

Threshold of Trichloroethylene Contamination in Maternal Drinking Waters Affecting Fetal Heart Development in the Rat

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Halogenated hydrocarbons such as trichloroethylene (TCE) are among the most common water supply contaminants in the United States and abroad. Epidemiologic studies have found an association but not a cause-and-effect relation between halogenated hydrocarbon contamination and increased incidence of congenital cardiac malformations or other defective birth outcomes. Avian and rat studies demonstrated statistically significant increases in the number of congenital cardiac malformations in those treated with high doses of TCE, either via intrauterine pump or in maternal drinking water, compared with controls. This study attempts to determine if there is a threshold dose exposure to TCE above which the developing heart is more likely to be affected. Sprague-Dawley rats were randomly placed in test groups and exposed to various concentrations of TCE (2.5 ppb, 250 ppb, 1.5 ppm, 1,100 ppm) in drinking water or distilled water (control group) throughout pregnancy. The percentage of abnormal hearts in the treated groups ranged from 0 to 10.48%, with controls having 2.1% abnormal hearts, and the number of litters with fetuses with abnormal hearts ranged from 0 to 66.7%, and the control percentage was 16.4%. The data from this study indicate not only that there is a statistically significant probability overall of a dose response to increasing levels of TCE exposure, but also that this trend begins to manifest at relatively low levels of exposure (i.e., < 250 ppb). Maternal rats exposed to more than this level of TCE during pregnancy showed an associated increased incidence of cardiac malformations in their developing rat fetuses. **Key words:** cardiac malformations, cardiac teratogenicity, environmental contaminants, halogenated hydrocarbon, heart defects, heart development, TCE, trichloroethylene. *Environ Health Perspect* 111:289–292 (2003). doi:10.1289/ehp.5125 available via <http://dx.doi.org/> [Online 31 October 2002]

TCE (trichloroethylene, ClHC=CCl₂), is a man-made chlorinated hydrocarbon in a ubiquitous class of pollutants used mainly as a solvent and is considered an animal carcinogen and a potential health hazard to humans. Halogenated hydrocarbons such as TCE and dichloroethylene (DCE) are among the most common water supply contaminants in the United States and abroad (1). Some products of these solvents are produced as a result of chlorination of municipal water supplies that contain natural organic material. TCE, because of its wide use and thoughtless disposal, is also a compound frequently detected at monitored sites. Its associations with adverse health effects have been studied, and it is one of the targets of bioremediation.

The possible link between environmental agents and human cardiac defects has long been known (2). Trichloroethane, a compound closely related to TCE, was investigated for its possible correlation with human congenital cardiac disease (3). Zierler et al. (4) showed that workers exposed to solvents experienced an associated increased prevalence of congenital heart disease. An epidemiology study by Goldberg et al. (5) determined that the distribution of patients with congenital heart disease in the Tucson, Arizona, basin was associated with an area of water supply contaminated with TCE, DCE, and chromium. Contamination of those

drinking water supplies, which had probably begun during the 1950s, was identified in 1981. After closure of the affected wells, the incidence of congenital cardiac disease fell dramatically in the contaminated area. This sequence of events and later statistical assessment established the higher prevalence of congenital heart disease in children whose parents were exposed to the contaminated water compared with children born to parents who were never so exposed (5). The cardiac lesions occurring in the area of contaminated water supply varied and showed no significant difference for lesion type when case and case control values were compared, although a cause-and-effect relationship could not directly be established in the experimental design.

Several other epidemiologic studies have examined the reported association between halogenated hydrocarbon contamination and increased incidence of major congenital cardiac malformations, other birth defects, and birth outcomes (3,6–12). Considerable information is available regarding the short- and long-term toxicity of these agents and, to a lesser extent, their general teratogenicity (13–18). Recent research has implicated halogenated hydrocarbons as specific cardiac teratogens in animal models and has indicated their significance to health issues in the United States and abroad (14,19–21).

Specific metabolites of TCE and DCE have now been identified for their role in defective cardiac development (22). These metabolites, especially trichloroacetic acid (19,22,23), have been discussed in detail by other researchers, and a discussion of these findings is beyond the scope of this article, particularly because the data from Boyer et al. (24) suggest that the cardiac valvular and septal malformations may be caused by TCE's inhibiting endothelial separation and early events of mesenchymal cell formation in the developing heart.

The goal of this research 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.

Methodology

All animals used in this study were maintained in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care International and in accordance with the established guidelines of the University of Arizona's Institutional Animal Care and Use Committee, the Animal Welfare Act, and U.S. Public Health Service policy standards. They were given access to food (4% rat diet; Teklad, Madison, WI) and water *ad libitum*. Each animal was identified individually by an ear notch code, and they were housed in groups of three or four, except for breeding males, which were individually housed.

According to the same animal model as in previous studies, various concentrations of TCE were administered in drinking water to pregnant Sprague-Dawley rats (5,19,21). Once pregnant, the rats were randomly placed in test groups. The animals were given fresh drinking water that contained the appropriate concentration of TCE *ad libitum* during the entire pregnancy (22 days). The test solutions were made daily to ensure the

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freshness of the solution. This provided a more consistent concentration in the solution to compensate for the amount of hydrocarbon lost because of environmental exposure, and allowed recording of amounts consumed over a 24-hr period. Treatment groups were distilled water controls, 2.5 ppb TCE (0.00045 mg/kg), 250 ppb TCE (0.048 mg/kg), 1.5 ppm TCE (0.218 mg/kg), and 1,100 ppm TCE (128.52 mg/kg). The amounts received by the rats per day were calculated by the average of the breakdown of TCE due to environmental exposure over a 24-hr period and the average amount of drinking water consumed by each group. For consistency and ease of reporting, we refer to the levels by the initial concentration. The groups tested are described in Table 1.

On the last day of pregnancy, each dam was euthanized. Dams and fetuses were examined for gross organ abnormalities. Placement of the fetuses, placental weights, and fetal crown-rump length, sex, and weights were recorded. The hearts and great vessels were examined *in situ* for external gross malformations. The hearts were then removed, flushed with 10% formalin, and placed in 10% formalin solution for later dissection. Each heart was given a code to comply with blind study requirements.

A Nikon SMZ-2T light microscope with television monitor (Nikon, Tempe, AZ) provided excellent visualization of the hearts for individual dissection. Hearts were examined by the strict protocol established for previous studies by the investigators. The course, caliber, and orientation of the aorta and pulmonary vessels were determined. The atrial appendages were removed and the atrial septum evaluated. After removal of the aorta, pulmonary vessel, and atrial appendages, the pulmonary, aortic, tricuspid, and mitral valves were examined and probed for patency. The formation of each leaflet was carefully evaluated. The ventricular septum was then visualized by removal of the left ventricular free wall. Any suspected abnormality was held for later observation by all three investigators. After unanimous agreement on an abnormality, it was photographed using a Nikon N2020 camera mounted on the light microscope.

Two outcomes (frequency of abnormal hearts in each group and frequency of litters with at least one fetus with an abnormal heart) were analyzed using a 5×2 chi-square test of homogeneity. Because both overall tests were statistically significant, pairwise comparisons of treated groups with control were made using 2×2 chi-square tests of homogeneity. A probit analysis of the frequency of abnormal hearts in each group was done to identify the dose-response curve—that is, the predicted probability of abnormal hearts for a specified TCE concentration.

Probit analysis was performed with logit transformation, and the natural response rate was calculated from the rate seen in the control group.

Results

As shown in Tables 1 and 2, 98 dams and 1,146 fetuses were examined. Maternal and fetal variables, including noncardiac congenital abnormalities, showed no significant differences between treated and control groups. In contrast, comparisons across groups of the incidence of heart abnormalities showed that there were significant differences between groups, both in the incidence of abnormal hearts per group [chi-squared (4) = 26.39, $p < 0.001$] and in the incidence of litters with one or more abnormal hearts [chi squared (4) = 17.82, $p = 0.001$]. As shown in Figures 1 and 2, the control group had a 2% rate of abnormal hearts and a 16% rate of litters with at least one fetus with an abnormal heart. This percentage is similar to that reported in human studies and in rat studies using this model (25). In comparison, rats exposed to the highest dose of TCE (1,100 ppm) had a 10.5% rate of abnormal hearts and a 67% rate of litters with one or more abnormal hearts, rates significantly higher than in the control group ($p < 0.001$). Intermediate exposure levels produced intermediate

response rates. As shown in Figure 3, probit analysis suggested that a concentration of 2,692 ppm (315 mg/kg dose) would be required to produce abnormal hearts in 50% of the fetuses (see Figure 3).

The variety of heart defects produced is consistent with previous studies, including those in the avian model, intrauterine mammalian exposure studies, and previous maternal drinking water studies (19–21). The types of defects found were as follows: absent coronary artery, enlarged coronary artery sinus, secundum-type atrial septal defects, aortic valve defect with fused leaflets creating aortic valvular stenosis, aortic valve defect with fenestrated leaflets, hypoplastic mitral valve annulus, hypoplastic tricuspid valve annulus, d-transposition, atrioventricular canal, and both membranous (subaortic) and muscular ventricular septal defects (Table 2). It is important to note that no litters in the treated or control groups had more than three abnormal fetuses (one litter in control, one in 1.5 ppm, and two in 1,100 ppm groups had three abnormal fetuses). All other litters had one or two abnormal fetuses only. Of interest, although not of individual statistical significance, is the fact that a similar percentage of defects was found in the 250 ppb and 1.5 ppm studies. These values contributed to the observed overall trend toward a dose-related

Table 1. TCE test groups.

Initial conc	Avg conc/24-hr drinking water (ppb)	Equivalent avg dose (mg/kg)	No. of maternal rats	Total no. of fetuses
1,100 ppm	918,500	129	9	105
1.5 ppm	1252.5	0.218	13	181
250 ppb	208.75	0.048	9	110
2.5 ppb	2.09	0.00045	12	144
Control	0	0	55	606

Abbreviations: Avg, average; conc, concentration.

Table 2. Types of heart malformations per 100 fetuses.

Type of defect/100 fetuses	Control	TCE dose group			
		1,100 ppm	1.5 ppm	250 ppb	2.5 ppb
Abnormal looping	0.33		1		
Coronary artery/sinus				1.82	
Aortic hypoplasia			0.55		
Pulmonary artery hypoplasia			0.55		
Atrial septal defect	1.16	6.67	2.21	0.91	
Mitral valve defect	0.17			0.91	
Tricuspid valve defect				0.91	
Ventricular septal defect					
Perimembranous (subaortic)	0.33	2.86	1.66		
Muscular	0.33	0.95	0.55		
Atrioventricular septal defect	0.17	0.95			
Pulmonary valve defect					
Aortic valve defects		1.9		0.91	
Fetuses with abnormal hearts (n)	13	11	9	5	0
Total fetuses (n)	606	105	181	110	144
Litters with fetuses with abnormal hearts/litters (n)	9/55	6/9	5/13	4/9	0/12
Litters with fetuses with abnormal hearts/no. litters (%)	16.4	66.7	38.5	44.4	0.0

Data were calculated on a per-100 fetus basis (i.e., 7/6.06 = normalized number for atrial septal defect in control group = 1.16).

effect and could indicate that a threshold effect exists at a level between 1.5 and 1,100 ppm (21,22).

Discussion

It is known that TCE is capable of placental transfer from mother to fetus (26). Small size and lipid solubility permit TCE to easily cross the placental barrier. Analysis of blood taken simultaneously from both mother and fetus has shown the presence of TCE in maternal and fetal blood. These studies, done in humans, found that the ratio of fetal concentration to maternal concentration of TCE varied from one subject to another (26). This has also been demonstrated in rodents administered TCE by gavage (27). Previous studies

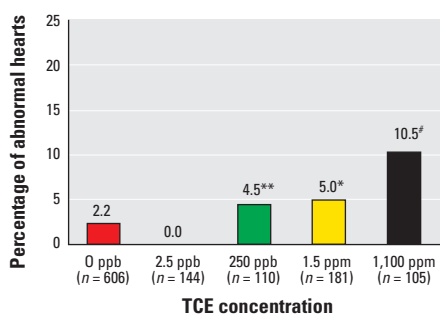


Figure 1. Dose–response pattern for treated and control groups.

*Compared to control, $p = 0.14$; **compared to control, $p = 0.04$; #compared to control, $p < 0.001$.

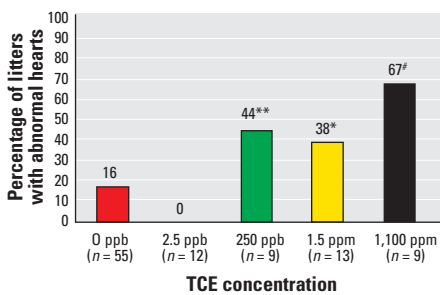


Figure 2. Dose–response pattern for treated and control litters.

*Compared to control, $p = 0.08$; **compared to control, $p = 0.05$; #compared to control, $p < 0.001$.

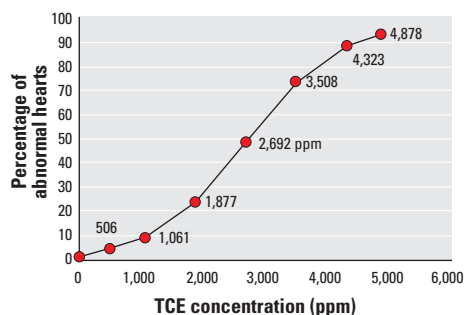


Figure 3. Results of probit analysis.

have identified organogenesis as the most vulnerable period of exposure, and although the experimental studies discussed here cannot be extrapolated directly to humans, many processes of cell division, migration, and differentiation are common to all mammals during fetal development.

TCE exposure *in vitro*, using a chick model, has been shown to affect several elements of epithelial–mesenchymal cell transformation. The endothelial cell–cell separation process that is associated with endothelial activation was blocked by TCE. TCE at concentrations ranging from 50 to 250 ppm also inhibited mesenchymal cell formation. The expressions of the transcription factors Mox-1 and extracellular matrix protein fibrillin 2 were also inhibited (24). This concentration range (50–25 ppm TCE) correlates well with our findings (Figure 1).

As a consequence of its solubility in water (maximum solubility of 1,100 ppm), a great deal of TCE has contaminated groundwater in aquifers and wells, often extending up to 10 km from its original dumping source (28). TCE and other chlorinated hydrocarbons are relatively stable in pure water at a pH of 7 and have extremely long half-lives unless degraded by chlorine removal, hydrolysis, and subsequent aerobic metabolism (28). The highest concentration chosen for this study was the maximum solubility of TCE. The next level was selected at a factor of 1,000 less. This was initially chosen to determine if this low-level dose would elicit a response in the rat model. When results from these two levels demonstrated a cardiac teratogenic potential in the rat model, we decided to test a level similar to that found in the Tucson basin's highest contamination area, 250 ppb. A concentration of 100-fold less was chosen to provide a very low exposure to TCE in drinking water and to attempt to establish a dose relationship.

There is a 35% reduction in the concentration of TCE over a 24-hr period in our study. Therefore, the amounts received by the rats per day (reported as either concentration or dose) were calculated by the average of the breakdown over a 24-hr period and the average amount of drinking water consumed by each group. The levels are given in Table 1. For consistency and ease of reporting, we refer to the levels by the initial concentration. Even the lowest concentration of 2.5 ppb TCE received by the rats is at least four times as high as the average received by humans in drinking water alone in the epidemiology studies, and direct extrapolation cannot be made. The recommended ambient water concentration for TCE is 27 ppb—that is, a factor of 10 lower than the level at which this study demonstrated a trend toward higher incidence of heart defects in fetuses (29).

Given that humans also have additional sources of uptake (e.g., respiratory), the evidence of an overall dose–effect response and a possible threshold level at less than 250 ppb, as shown in this study, should indicate the importance of restricting contamination by this compound to the lowest possible level.

Care must always be taken in extrapolating rodent experimental data to humans. As suggested by other researchers in this field, instead of a straight-line extrapolation model, a threshold model (from high doses in rodents to low doses in humans) may be more appropriate (30). The concentration of TCE range used in this study is large, and dose increases in smaller increments would further delineate the threshold or dose response to TCE. It would be of interest to look closer at the lower concentration levels and use larger numbers of animals to improve statistical significance.

Conclusion

In summary, we present further evidence that drinking water contaminated by TCE is associated with increased incidence of congenital cardiac malformations. This study confirms our previous studies regarding the cardiac teratogenesis, but not general teratogenesis, of TCE when administered in drinking water. The data from Boyer et al. (24) suggest that the cardiac valvular and septal malformations may be caused by TCE inhibiting endothelial separation and early events of mesenchymal cell formation in the developing heart. Moreover, the data from this study reveal a threshold level of less than 250 ppb TCE above which rats exposed to increasing levels of TCE during pregnancy have increasing incidences of cardiac malformations in their fetuses. This information reinforces the importance of adhering to ambient levels of TCE recommended by the U.S. Environmental Protection Agency, which will help to minimize the potential health risks associated with these chemicals.

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