anomalies were reported. The authors found a positive association with exposure to water from the contaminated wells and leukemia, but noted that the number of cases was too low to definitively state that the contaminated wells were the cause. A major confounding factor for this study is the likelihood of bias because interviews were sometimes performed by community members who were involved in a lawsuit against the company responsible for the TCE contamination [18].

4.4. Camp Lejeune Marine Corps Base, Onslow County, NC

In early 1982, TCE at concentrations as high as 1400 ppb was found in tap water samples from one water distribution system on Camp Lejeune. By July, the concentration in that distribution system had dropped to a maximum level of 20 ppb. In 1985, however, the TCE concentration in another water distribution system at the base was 1148 ppb. Thus, it is assumed that residents of Camp Lejeune using water from these two distribution facilities were exposed intermittently to TCE between 1982 and 1985. It is thought that exposure was likely prior to 1982, but there is no sampling information to confirm this assumption [19]. In order to determine if there was a link between TCE exposure and adverse birth outcomes a retrospective cohort study of infants born between January 1, 1968 and December 31, 1985 was performed based on birth and infant death certificates [19]. The investigators controlled for sex of the infant, maternal and paternal ages, parity, maternal race, maternal and paternal education, military pay grade, adequacy of maternal care, marital status, and year of birth. Short- and long-term exposure durations were defined based upon the water distribution sources to particular housing areas and residence therein of a pregnant woman for a minimum of 1 week at any time during her gestation. The investigators identified a cohort of 141 infants born to women with short-term exposure to TCE and a second cohort of 31 infants born to women with long-term exposure to TCE. The only adverse birth outcome correlated with TCE exposure was an increased number of low birthweight males born to mothers with long-term exposure to TCE (OR = 3.9; 90% CI = 1.1–11.8). This association, which is based on a small number of long-term exposure pregnancies, is very weak. It should be noted that the investigators reported the 90% CI; had they used the 95% CI, the range would likely have included 1.0, indicating the lack of a statistically significant association. No link between TCE exposure and CHD was reported [19]. Considerable shortcomings of this study include that TCE exposure could not be quantified and that exposures during gestational periods other than the first trimester were included.

4.5. Milwaukee, WI

In 2004, a case-control study that examined the incidence of CHD in the offspring of mothers residing close to any of 21 TCE-emitting facilities was published by Yauck et al. [35]. A total of 4025 infants born from 1997 to 1999 in Milwaukee, WI, were identified from hospital and birth records. Of this group, 245 infants had a CHD and 3780 did not. Using information on the birth records, the maternal residence was identified. Pregnant women were considered to be exposed if they resided within 1.32 miles of at least one TCE-emitting facility. Older mothers were classified as women who were pregnant at ≥38 years of age; younger women were <38 years old. Of the 245 infants born with a CHD, 8 (3.3%) were born to older exposed mothers. In comparison, of the 3780 control cases (who bore infants without a CHD), only 19 (0.5%) were born to older exposed mothers. Based on this information, the authors concluded that TCE exposure makes older women more likely to give birth to a baby with a CHD compared to non-exposed older women (OR = 6.2, CI = 2.6–14.5). Younger exposed mothers were no more likely to give birth to a child with CHD than similarly aged non-exposed mothers. Limitations of this study are that the amount of TCE was not quantified, maternal residence at the time of delivery was assumed to be the residence during pregnancy, and the sample size for older exposed mothers was extremely small. Though the authors claim that advanced maternal age (defined as ≥38 years of age) can make women more susceptible to adverse effects of TCE on the developing heart compared to younger women, advanced maternal age in and of itself is associated with an increased risk of CHD [20]. When one considers the potentially confounding effect of advanced maternal age in conjunction with the small number of cases, it is improbable that one could establish the relative roles that TCE exposure and maternal age might play in the increased risk of CHD. Furthermore, as pointed out by Scialli and Gibb [21], the authors failed to evaluate gradients of risk associated with either increasing distance from the facilities or with increasing maternal age.

4.6. Santa Clara, CA

In November 1981, a leak of organic chemicals resulting in contamination of a drinking water well for residents of Santa Clara County was discovered [22]. Trichloroethane, an organic solvent similar to TCE, was reported to be the predominant contaminant. Although the concentrations of trichloroethane were as high as 8800 ppb at the well head shortly after the leak, it is likely that the public was not exposed to that high of a level in the drinking water due to mixing [22]. Lesser amounts of dichloroethylene (DCE), isopropyl alcohol, and freon were also present. Prior to 1981, the amount of trichloroethane is unknown.

Several epidemiological studies were conducted to determine whether the contaminated water contributed to adverse pregnancy outcomes, as informal reports had suggested. First, a cohort study was performed by Deane et al. [23] in which women pregnant between January 1, 1980 and December 31, 1981 were interviewed by telephone. The cohort group consisted of 191 mothers residing in a census tract that was known to be served by contaminated water supply at the time of pregnancy; the control group consisted of 210 mothers residing in non-contaminated census tracts during pregnancy. After correcting for differences in maternal risk factors including age, alcohol consumption, smoking and prior fetal loss, residence in a contaminated census tract was associated with an increased incidence of spontaneous abortion (OR = 2.3, CI = 1.3–4.2) and total congenital malformations (specific types not indicated; OR = 3.1, CI = 1.1–10.4). The authors cautioned that results of their study cannot be used to support or refute a causal inference because of the small number of cases and the lack of information on the timing and extent of contamination.

In a re-evaluation of the link between trichloroethane and adverse pregnancy outcomes in the Santa Clara region, Wrensch et al. [24] examined both the contaminated and non-contaminated regions presented in Deane et al. [23], as well as a second, previously unexamined contaminated census tract in the Santa Clara region with a new non-contaminated area control. Interviews with households in which a woman gave birth between 1980 and 1985 were conducted. For all areas, the authors considered 1980–1981 to be the exposure period, and 1982–1985 to be the post-exposure period. To re-evaluate the original contaminated area, a cohort of 266 pregnancies in the contaminated area from 1980 to 1985 were compared with a 293 control pregnancies over the same time period in the original non-contaminated control area used in [23]. Many of these cohorts and controls are likely to be the same as those examined in [23]. A cohort of 299 pregnancies in the new contaminated study area was compared with a control group of 180. Women were interviewed over the telephone or in person about their pregnancies. Consistent with the findings reported by Deane et al. [23], significant increases in spontaneous abortions (OR = 3.5, CI = 1.2–10.3) and congenital malformations (OR = 4.3, CI = 1.2–4.7) reported during the exposure period were seen in the original contaminated area compared to the original control area. In contrast, in the second contaminated area, exposure was not associated with an increase in spontaneous abortion (OR = 0.3, CI = 0.1–1.1) or congenital malformations (specific types not indicated; OR = 0.9, CI = 0.1–6.6) during the exposure period. Adjustment for maternal risk factors, such as maternal age, previous spontaneous abortion, mother's ethnicity, alcohol, and cigarette smoking did not significantly alter these findings. No increase in adverse pregnancy outcomes was observed in either the original or the new study groups during the post-exposure period. These findings are particularly interesting because the new study area was likely to be more heavily contaminated: 60–100% of the residential water was serviced by the contaminated well versus 20–50% in the original contaminated tract evaluated. Therefore, fewer adverse pregnancy outcomes were reported with water containing higher levels of trichloroethane, weakening the claim that adverse pregnancy outcomes were associated with trichloroethane exposure.

A third evaluation by Hertz-Picciotto et al. [25] underscored the importance of the type of survey used in these epidemiological studies. Cases and controls were sampled from a cohort of pregnancies that were medically confirmed between September 1, 1981 and June 30, 1982. Of this group, 1933 were selected for further evaluation. Out of this group of 1933 infants, parents of 1697 infants provided further information about the health of their child and likely exposure to contaminated water. The authors received 48% (829) surveys by mail and 52% (868) by telephone. The ORs associated with exposure to contaminated water and an increased risk of spontaneous abortion were 1.3 (CI = 0.8–2.0) for the group responding by mail, and 2.2 (CI = 1.4–3.6) for the group responding by the telephone. Thus, a positive association was only seen in the group interviewed over the telephone. This finding strongly calls into question the validity of the initial positive association between TCE and CHD indicated by Deane et al. [23], and suggests that the results of that study are likely to have been influenced by reporting bias. It cannot be said for certain whether telephone or mail interviews are more accurate, but two considerable advantages of the mail questionnaire are that the respondents have more time to reflect upon whether or not exposure to the contaminated water is likely to have occurred, and it is less likely that the response would be influenced by an interviewer's input.

Only two studies, Swan et al. [22] and Shaw et al. [26], examined the possible impact of trichloroethane on CHD rather than on spontaneous abortion or congenital malformations in general. In Swan et al. [22], a case-control study was conducted based on medical records of children born in the area between 1981 and 1983. The authors considered the exposed period to be January 1, 1981 to August 31, 1982. Births in the exposure period include births in which the mother could have been gestationally exposed to trichloroethane. The non-exposed period was determined to be September 1, 1982 to December 31, 1982; births during this period were conceived after the contaminated wells were

closed. Birth records of 2151 infants born in the previously contaminated water area during the exposed period and birth records of 36,592 infants born in non-contaminated areas during the same time were reviewed. During this period, 12/2151 (5.6/1000) children in the contaminated area had a CHDs compared to 94/36,592 (2.6/1000) in the non-contaminated area. Thus, birth in the contaminated area during the exposure period is associated with an OR of 2.2 (CI = 1.2–4.0) for CHD. Birth records of 1744 infants born in the contaminated area and 28,317 births in the non-contaminated area during the non-exposure period were reviewed. Birth in the contaminated area during the non-exposure period is not associated with an increased risk of CHD (OR = 0.5; CI = 0.2–1.7). These data appear to indicate that the exposure period is linked to an increased risk of CHD, but when the authors evaluated CHDs by month between 1981 and 1983, they noted that the incidence of CHD in the first part of 1982 was much less than that of 1981. If trichloroethane was the cause of the increased incidence of CHD during the exposure period, then one would expect to see a similar incidence of CHD during the first half of 1982 compared to 1981. The leak is assumed to have occurred in 1981; therefore, increased contamination prior to 1981 is not likely. Thus, the authors concluded that temporal association of trichloroethane exposure is inconsistent with a link between trichloroethane and CHD.

Similarly, Shaw et al. [26] shows that the relationship between exposure to trichloroethane and CHDs is strongest in 1981. Shaw et al. [26] conducted a case-control study specifically examining the prevalence of CHD in infants of women who drank either bottled water or Santa Clara tap water between 1981 and 1983. Data were gathered through detailed telephone interviews of 145 mothers whose children presented with a CHD and 176 mothers whose children did not. The investigators asked about the approximate amount of tap or bottled water consumed during pregnancy. The authors reported a higher prevalence of CHDs in women who drank tap water; the prevalence increased as the number of daily glasses of water increased [26]. The relationship was strongest in 1981, the only year in which there was a clear dose–response relationship between the amount of tap water drunk and the prevalence of CHDs. It is known that the water was contaminated from November to December 1981, and if the solvent had contributed to CHD, one would have expected to see an increase in 1982, when the women who would have been exposed during the critical period of pregnancy would have given birth. The fact that this strong positive association is primarily limited to 1981 indicates an inconsistent temporal relationship similar to that described by Swan et al. [22].

Taken together, the studies in Santa Clara do not support a strong association between trichloroethane and CHD. The positive correlations reported are weakened by dependence upon mode of interview, lack of a consistent dose–response relationships, and in some cases, the absence of the type of temporal relationships expected if trichloroethane contributes to CHD. Given the absence of a clear correlation between trichloroethane and CHD, one certainly cannot use these studies to support a causal relationship between TCE and CHD.

4.7. San Francisco, CA

Shaw et al. [27] conducted a case-control study of the prevalence of 10 different types of congenital malformations in regions of San Francisco where pregnant women were potentially exposed to various contaminants from 1983 to 1985. Of a database of 214,499 births in the San Francisco vital statistics files during this time period, there were 5046 CHDs and 20,882 randomly selected non-CHD infants were chosen as controls. Births were then assessed for likely exposure to various contaminants-based maternal residence at birth. Exposure to hydrocarbon solvents was associated with an OR of 1.4 (CI = 0.89–2.3) for heart and circulatory malformations, and an OR of 2.2 (CI = 0.93–5.2) for conotruncal malformations. The authors are forthcoming about the limitations of this study, including the inability to assess individual exposure, and the fact that the census data used in this study cannot guarantee that a woman lived in a contaminated or uncontaminated area during the critical period of her pregnancy. Considering these limitations, in conjunction with the facts that TCE in particular was not examined and the CIs of the ORs linking hydrocarbon solvent exposure to heart and circulatory defects includes 1.0, a positive correlation between cardiac malformations and TCE was not supported by this study.

4.8. Baltimore, MD/Washington, DC area

The incidence of CHDs in relation to exposure to solvents and/or degreasing agents was investigated in a case-control study of infants born in Baltimore, MD/Washington, DC and surrounding counties in Maryland and Virginia between 1981 and 1989 [20]. The case group consisted of 4296 infants with one or more CHDs. The control group consisted of 3572 infants born in the same area and at the same time with no CHDs. At least one parent was interviewed within 1 year after birth. Parents were asked about medication use, socioeconomic factors, and exposure to occupational and household materials including pesticides, dyes, metals, solvents, and degreasing agents. The authors made an effort to correct for several confounding factors including family history of CHDs, maternal age, maternal diabetes, maternal alcohol and smoking, maternal exposure to radiation, race, and socioeconomic status. Attributable fractions calculated in this study indicated that solvent or solvent/degreasing agent exposure was linked to three types of heart defects: transposition of the great arteries with an intact ventricular septum, hypoplastic left heart, and coarctation of the aorta [20]. The AFs associated with exposure to solvents and/or degreasing agents were 4.6% ($p < 0.01$; CI = 3.2–6.0) for hypoplastic left heart, 4.8% ($p < 0.01$; CI = 3.0–6.6) for transposition of the great arteries with intact ventricular septum; 3.0% ($p < 0.05$;

CI = 1.6–4.5) for coarctation of the aorta. While these data suggest a stronger link than was found in the previously discussed epidemiological studies, they are not specific for TCE, and both interviewer bias and weak estimates of exposure are likely confounding factors.

4.9. Finland

Finland has an impressive countrywide registry of health data, which facilitates evaluations of associations between various types of exposures and adverse health effects. The Finnish data do not include information specifically for TCE, but they do examine the effects of organic solvents in general. In a case-control study by Tikkanen et al. [28], parents of 160 infants with a CHD and 160 healthy infants were studied. Mothers were considered to be "substantially" exposed to organic solvents if the estimated continuous exposure was at least one-third of the ACGIH TLV, or if the estimated short-term exposure reached the TWA STEL. Only exposures that took place in the first trimester were included. No significant difference was observed in the incidence of children with a CHD born to mothers who were or were not exposed to organic solvents. In a more extensive follow-on case-control study, Tikkanen and Heinonen [29] interviewed 569 mothers of children with CHDs and 1052 controls. All mothers were interviewed approximately 3 months after delivery, minimizing inaccurate recall of exposure. The controls did not vary significantly from cases with regard to mean maternal age, smoking habits, and alcohol consumption. Exposure to organic solvents was not associated with a significant increase in the risk of CHD (OR = 1.3; CI = 0.8–1.3). When the authors categorized the CHDs by type, they reported an adjusted OR of 1.5 (CI = 1.0–3.7) for VSD. Although the authors of this study suggest that exposure to organic solvents increases the risk of CHDs and VSD in particular, the ORs relating exposure to organic solvents with these defects have confidence intervals including 1.0, indicating that there is not a significant increase in cardiac problems associated with organic solvent exposure.

A third Finnish case-control study evaluated the effects of paternal exposure to organic solvents on the occurrence of CHD in offspring [30]. No correlation was found between exposure and the incidence of CHDs. Of the total number of fathers of children with CHD, 72% were exposed to solvents, and of the total number of fathers of children without CHD, 73% were exposed to solvents. Overall, the bulk of Finnish data either fail to support a link between organic solvents and CHDs or the strength of this association is weak.

4.10. France

In a case-control study published by Cordier et al. [31], mothers of 325 children with major malformations and 325 healthy children were identified from 15 maternity hospitals in France and interviewed to determine their gestational exposure to various chemicals. The interview reports were reviewed by an industrial hygienist who assessed the presence of various chemicals and the probability of exposure. Their analysis showed that exposure to organic solvents or products containing organic solvents resulted in no significant increase in CHD (OR = 1.4; 90% CI = 0.3–6.2), based on the finding that mothers of 6/30 infants with CHDs and 4/30 control infants were exposed to organic solvents. Overall, this study is based on a very small data set and is beset with the limitations of weak dosimetry and the absence of data specifically about TCE.

4.11. Conclusions from epidemiological studies

There are several important confounding factors in the interpretation of the epidemiological data. The first is the extreme difficulty in quantifying TCE exposure of pregnant women during the first trimester when organogenesis is underway and the developing heart is most susceptible to environmental insult. To quantify exposure, one needs to know the amount of tap water drunk by the women on study and have solid information about the concentration of TCE in the drinking water. A second major problem is that the majority of epidemiology studies conducted examined solvents in general; data pertaining specifically to TCE is sparse. If TCE had been the predominant solvent in a mixture, or if the effect of TCE-containing organic solvents on the incidence of CHD had been particularly marked, this would be less of a problem; however, the proportion of TCE present in mixtures of organic solvents is not known, and there is no strong evidence linking TCE-containing mixtures to CHDs. Thus, drawing a conclusion about TCE based on studies of organic solvents in general is inappropriate.

In all of the epidemiological studies reviewed, regardless of the type, the validity of the data relies on the quality of the parental interview or on the rigor with which CHDs were detected and reported in birth defects registries. When relying on interviews, an intrinsic problem is that the validity of the findings is limited by the recall of the subjects. It is probable that the parents of children with a CHD would be more eager to participate in a study evaluating possible reasons for their child's condition and/or may have already given considerable thought to how maternal exposure might have influenced their child's condition. In some of the studies reviewed, the interview process itself was biased either because interviewers had a stake in the outcome of the study (as discussed in [18] for the Woburn, MA, study), or because a positive association was only obtained when data were gathered over the telephone, and not through a mail-in questionnaire [23,25].

It is important to note that there have been reports indicating changes in the prevalence of CHDs in areas not contaminated with TCE. Based on a registry of 937,195 births in the Atlanta region from 1968 to 1997, the prevalence of CHDs was 6.2/1000 [32]. Within this group, the prevalence of CHDs in the subgroup between 1995 and 1997 was 9.0/1000. The underlying reason for this increase in CHDs in the Atlanta region is unclear, but there was no distinct contaminant in the

region that could explain this change in incidence. In general, the prevalence of VSDs, tetralogy of Fallot, atrioventricular septal defects (ASDs), and pulmonary stenosis increased, and transposition of the great arteries decreased [32]. Differences in case ascertainment and reporting, as well as changes in the racial profile of the population and advances in CHD detection technology, are possible explanations for this [32]. Additionally, different studies report different estimates of the prevalence of CHD. In Blackpool, UK, the incidence of CHD in 57,979 births from 1957 to 1971 was 6.8/1000 total births, and the authors stated that the incidence of VSD and endocardial cushion defects seemed to be increasing slightly [33]. In the U.S. during the 1970s, an 8.8/1000 incidence of CHD was calculated from 19,979 births among families who participated in a Kaiser Foundation Health Plan [34]. This rate is approximately equivalent to the purportedly elevated incidence found in Atlanta [32]. Given that changes in CHD prevalence have been identified in the absence of TCE contamination and that the background prevalence of CHD varies from study to study, it is plausible that many of the changes in CHD prevalence attributed to TCE might instead be the result of normal variations. None of these estimates differ drastically from the current estimate of 8/1000 provided by the American Heart Association [6].

The five studies that investigated the effects of TCE specifically were Goldberg et al. [13], Bove et al. [14] Lagakos et al. [17], ATSDR [19] and Yauck et al. [35]. Because of the limitations of these studies, they are insufficient to support the hypothesis that TCE contributes to CHD. Notably, both the Goldberg and Bove studies alleged an increase in CHD among exposed populations when the prevalence of CHD in these groups was well within the expected range. Lagakos et al. [17] and ATSDR [19] did not find an increased risk of CHD. The study published by Yauck et al. [35] did not find a link between CHD and TCE in mothers younger than 38 years, and for exposed older mothers there were too few cases (only 8) to determine the relative impact of CHD and age. Also, few studies identified an increase in any one particular type of CHD, which one might expect if TCE had a specific adverse effect on the developing heart. While we have no evidence supporting causality, and the results against causality are not very strong by themselves. It is possible that studies in which chemical mixtures were examined were not powerful enough to detect an effect specifically attributed to TCE. Taken together, however, these epidemiological studies provide no convincing evidence that TCE exposure during early pregnancy is associated with CHD in offspring.

5. Animal studies

In order to investigate whether exposure to TCE can adversely impact normal heart development, research has been conducted using various experimental animal models. Although such studies are necessary to gauge the risk TCE poses to humans, especially in light of the equivocal epidemiological information, there are important caveats involved with extrapolating results of experimental animal exposures to humans. To begin with, there are notable differences in how rodents and humans metabolize TCE [36]. Mice and rats metabolize TCE more efficiently than humans; the maximum rate of TCE metabolism in humans is one-third that of the rat and one-fourth that of the mouse [37]. In rodents, a greater proportion of TCE is metabolized to DCA, mercapturic acid and a reactive thiol, whereas humans metabolize a greater proportion of TCE to TCA [38]. TCE induces peroxisomal proliferation and mutagenicity in rodent hepatocytes, while neither of these adverse effects is seen in TCE-treated human hepatocytes [39]. Lastly, when considering the relevance of these studies to human health, one must determine if the experimental exposure concentration and route of exposure are relevant to humans. Many of these studies have been performed at doses far exceeding what would be expected from environmental exposure, and often one cannot reasonably extrapolate data at these high doses to human health risk.

5.1. Animal studies reporting positive effects

Animal studies reporting a positive relationship between TCE and CHD are presented in Table 3. The first animal study suggesting a positive link between TCE and CHD was performed by Loeber et al. [42] using the White Leghorn chick model. In this study, concentrations ranging from 5 to 25 1-M TCE were injected into the air space of chicken eggs. TCE was linked to an increase in various types of CHDs including, but not limited to, atrial defects of the septa primum and secundum, VSDs, malposed truncus arteriosus, and abnormal cardiac muscle. No particular type of defect predominated. The increase in abnormal hearts peaked at 21% of afflicted live embryos following administration of 20 1-M TCE. At 25 1-M TCE, there was no increase in the percentage of abnormal hearts compared to controls, and the lack of defects at this concentration was not attributed to an increase in chick death. No clear pattern in the prevalence of particular types of heart malformations was attributed to TCE. Similar results were obtained in a study in which doses of 30 and 40 1-M TCE were directly injected onto the chorioallantoic membrane [40]. Cardiac malformations were found in 46/185 (25%) of the 40 1-M TCE-treated group, and in 35/195 (18%) of the 30 1-M TCE-treated group, compared to 1/47 (2%) in both the saline and mineral oil control groups. The various cardiac anomalies included large VSDs, endocardial cushion defect, single ventricle, double outlet right ventricle and truncus arteriosus. No distinctive type of cardiac anomaly predominated. Han et al. [41] conducted an experiment investigating the effects of DCE on the cardiovascular system of the developing chick. DCE was injected directly onto the chorioallantoic membrane at concentrations of 15 and 20 1-M. Cardiovascular anomalies were reported in 50/210 (24%) of the chicks treated with 15 1-M DCE, and in 63/210 (30%) of the group treated with 20 1-M DCE, compared with 3/73 (4%) in the

Table 3
Summary of animal data demonstrating a positive correlation between trichloroethylene or related compounds and congenital heart defects

Reference	Concentration of TCE[a] or related substance	Animal model and *N* pregnant rodents (or chick eggs)	Route/duration of exposure	Findings	Predominant type(s) of CHD[b]	Comments
Loeber et al. [42]	5–25 1-M TCE (gas chromatography indicates that only 0.2% was absorbed by the embryo)	White Leghorn chick *N* = 86–128 in treatment groups *N* = 266–267 in controls	Injection into air space of eggs starting at Hamburger-Hamilton stages [83] 6, 12, 18, or 23 and ending at stage 29, 34, or 44	• Dose–responsive increase in CHD to 20 1-M • Percent fetuses with CHD by group: -5 1-M: 2.3% (3/128) -10 1-M: 6.6% (6/91)* -15 1-M: 7.2% (7/97)* -20 1-M: 21% (18/86)* -25 1-M: 3.3% (4/120) -Saline: 2.3% (6/267) -Mineral oil: 1.5% (4/266)	• No specific type predominated.	• No increase in CHD compared to control was observed in the highest dose group, 25 1-M • Hamburger and Hamilton stage-specific general dissection of chick heart
Hong et al. [40]	30 and 40 1-M TCE in mineral oil	White Leghorn chick *N* = 185–195/treatment groups *N* = 46–47/mineral oil and control groups White Leghorn chick	Injection into chorioallantoic membrane during Hamburger-Hamilton stages 27–28	• Percent fetuses with CHD by group: -30 1-M: 18% (35/195)* -40 1-M: 25% (46/185)* -Control (saline): 2% (1/47) -Control (mineral oil): 2% (1/47)	• No specific type predominated.	• Hamburger and Hamilton stage-specific general dissection of chick heart.
Han et al. [41]	15 and 20 1-M dichloroethylene (DCE)	*N* = 210/treatment groups *N* = 73/saline control group *N* = 69/mineral oil control	Injection into chorioallantoic membrane during Hamburger-Hamilton stages 27–28	Percent fetuses with CHD by group: -15 1-M: 24% (50/210) -20 1-M: 30% (63/210) -Control (saline): 4% (3/73) -Control (mineral oil): 4% (3/69)	Degeneration of the embryonic myocardium, particularly in the primitive ventricular area	Hamburger and Hamilton stage-specific general dissection of chick heart
Dawson et al. [43]	15 and 1500 ppm TCE (0.036 and 36 1-g/kg/day), 1.5 and 150 ppm DCE (0.0036 and 3.6 mg/kg/day)	Sprague–Dawley rats *N* = 10–17/group	Intraperitoneal osmotic pump inserted into uterus on GD 7 and left operational until sacrifice on GD 22	Percent fetuses with CHD by group: -15 ppm TCE: 9% -1500 ppm TCE: 14%** -0.15 ppm DCE: 12%** -150 ppm DCE: 21%** -Saline: 3%	No specific type predominated.	• 1500 ppm is a very high dose of TCE, especially considering the solubility limit in water is approximately 1100 ppm. • Statistical significance is based only on a per-fetus analysis, none of these groups exhibited a statistically significant increase in CHD when analyzed on a per-litter basis • Fresh dissection technique [45,44]

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Dawson, et al. [46]	1.5 and 1100 ppm TCE (0.218 and 129 mg/kg/day) 0.15 and 110 ppm DCE (0.0218 and 12.9 mg/kg/day)	Sprague–Dawley rats $N = 12$–20/group	Maternal exposure to drinking water before (pre) and/or during pregnancy (preg)	• Percent fetuses with CHD by group: -1.5 ppm TCE pre only: 6.6% -1100 ppm TCE pre only: 6.5% -1.5 ppm TCE preg only: 5.5% -1100 ppm TCE preg only: 10.4%*** -1.5 ppm TCE pre + preg: 8.2%** -1100 ppm TCE pre + preg: 9.2%** -0.15 ppm DCE pre only: 1.9% -1100 ppm DCE pre only: 3.6% -0.15 ppm DCE pre + preg: 11.6%** -110 ppm DCE pre + preg: 13%** Control: 3%	No specific type predominated.	• Statistical significance is based only on a per-fetus analysis, none of these groups exhibited a statistically significant increase in CHD when analyzed on a per-litter basis. • Dawson dissection technique [46]
Johnson et al. [48]	2730 ppm (291 mg/kg/day) trichloroacetic acid	Sprague–Dawley rats $N = 11$/treatment group; pregnant rats control $N = 55$/control group	Maternal exposure to drinking water throughout pregnancy	• Percent fetuses with CHD by group: -Per-fetus data: 2730 ppm TCA (291 mg/kg/day): 10.53%* -Control: 2.15%	No specific type predominated.	Dawson dissection technique
Johnson et al. [63]	2.5 ppb TCE (0.00045 1-g/kg/day) 250 ppb TCE (0.048 1-g/kg/day) 1.5 ppm TCE (0.218 mg/kg/day) 1100 ppm TCE (129 mg/kg/day)	9–13 Sprague–Dawley rats/treated group 55/control group	Maternal exposure to drinking water throughout pregnancy	• Percent litters with abnormal hearts -2.5 ppb: 0% -250 ppb: 44%* -1.5 ppm: 38% -1100 ppm: 67%* -Control: 16%	No specific type predominated.	• Some of the data presented in Dawson et al, 1993 was again presented 10 years later here • Lack of dose–response relationship • Following publication of this manuscript, the author has presented the 250 ppb concentration as NOT significantly linked to CHD. • Dawson dissection technique.

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Table 3 (*Continued*)

Reference	Concentration of TCE[a] or related substance	Animal model and *N* pregnant rodents (or chick eggs)	Route/duration of exposure	Findings	Predominant type(s) of CHD[b]	Comments
Smith et al. [49]	330–1800 mg/kg/day trichloroacetic acid (TCA)	Long Evans rat *N* = 20–26/group	Maternal oral intubation during GD 6–15	• Percent fetuses with CHD by group: -330 mg/kg/day: 5.4%; 6 litters* -800 mg/kg/day: 23.6%; 12 litters* -1200 mg/kg/day: 46.8%; 11 litters* -1800 mg/kg/day: 94.8%; 8 litters* -Control: 0.96%	Levocardia VSD	Free-hand sectioning technique [47]
Smith et al. [50]	14–2400 mg/kg/day dichloroacetic acid (DCA)	*N* = 19–21/group Long Evans rat	Maternal oral intubation during GD 6–15	• Percent Fetuses with CHD by group: -14 mg/kg/day: 0.69%; 1 litter -140 mg/kg/day: 1.02%; 2 litters -400 mg/kg/day: 8.07%; 6 litters* -900 mg/kg/day: 8.15%; 6 litters* -1400 mg/kg/day: 23.91%; 12 litters* -1900 mg/kg/day: 43.67%; 15 litters* -2400 mg/kg/day: 68.75%; 16 litters* -Control: 0%	Levocardia, VSD, interventricular septal defect, and defects found between the base of the ascending aorta and right ventricle.	• Study reports no malformations in the 507 control fetuses from 39 litters. • Free-hand sectioning technique
Epstein et al. [51]	1900 mg/kg DCA on GD 6–8, 9–11 or 12–15 Single day doses of 2400 and 3500 mg/kg DCA given on GD 9, 10, 11, 12, or 13	Long Evans rat *N* = 7–10/group	Maternal oral intubation daily from GD 6 to 8, 9 to 11, 12 to 15, or a single intubation on GD 9, 10, 11, 12, or 13	Precent heart defects per-litter: -1900 mg/kg/day on: -GD 6–8: 0% -GD 9–11: 7.2%* -GD 12–15: 15.1%* -Control: 0% -2400 mg/kg/day on -GD 10: 2.5%* -GD 11: 0% -GD 12: 3.3%* -GD 13: 0% -Control: 0% -3500 mg/kg/day on -GD 9: 3.6% -GD 10: 2.9% -GD 11: 0% -GD 12: 2.9% -GD 13: 0% -Control: 0.5%	High interventricular defects caudal to the semilunar valves, with the anterior right wall of the aorta communicating with the right ventricle	• Free-hand sectioning technique

[a] TCE: trichloroethylene.
[b] CHD: congenital heart defect.
* Statistical significance ($p \leq 0.05$), correctly expressed in a per-litter basis in mammals, or in the case of injected chick embryos, in a per-fetus basis.
** Statistical significance ($p \leq 0.05$), based on a per-pup or per-fetus basis, when this should be evaluated by a per-litter basis.

saline controls and 3/69 (4%) in the mineral oil-treated controls. Predominant common intracardiac anomalies reported were ASDs, VSDs, and double-outlet right ventricles.

Following the positive results in the avian model reported by Loeber et al. [42], a group at the University of Arizona examined the effects of TCE and its metabolites in a series of papers using the Sprague–Dawley rat model. Concentrations of 15 ppm TCE (~0.036 1-g/kg/day), 1500 ppm TCE (~36.0 1-g/kg/day), 1.5 ppm DCE (0.0036 1-g/kg/day), and 150 ppm DCE (0.36 1-g/kg/day) in saline were pumped into the uterine lumen using osmotic pumps inserted into each uterine horn from gestational days (GD) 7 until sacrifice at GD 22 [43]. Fetal hearts were dissected approximately 1 day before parturition using the fresh dissection technique [44,45] wherein incisions into the heart follow the route of blood through the chambers and great vessels. A CHD was observed in 3% of control animals, 9% of animals exposed to 1.5 ppm TCE, and 14% of animals exposed to 1500 ppm TCE, 12% of animals exposed to 0.15 ppm DCE, and 21% of animals exposed to 150 ppm. The increase in the percentage of CHD in the TCE-treated animals was statistically significant on a per-fetus basis. Their second study conducted in rodents [46], exposed rats to 1.5 ppm TCE, 1100 ppm TCE, 0.15 ppm DCE, or 110 ppm TCE in the drinking water under three exposure regimens: prior to mating only, prior to mating and during pregnancy, and during pregnancy only. For this study, and for all subsequent studies performed in this laboratory that focus on the effects of TCE on the rodent heart, the Dawson dissection technique was used. The Dawson technique differs from typically employed methods for examining the heart (as described in Stuckhardt and Poppe [44] and Wilson [47]). Using the Dawson method, incisions are made ventrally through the tricuspid and pulmonary valves toward the heart apex. Next, an incision is made at each edge of the mitral valve toward the heart apex. Abnormalities were determined independently, and then collectively by a pediatric cardiologist, a pathologist, and a veterinarian. Using this method in the [46] study, the investigators reported a significant increase (on a per-fetus basis) in the incidence of CHDs in the following treatment groups: 1100 ppm TCE during pregnancy (10.4%), 1100 ppm TCE before and during pregnancy (9.2%), 1.5 ppm TCE before and during pregnancy (8.2%), 0.15 ppm DCE before and during pregnancy (11.6%), and 110 ppm DCE before and during pregnancy.

Johnson et al. [48] gave pregnant rats drinking water with various metabolites of TCE or DCE including 2730 ppm TCA, 1570 ppm monochloroacetic acid, 2349 ppm trichloroethanol, 473 ppm carboxy methylcysteine, 1232 ppm trichloroacetaldehyde, 174 ppm dichloroacetaldehyde, and 50 ppm dichlorovinyl cysteine. Concentrations of these metabolites were based on the dosage equivalent to that expected if all of the high dose of TCE at 1100 ppm, the limit of solubility, was to completely break down to that particular metabolite [48]. Of these compounds, only administration of 2730 ppm TCA led to an increase in CHD; 10.5% of pups born had malformed hearts, an incidence that was significantly higher than the 2.15% incidence in the control pups on a per-pup and per-litter basis (though the number of affected liters was not indicated). In all the studies performed at the University of Arizona, no specific type of CHD was linked to TCE or its metabolites.

A study/review published by Johnson et al. [63] summarized the studies emanating from this laboratory. In this paper, the authors contend that their results point toward a correlation between TCE and CHDs. Using their previous data, they attempt to identify a threshold concentration of TCE at which an increased risk to the developing heart would be expected. The percentage of abnormal hearts reported in their various studies over a 10 years span were reported to be 2.2, 0, 4.5, 1.5, and 10.5% at concentrations at 0 ppb, 2.5 ppb, 250 ppb, 1.5 ppm, and 1100 ppm TCE, respectively. The authors state that when analyzed on a per-fetus and per-litter basis, the 2.5 ppb and 1100 ppm concentrations led to a statistically significant increase in the number of abnormal hearts, though the marked absence of a dose–response relationship should be noted. For each treatment group, there were 9–13 litters, and for the control group (consisting of animals used in 1993 and 2003) contained 55 litters. To calculate the per-litter statistics the authors appear to have divided the number of litters with at least one CHD by the total number of litters in the group. In contrast, the correct way to conduct per-litter statistics is by examining the proportion of pups per-litter.

Although the University of Arizona group is the only laboratory to report a positive association between TCE and CHD in mammals, positive correlations with CHD have been reported for the TCE metabolites TCA and DCA by other laboratories. TCA administered to Long Evans rats by oral intubation during gestational days 6–15 (includes the sensitive period of organogenesis) at doses of 330, 800, 1200, or 1800 mg/kg/day was associated with a significant increase in the number of CHDs observed in offspring [49]. The primary defects observed were levocardia (displacement of the heart towards the left side of the chest) and interventricular defects. Smith et al. [50] reported statistically significant increases in soft tissue malformations, including those affecting the cardiovascular system, and particularly those between the base of the ascending aorta and the right ventricle, at doses of DCA ranging from 140 to 2400 mg/kg/day administered by oral intubation to Long Evans rats during GD 6–15. Epstein et al. [51] also reported a positive association between DCA treatment and the prevalence of CHDs. In this study, effort was made to identify sensitive windows during prenatal development when DCA exposure would be most harmful. Statistically significant increases in the percentage of CHDs were seen in the pups of Long Evans rat dams that had been orally intubated with 1900 mg/kg DCA on GD 9–11 or 12–15, with a higher incidence occurring on days 12–15. Single doses of 2400 mg/kg, but not 3500 mg/kg of DCA led to an increase in CHDs when given on GD 10 and 12. The main types of defects were characterized as high interventricular septal defects, including defects caudal to the semilunar

valves, with the anterior right wall of the aorta communicating with the right ventricle.

5.2. Animal studies reporting negative findings

Studies that did not find an association between TCE and CHD are summarized in Table 4. In contrast to some of the studies reporting positive findings, statistics in the studies reporting negative findings were always performed on a per-litter basis. All studies investigating exposure to TCE vapors failed to detect any negative impact on the developing heart. Schwetz et al. [52] exposed Sprague–Dawley rats and Swiss Webster mice to 300 ppm TCE vapors for 7 h daily throughout GD 6–15. The authors looked for CHDs using the freehand razor sectioning technique described by Wilson [47]. No significant maternal, embryonal or fetal toxicity was reported at this concentration. Dorfmueller et al. [53] exposed Long Evans rats to even higher concentrations of TCE vapors (1800 ± 200 ppm), and examined the effects of exposure to TCE for 2 weeks before mating and/or during pregnancy. Soft tissues were examined using the freehand razor sectioning technique. The presence of skeletal and soft tissue anomalies indicated developmental delay in the animals treated with TCE during pregnancy only. Again, no treatment-related CHDs or any other developmental anomaly were reported. A study examining the effects of inhalation exposure of 500 ppm TCE in rats and rabbits on GD 1–19 and 1–24, respectively, provided further experimental evidence that TCE did not contribute to CHDs [54]. An increase in fetal hydrocephaly in rabbits exposed to 500 ppm was reported, but it is thought that this increase is likely to have been the result of fixation in Bouin's solution, which was done prior to brain evaluation. Healy et al. [55] exposed pregnant Wistar rats to 100 ppm TCE for 4 h daily from GD 8 to 21. On GD 21, fetuses were examined for developmental abnormalities, including, but not limited to CHDs. No significant increase in abnormalities was observed as a result of TCE exposure.

In an evaluation by Carney et al. [56], a definitive developmental toxicity study was conducted, compliant with USEPA Office of Pesticides and Toxic Substances Guideline 870.3700 for prenatal and developmental toxicity studies, as well as the Organization for Economic Co-operation and Development Guideline No. 414 for developmental toxicity studies. Pregnant Sprague–Dawley rats were exposed to 50, 150, or 600 ppm TCE vapors for 6 h a day during GD 6–20. At least half of all fetuses in each litter were randomly chosen for complete visceral examinations, including a thorough dissection of the heart and great vessels. Dams treated with 600 ppm TCE exhibited a significant decrease in body weight gain; however, no indications of developmental toxicity (including any alterations in the heart) were observed at any dose level.

Two rigorous National Toxicology Program (NTP) studies were performed in Swiss CD-1 mice and Fischer 344 rats treated by oral gavage throughout pregnancy [57,58]. Mice were administered 100, 300, or 700 mg/kg/day throughout pregnancy, and rats were given 76, 156, or 289 mg/kg/day. Again, free-hand sectioning of the fetal hearts was performed. No correlation between TCE and CHDs was identified in the offspring of any treatment group.

In a large study performed by Fisher et al. [59], 20 presumed-pregnant rats per group were given a daily oral bolus of 500 mg/kg TCE, 300 mg/kg TCA, or 300 mg/kg DCA from GD 6 to 15. As a positive control, 12 pregnant dams were administered a daily dose of 15 mg/kg retinoic acid, a known cardiac teratogen. Soybean oil and water controls were conducted with 25 and 19 pregnant dams, respectively. Fetuses were subjected to body weight and sex evaluation as well as a comprehensive cardiac examination by persons experienced in developmental and reproductive toxicity studies. Hearts were dissected according to the Dawson method previously described to have been used by the University of Arizona group, and the team of observers included members of the University of Arizona laboratory. All observers were blinded to treatment. Although gestational treatment with TCA and DCA led to a statistically significant decrease in fetal body weight, neither the percentage of fetuses with cardiac anomalies nor the percentage of litters with a CHD was higher in the TCE, TCA or DCA groups compared to water or soybean oil controls. As expected, retinoic acid administration to dams led to a statistically significant increase in CHD compared to both control groups.

5.3. Analysis of the conflicting animal studies

The animal studies reviewed were performed on a variety of experimental models using TCE, TCA and DCA. In comparing and contrasting the results of these studies, one must be mindful of which studies are not germane to human environmental exposure.

The three studies performed with TCE and DCE in the chick model indicate a positive relationship with CHD, though this is based on only a few studies and two of these were performed in the same laboratory. The relevance of these findings to humans is unclear; data in the chick model is not directly applicable to human risk due to significant developmental differences between chickens and humans and the absence of a maternal influence in the chick model system. Additionally, the chicks were injected with high concentrations of test articles administered directly to the chorioallantoic membrane, a route of exposure that it not at all representative of how pregnant women are likely to be exposed to these substances.

Assessments of risk should be based on hazard data from mammalian species; a brief review of the positive and negative studies performed in mammalian species and analyzed in this paper revealed the following. Nearly all studies used one of two dosing schedules throughout gestation, or starting on gestational day 6 and continuing through major organogenesis. Both of these regimens encompass the critical period for heart development, from gestational days 7.25 to 14 [60]. The lone example of a (negative) study that missed the earliest

Table 4
Animal studies reporting negative findings

Reference	Concentration of TCE[b] or related substance	Animal model and *N* pregnant rodents	Route/duration of exposure	Findings	Comments
Schwetz et al. [52]	300 ppm TCE vapors	Sprague–Dawley rats *N* = 20–35/group Swiss Webster mice *N* = 30–40/group	Maternal inhalation of fumes 7 h/day during GD[a] 6–15.	No correlation was found between TCE administration and CHD[c]	Free-hand sectioning technique [47]
Dorfmueller et al. [53]	1800 ± 200 ppm TCE vapors	Long Evans rats *N* = 30/group	Maternal inhalation of fumes 2 weeks before pregnancy, during pregnancy, or before and during pregnancy.	Some soft tissue anomalies indicative of developmental delay were seen, but there was no link between CHD and TCE exposure during, before, or during and before pregnancy.	Free-hand sectioning technique
Hardin et al. [54]	500 ppm TCE vapors	Sprague–Dawley and Wistar rats *N* = 20–35/group New Zealand rabbits *N* = 15–20/group	Maternal Inhalation for 6–7 h/day during GD 1–19 in the rat and GD 1–24 in the rabbit.	No correlation was found between TCE and CHD in rabbits or either strain of rat. 4 cases of hydrocephaly (2 ea. in 2 litters) were noted in the rabbits.	Free-hand sectioning technique
Healy et al. [55]	100 ppm TCE vapors	Wistar rats *N* = 31–32/group	Maternal inhalation for 4 h daily from GD 8 to 21	No correlation was found between TCE and CHD or any other organ abnormality.	Free-hand sectioning technique
NTP [57]	100, 300, and 700 mg/kg/d TCE	Swiss CD-1 mice *N* = approximately 20/group	Maternal oral exposure to breeding pair during pregnancy.	No correlation was found between TCE and CHD.	Free-hand sectioning technique
NTP [58]	76, 156, and 289 mg/kg/d TCE	Fisher 344 rats *N* = approximately 20/group	Maternal oral exposure during pregnancy.	No correlation was found between TCE and CHD.	Free-hand sectioning technique
Carney et al. [56]	50, 150, and 600 ppm TCE vapors	CD rat	Maternal inhalation during pregnancy (6 h/day, GD 6–20)	No correlation was found between TCE and CHD.	Visceral exam by fresh microdissection including visualization of the internal structures of the heart
Fisher et al. [59]	500 mg/kg/day TCE, 300 mg/kg/day TCA 300 mg/kg/day DCA	Sprague–Dawley rats *N* = 19–20/group	Maternal oral bolus (gavage) once daily GD 6–15	No correlation was found between TCE, TCA, or DCA and CHD.	This author was working with the Goldberg/Dawson/Johnson laboratory Dawson dissection technique.

These studies should be listed in the table in the same order as they are discussed in the text.

[a] GD: gestational day.

[b] TCE: trichloroethylene.

[c] CHD: congenital heart defect.

phase of heart development is the inhalation study of Healy et al. [55], which began exposure on gestational day 8. The impact of the results of Healy et al. on our assessment is small because the GLP-compliant, exposure-response inhalation study by Carney et al. [56], which began exposure on gestational day 6, was also negative.

While investigations of the potential health effects attributed to TCE and its metabolites in the rodent model are more likely to be more predictive of human risk, most of the available studies, including those that reported no link between TCE and CHD, were performed at concentrations far exceeding the highest concentration of TCE ever detected in the drinking water (roughly 1400 ppb). For example, a 1100 ppm (~129 mg/kg/day) concentration of TCE was given to rats throughout pregnancy in [46], and in Fisher et al. [59], a dose of 500 mg/kg/day was given from GD 6 to 15. A concentration of 1500 ppm TCE (in saline) was injected directly into the pumps inserted into the rodent uterine horns [43]. The ~300 mg/kg dose of TCA used in [48,51,59] is the dosage equivalent to that expected if all of a 1100 ppm TCE dose were to break down completely into that metabolite [48]. In comparison, the solubility limit of TCE in water is 1070 ppm at 20 °C [61] and 1366 ppm at 25 °C, and the odor threshold is approximately 28 ppm [62]. Thus, the bulk of these studies were performed at malodorous concentrations of TCE that humans would likely avoid, and are far above concentrations that should be used to estimate human risk from environmental exposure.

The most controversial studies are those in which rodents are exposed to TCE itself (not a metabolite). It is striking that all of the studies alleging that TCE plays a causal role in CHD were conducted at the same laboratory at the University of Arizona [43,46,63]. The consistent positive findings at only this laboratory cannot be explained by the use of extremely high concentrations of TCE because Fisher et al. [59] also used a high dose of TCE (500 mg/kg/day) during GD 6–15 and found no effect. The mode of exposure at the University of Arizona laboratory (via drinking water throughout pregnancy) rather than limiting exposure to GD 6–15 (the sensitive period of organogenesis) cannot explain the differences. The heart is formed during the period of organogenesis; exposure to TCE prior to or after this period would not increase the likelihood of a CHD. Dorfmueller et al. [53] and Hardin et al. [54] exposed animals to high concentrations of TCE for all or most of pregnancy and still reported negative results. Possible reasons for the laboratory-specific positive link between TCE and CHD include their unique dissection technique and the use of non-standard statistical evaluations for developmental toxicity tests.

With regard to the different modes of administration, the distinction between oral gavage and drinking water exposures is that the former results in rather high blood concentrations shortly after administration; these elevated blood concentrations decay relatively quickly. In contrast, drinking water exposure provides a moderate blood level that remains relatively constant. Because congenital malformations are considered to be a threshold phenomena [64,65] exposure regimens that result in high peak blood levels are more likely to exceed the threshold concentration. This indicates that the gavage studies are more likely to cause malformations than drinking water studies.

All rodent studies performed at the University of Arizona laboratory in 1993 or later used the Dawson dissection technique. The authors state that this method is more sensitive for detecting lesions, such as adhered valve cusps because they separate fossa ovalis defects from the septum secundum atrial defects [48]. Further, they note that the method allows for the detection of abnormal valve dimensions. However, it does not appear that this dissection method in and of itself is the reason for the unique findings in this laboratory. The particular defects that are said to be more easily detected using the Dawson method are not predominant findings in the studies in which the Dawson method was used. Also, when the University of Arizona collaborated with investigators outside of the University of Arizona for the robust study described in Fisher et al. [59], the Dawson technique was used, the dissections were videotaped, and the treatment status of dissected animals was blinded to the observer. A positive link between TCE and CHD was not detected in this study, despite the fact that this study used even larger doses than had been used in the previous studies performed at the University of Arizona [59].

The second possible reason for the positive results reported only in the University of Arizona studies is that the statistics were performed in a different manner than those performed in studies not reporting a TCE–CHD correlation. Statistics were performed on a per-fetus basis, rather than on a per-litter basis [43,46]. Per-litter analysis is the accepted method of analysis for developmental effects related to chemical exposure during pregnancy, as recommended by the EPA Office of Research and Development [66]. As first described by Haseman and Hogan [67], 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. In other words, the pups are not statistically independent units. Performing statistics in a per-fetus manner artificially inflates the significance of the findings by making the N appear to be greater than it actually is. Had the correct statistical unit been used in these studies, a positive correlation between TCE and CHD probably would not have been reported by Dawson et al. (1990, 1993) studies. Furthermore, Johnson et al. [63] study re-published data from the 1.5 and 1100 ppm concentration groups originally published by Dawson et al. [46], and pooled controls from the 1993 and 2003 studies in their statistical evaluation. This re-publication of data was heavily criticized in a comment to *Environmental Health Perspectives* by Hardin et al. [68]. Pooling of controls is not an appropriate statistical practice and is likely to have exaggerated the alleged statistical significance. Johnson et al. [63] reported a statistically significant increase in CHD by a per-litter basis following administration of 250 ppb TCE. This finding appears to be worrisome because it is within range of the highest concentration found in the drinking water,

but it is peculiar that the 1.5 ppm concentration (a six-fold increase) did not contribute to CHD, indicating a lack of a dose–response relationship. Another puzzling aspect of the review by Johnson et al. is the insertion of a dose–response graph obtained through probit analysis in which the percent of *expected* CHDs as a result of TCE exposures at concentrations up to 4870 ppm (a dose at which the authors expect 100% of the offspring to be affected). The 4870 ppm concentration is more than four times above the 1100 ppm water solubility limit for TCE, and it is unclear how such a dose–response curve could be generated when the highest dose for which data exist is 1100 ppm.

Although one cannot make a causal link between TCE and CHD from the animal studies evaluated, studies evaluating the effects of large concentrations of DCA and TCA do seem to indicate that gestational administration of large amounts of these substances might be linked to an increased risk of CHD. Smith et al. [49] and Johnson et al. [48] both establish a statistically significant per-litter increase in CHD frequency. Smith et al. [49] reports an increased risk of CHD in Long Evans rats starting at doses of 330 mg/kg/day TCA given by oral gavage, with a dose–response increase up to the high dose of 1800 mg/kg/day. In this study, the authors reported an increase in VSDs and levocardia. Long Evans rats are said to be more sensitive to levocardia than other rat strains [49], and for this reason this study cannot be directly compared to studies using the Sprague–Dawley rat. Both Johnson et al. [48] and Fisher et al. [59] examined the impact of 300 mg/kg/day TCA administered to Sprague–Dawley rats administered during pregnancy. Johnson et al. [48] reported a variety of defects attributed to this concentration of TCA, with no particular defect predominating. In contrast, Fisher et al. [59] study found no increase in the risk of CHD at 300 mg/kg TCA. Therefore, although TCA appears to increase CHD in the [49] study, this is the only study available in which this experiment was performed. Results in the Sprague–Dawley were equivocal. Thus, there is slight evidence that high concentrations of TCA can contribute to CHD, but the relevance of this, too much lower concentrations of the parent compound TCE is unknown.

Smith et al. [50] and Epstein et al. [51] report increases in CHDs in pups born to pregnant Long Evans rats treated with DCA. The lowest dose that led to a statistically significant increase in the percentage of pups/litter that had CHDs was 400 mg/kg/day [50]. Epstein et al. reported that the rats were most likely to give birth to pups with CHD when treated with single high doses of DCA ranging from 1900 to 3500 mg/kg/day given by gavage on single day periods between GD 9 and 15. In both studies, the predominant defects were VSDs and defects between the ascending aorta and the right ventricle. Though the studies assessing the risk of DCA were well designed with a sufficient number of animals and the use of the correct statistical unit, these studies were performed by the same laboratory and should be replicated elsewhere before a concrete claim is made that DCA contributes to an increased risk of CHD.

Overall, the animal studies do not support an association between TCE and CHDs. Positive results from high concentrations of TCE administered to the chick are not relevant to environmental exposure to humans. The studies performed with TCE in the rodent were the most conflicted. Animal studies proposing a relationship specifically between TCE and CHDs were flawed in design and/or statistical analyses. Studies on TCA and DCA studies hinted at a positive association, but few studies have been performed, and those that are available were performed at very high doses that are at least 5000 times greater than the amount of these substances produced in the body from metabolism of water with 300 ppb TCE (the approximate maximum concentration reported). Therefore, none of these data from the animal studies are adequate or appropriate for extrapolating potential risks associated with developmental TCE exposures in humans.

6. Possible mechanisms of action of TCE on the heart

6.1. An examination of possible biological mechanisms of TCE in the heart

Only a handful of studies have attempted to identify a biological mechanism underlying the purported cardiac-specific teratogenic effect of TCE (Table 5). Boyer et al. [69] examined TCE's impact on epithelial-mesenchymal cell transformation in an in vitro system wherein cardiac endothelial cells were isolated from chicken embryo hearts and exposed to 50–250 ppm of TCE. In this model, TCE inhibited epithelial-mesenchymal cell transformation in a dose-dependent manner at all concentrations, and the 250 ppm concentration also decreased gene expression of *Mox-1* and *fibrillin-2*. These genes are involved in the differentiation of mesenchymal derivatives and extracellular matrices, respectively. It should be noted that this report was criticized for failing to use doses relevant to in vivo exposure. In a comment to *Toxicological Sciences*, it was noted that in order to achieve an internal level of 250 ppm TCE in the body fluids, one would have to inhale an atmosphere of approximately 180,000 ppm [70]. At the lowest dose examined, 50 ppm, the authors observed only a very modest (~5%) loss in mesenchymal cells. Even that concentration is much higher than what is likely to reach the embryo based on amounts of TCE found in the water supply. Therefore, although this study indicates a possible mechanism of action at a very high concentration under artificial circumstances, it fails to shed light on a mechanism likely to occur in people at environmentally relevant exposures.

In a study by Collier et al. [71], Sprague–Dawley rats were given drinking water with 110 and 1100 ppm TCE throughout pregnancy. The stress response gene *Hsp 70* was up-regulated in TCE-treated animals, and treatment was associated with the down-regulation of *GPI-p137* and *vimentin* (both involved in extracellular matrix formation), as well as *β-catenin* and *Serca2-Ca^{2+}-ATPase* (genes encoding Ca^{2+} responsive proteins). In further experiments, *GPI-p137* and

Table 5
Summary of animal mechanistic data

Reference	Concentration of trichloroethylene	Experimental model (in vitro vs. in vivo)	Parameter evaluated	Findings	Comments
Boyer et al. [69]	50–250 ppm	In vitro chick atrioventricular canal model	TCE[a]-induced changes in epithelial-mesenchymal cell interaction	Dose-dependent decrease in epithelial-mesenchymal cell transformation of endothelial cells between 50 and 250 ppm; at 250 ppm, TCE decreased expression of *Mox-1* and ECM protein *fibrillin 2*	Concentration is extremely high and not relevant to human exposure levels
Collier et al. [71]	110 and 1100 ppm given to pregnant rats	In vivo Sprague–Dawley rats	TCE-induced changes in gene expression	The authors claim that genes associated with stress response and homeostasis were upregulated, and genes associated with extracellular matrix components were down-regulated	• Very few clones had up or down-regulated genes (typically one to two) • No statistical analysis was performed
Ou et al. [72]	0.05–100 1-M (6.5–13,000 ppb)	In vitro endothelial cell culture	TCE-induced changes in eNOS induction of NO and interaction with hsp90	• At 0.05–100 1-M (1.3–13,000 ppb) TCE, VEGF-stimulated endothelial cell growth was inhibited. • At 5 1-M (650 ppb), TCE inhibits the interaction between eNOS and hsp90.	• Concentrations used in this study were not unreasonably high, but the approximate concentration of TCE in the drinking water to achieve exposure of these concentrations of TCE to the cardiac endothelial cells would be approx. 42 ppm.

[a] TCE: trichloroethylene.

Serca2-Ca^{2+}-ATPase were chosen as putative markers of TCE exposure. The choice of these particular genes as markers is questionable because the function of *GP1-p137* is largely unknown, and in the case of *Serca2-Ca^{2+}-ATPase*, expression changes were seen in only two clones from the 110 ppm dose and in no clones from the 1100 ppm dose. Nevertheless, further analysis of tissue from rats treated with a wider range of doses indicated a dose–response decrease in expression levels of *GPI-p137* and *Serca2-Ca^{2+}-ATPase* at concentrations as low as 100 ppb, though it is unclear how many animals were used for this analysis and what the impact would be of this amount of down-regulation of these genes.

In a third mechanistic study, TCE was tested for its ability to inhibit nitric oxide (NO) by blocking interactions between heat shock protein 90 and endothelial nitric oxide synthase [72]. The authors propose that this is a probable mechanism behind TCE-induced decreases in endothelial cell proliferation. TCE exposures ranging from 0.5 to 100 1-M (6.5–13,000 ppb) were examined for an effect on endothelial cell growth. The authors noted a distinct dose–response relationship, with a statistically significant decrease in endothelial cell growth commencing at 13 ppb. In order to gain some perspective regarding the environmental exposure, it is important to consider what concentration of TCE would have to be ingested to produce an internal concentration of 6.5 ppb. Substances in the environment that are absorbed into the body are distributed to tissues according to principles of pharmacokinetics. Using the pharmacokinetic model for distribution of TCE in pregnant rats developed by Fisher et al. [73], the concentration of TCE in drinking water that rats must ingest daily (assuming standard intake of water) to achieve the lowest concentration of TCE bathing the cardiac endothelial cells in this study (6.5 ppb) is approximately 175 ppm (J. Fisher and T. Sterner via e-mail communication). This calculated concentration of TCE greatly exceeds (by more than six-fold) the odor threshold of approximately 28 ppm in water [62] and is far greater than any environmental concentration reported.

The mechanistic studies we evaluated provide hypotheses of possible mechanisms by which TCE could be speculated to act in the heart. For this type of study, the use of high concentrations of the test article is often intentional, with an eye to performing the studies at lower concentrations once a reasonable hypothesis has been generated. However, these studies cannot be used to extrapolate risks to humans at environmentally relevant doses. Further studies investigating these hypotheses would have to be conducted before any conclusions can be drawn regarding their relevance.

6.2. Does TCE appear to affect a specific developmental process?

One of the difficulties in attempting to link a particular CHD with exposure to an agent is the great number of individual defects that compose the family of CHDs. Because it has been recognized for years that toxicants interact with developing organisms by means of specific mechanisms [74,75], we attempted to increase the likelihood of discerning a potential effect of TCE exposure by combining the reported cardiac defects according to the underlying developmental process(es) that would have been perturbed. If TCE does cause CHDs, it is anticipated that the prevalence of CHDs caused by one of the developmental processes would be increased causing a skewing of the expected distribution from control populations. As previously mentioned, the major developmental mechanisms involved in heart development include: (1) cellular migration, particularly of the neural crest cells, (2) hemodynamics, (3) cell death, (4) actions of the extracellular matrix, (5) targeted growth, and (6) establishment of visceral situs and cardiac looping [7,8]. Perturbations of any of these developmental mechanisms can result in CHDs. If TCE exposure did indeed increase the incidence of CHDs, then one would expect to observe an increase in particular types of CHDs associated with a specific embryological target. Of the studies discussed, only 3 of 15 epidemiological studies, and 3 out of 18 animal studies indicate an increase in a particular type of CHD. None of these studies were performed specifically on TCE, but instead focused on organic solvents and/or degreasing agents in general (which may or may not have included TCE). Results of studies performed with the White Leghorn chick model are not included in this analysis due to the significant differences between mammalian and avian development.

A shared developmental mechanism among the CHDs reported in the studies would provide some strength to the theory that TCE exposure played a role in their development. For studies alleging a particularly marked increase in VSD incidence related to exposure to TCE (or similar substances), specific information on the type and location of the VSD was often not provided; therefore, several possible developmental processes are listed. As indicated in Table 6, the CHDs indicated in the reviewed studies emanate from the disturbance of a variety of developmental mechanisms. Results from Smith et al. [49,50] indicate an increased prevalence of levocardia, a defect specifically linked to errors in positional information or looping. Other studies report increases in hypoplastic left heart [20], coarctation of the aorta [20], and VSD Type II [51], all defects linked to errors in hemodynamic mechanisms. Four defects were linked to cell migration including conotruncus defects [27], transposition of the great arteries [20], defects between the ascending aorta and the right ventricle [50], and VSD type I [51].

One might assume from this evaluation that if TCE-related substances are likely to affect the developing heart, cell migration and/or hemodynamics are the developmental processes targeted. However, the distribution of defects and underlying developmental processes likely to have been disturbed mirrors that found in the distribution of CHDs in the U.S. (Table 7). VSDs are the most frequent forms of CHDs in the U.S., and the most prevalent types of VSDs are likely to derive from alterations in cell migration and hemodynamics [76,77]. The incidence of VSD varies widely because

Table 6
Summary of epidemiological and animal studies suggesting a predominant type of congenital heart defect attributed to trichloroethylene

Reference	Concentration of TCE[b] or related substance	Type of study	Predominant type of CHD[c] reported	Developmental process likely to be disturbed[a]
Shaw et al. [27]	Specific concentrations not indicated; study examines effect of hydrocarbon solvents and related substances	Epidemiological; case-control	Conotruncus defects	Cell migration
Wilson et al. [20]	No specific concentrations indicated; study examines effect of solvents and/or degreasing agents	Epidemiological; case-control with attributable fraction analysis	Hypoplastic left heart Transposition of the great arteries Coarctation of the aorta	Hemodynamics Cell migration Hemodynamics
Smith et al. [49]	300–1800 mg/kg/day trichloroacetic acid	Animal study: Long Evans rat	Levocardia VSD (specific type not indicated)	Positional information and cardiac looping Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics
Smith et al. [50]	14–2400 mg/kg/day of dichloroacetic acid (DCA)	Animal study: Long Evans rat	Levocardia Defects between ascending aorta and right ventricle VSD (specific type not indicated)	Positional information and cardiac looping Cell migration and/or extracellular matrix formation Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics
Epstein et al. [51]	1900–3500 mg/kg/day DCA	Animal study: Long Evans rat	VSD type I VSD type II	Cell migration Hemodynamics

[a] Categories are those described by Clark [7].
[b] TCE: trichloroethylene.
[c] CHD: congenital heart defect.

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Table 7
Most prevalent types of congenital heart defects in the U.S. and the developmental and the predominant developmental process underlying the defect[a]

Congenital heart defect	Percentage of CHD patients with this defect	Developmental process disturbed
Ventricular septal defects	15–50[b]	Cell migration, cell death, extracellular matrix formation, and/or hemodynamics
Tetralogy of Fallot	9–14	Cell migration
Transposition of the great arteries	10–11	Cell migration
Atrioventricular septal defects	4–10	Extracellular matrix formation
Coarctation of the aorta	8–10	Hemodynamics
Hypoplastic left heart syndrome	4–8	Hemodynamics

[a] Information from this table was obtained from statistics provided by the American Heart Association [76,77].
[b] The percentage of CHDs that are VSDs varies according to when the statistics were taken and the number of asymptomatic children screened for CHDs. The increased use of echocardiography has led to increased detection of these defects in recent years [77].

of an increased use of improved diagnostic tools, such as echocardiograms available to detect this defect [77]. Given that the distribution of developmental mechanisms purportedly disturbed by TCE and related substances is not markedly different from the distribution of developmental processes anticipated to be involved in CHDs not attributed to these substances, as well as the fact that these studies did not specifically evaluate TCE, there is insufficient evidence to state that TCE has a specific effect on the heart.

7. Does TCE exposure cause an increase in CHDs?

As previously noted, none of the epidemiological studies was sufficiently robust to indicate a link between TCE exposure and CHDs. A major obstacle in interpreting most of the animal and in vitro studies was the lack of data at the concentrations of TCE that have been reported in contaminated water. The need for generating toxicological data at "real-world" exposure levels in order to provide information

Table 8
Application of Hill's guidelines of causation to trichloroethylene/congenital heart defect data

Criterion	Extent to which data supports or does not support guideline	Comments
Temporality	All studies fulfill this requirement except for the epidemiology studies in Santa Clara, CA, where a temporal relationship is inconsistent with a link between TCE[c] and CHD[d] as discussed in [22], and as is a likely confounding factor in [26]	
Strength of association[a]	Fulfilled by Wilson et al. [20] epidemiology study	Wilson et al. [20] examines solvents in general, not only TCE
	Fulfilled in the following animal studies: Smith et al. [49] (TCA); Smith et al. [50] (DCA); Epstein et al. [51]	Johnson et al. [63] has several significant flaws.
	Fulfilled in the following mechanistic studies: Boyer et al. [69] and Ou et al. [72]	
Dose–response relationship[b]	Epidemiology studies did not address dose–response issues.	A dose–response relationship was not examined in any animal study examining the specific effect of TCE
	Fulfilled in the animal studies by Smith et al. [49,50] (TCA, DCA), respectively	
	Fulfilled in the following mechanistic studies: Boyer et al. [69] and Ou et al. [72]	
Specificity	No studies fulfill this requirement	
Consistency	Conclusions made in TCE studies are inconsistent between investigators, as been as within the same group in the case of the contradictory findings of Fisher et al. [59] compared to Dawson et al. [46]	
Plausibility	The plausibility requirement states that the proposed causal relationship would not conflict with the currently accepted understanding of pathological processes. At very high doses of TCE, it is plausible that the developing heart may be adversely affected. However, as discussed previously, the cardiac-specific effects of TCE that were purportedly demonstrated in the animal studies have not been shown to occur at environmentally relevant doses	

[a] An association is deemed to be strong if statistical analysis was performed on the appropriate statistical unit, yields a p-value ≤ 0.05, or if the 95% CI for an OR does not include 1.0.
[b] A dose–response relationship is deemed to be present if at least 3 doses of TCE or a related substance were analyzed, and there was a clear dose–response effect.
[c] TCE: trichloroethylene.
[d] CHD: congenital heart defect.

useful in making regulatory decisions is paramount for several dimensions of risk assessment, including evaluation of the toxicity of complex mixtures [78], as well as for choosing appropriate doses to employ in carcinogen bioassays [79]. Most of the animal and in vitro studies performed specifically on TCE indicate that doses larger than the largest concentration reported in the water do not harm the developing heart. Nevertheless, if one were to design additional studies on the risks of TCE it is hoped that these studies would be performed at relevant doses/concentrations so that the data would be directly applicable to human risk.

In a final evaluation of the data, Hill's guidelines of causation [80] were applied to all epidemiological and mammalian animal studies (Table 8), despite previously described reservations regarding the data. Studies using the chick model were omitted because they are not representative of mammalian toxicity. Hill's guidelines were originally intended for use in analyzing epidemiological research, but the principles can be applied to evaluate animal and in vitro studies as well. Hill's guidelines of causation dictate that as more guidelines are met, the likelihood of a causal association increases. In order to state confidently that an agent is the cause of a given effect, most (and ideally all) of the following requirements should be met:

1. *Temporality:* For causality to exist, the exposure must precede the health effect under investigation. This guideline is met by most of the TCE studies discussed, with the exception of Santa Clara studies (Swan et al. [22]; Shaw et al., 1990 [26]) in which the purported positive correlation did not make sense based on the time of exposure.
2. *Strength of association:* The strength of the association is commonly measured using appropriate statistical tests. In general, the stronger the association, the more likely that the relationship is causal. The requirement for a strong association is deemed to have been met if (1) in humans, the incidence of CHD is above the normal background incidence, or in animal studies, the incidence is greater than that in the controls, (2) the correct statistical unit is used (i.e. the litter, rather than the pup or fetus in the animal studies), and (3) the p-value < 0.05 (if a 95% CI is reported, it does not include 1.0). In general, the stronger the association, the more likely the relationship is causal. Based on these conditions, Wilson et al. [20] study examining the effects of organic solvents was the only epidemiological study fulfilling this criterion. Animal studies showing strength of association include the Johnson et al. [48] rodent study on TCA, Epstein et al. [51] study on DCA, and Smith et al. [49,50] studies on TCA and DCA, respectively. While the Johnson et al. [63] study appears to meet the requirements, this study is significantly flawed due incorrect statistical evaluation and the inclusion of repeated and reclassified data, as previously discussed. Therefore, it cannot be concluded that an association of TCE exposure with CHDs was clearly demonstrated by this study. In the case of the in vitro studies, both Boyer et al. [69] and Ou et al. [72] demonstrated strength of association, but no statistical analysis was performed in the third mechanistic study by Collier et al. [71].
3. *Dose–response relationship:* A dose–response correlation is likely to be present in a causal relationship, although there may be cases in which a threshold amount of the substance changes the response and a clear dose–response relationship is not evident. For the purposes of this evaluation, a genuine dose–response relationship will be considered to be one in which a dose–response is seen in at least three consecutive doses. No epidemiological studies were sufficiently robust to investigate the presence of a dose–response relationship. In the case of the animal studies, this guideline is fulfilled by the Smith et al. [49,50] studies, as well as by the two mechanistic studies by Boyer et al. [69] and Ou et al. [72].
4. *Specificity:* Specificity is established when a putative cause is associated with only one or a few specific effects. This is a particularly difficult guideline to assess, and while it supports a causal relationship, absence of specificity does not necessarily negate the presence of a causal relationship. For example, the diseases caused by smoking do not meet this criterion, because smoking contributes to an increased risk of several different diseases, including emphysema and bladder cancer, not just lung cancer [81,82]. As has already been discussed, the epidemiology studies specifically evaluating TCE failed to detect a statistically significant increase in CHDs or any other adverse effect above background levels. The animal studies alleging a positive association between TCE and CHD are unreliable due to flawed design and/or incorrect statistical evaluation of data.
5. *Consistency:* A consistent effect means that the association is seen in several different studies, preferably by a number of different laboratories under a variety of different conditions. Taking into consideration the aggregate of studies, which provide various contradictory results, this criterion is obviously not met.
6. *Biological plausibility:* The plausibility requirement states that the proposed causal relationship would not conflict with the currently accepted understanding of pathological processes. At extremely high doses of TCE, it is plausible that the developing heart may be adversely affected. However, as discussed previously, the cardiac-specific effects of TCE that were purportedly demonstrated in the animal studies have not been shown to occur at environmentally relevant doses.

At most, a single individual study fulfilled three of the Hill's Guidelines, and the majority of the studies only supported one. None of the studies fulfilled the specificity, consistency, or biological plausibility criteria. Thus, the overall body of the data does not support the hypothesis that TCE is a causal factor in CHDs.

8. Conclusion

CHDs are the most frequent form of birth defects, affecting nearly 1% of newborns [6]. Because the underlying cause of the CHDs is rarely understood, any indication that an environmental contaminant might increase the prevalence of CHDs warrants further investigation. Several studies attempted to address the question of whether TCE is likely to contribute to CHDs; however, in no case was a strong, reliable correlation substantiated.

The epidemiology studies alleging a possible link between TCE exposure and the development of CHDs all suffer from one or more weaknesses. All the positive studies suffer from non-robust design, which limits the conclusions that can be drawn from them regarding causality. Many studies present results that were not statistically significant (i.e., the 95% CI of the OR included 1.0), many do not examine the effects of TCE exposure specifically, and as a whole, the positive studies are inconsistent regarding the specific type of CHD that was observed following exposure. Thus, while the body of epidemiology studies provides no causal link between TCE and CHD, the data are too weak to draw firm conclusions about the lack of a possible effect.

Animal study results indicate that TCE is not a causal factor in CHD, although high concentrations of the TCE metabolites TCA and DCA are likely to adversely affect the developing heart. We note that the exposure concentrations of TCE needed to obtain the toxic concentrations of TCA and DCA are several orders of magnitude higher than those found in the environment. When all CHDs reported after exposure to TCE were categorized by the underlying perturbed morphogenetic processes, there was no shift in the expected distribution. This indicates the absence of a specific mechanism, which reduces the probability that TCE causes CHD.

Finally, application of the Hill's Guidelines for causation failed to support an underlying link between TCE exposure and CHD. Thus, the overall results of our analysis prompt us to conclude TCE is not a specific cardiac teratogen at environmentally relevant exposures and that the MCL for TCE (5 1-g/L) is protective.

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