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, 25 ppb, 1.5 ppm, 1.0 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.


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 fetuses 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 rat studies using this model (29). 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 maternal 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 response.

### Table 1. TCE test groups.

<table>
<thead>
<tr>
<th>Initial conc</th>
<th>Avg conc/24-hr drinking water (ppb)</th>
<th>Equivalent avg dose (mg/kg)</th>
<th>No. of maternal rats</th>
<th>Total no. of fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,100 ppm</td>
<td>918,500</td>
<td>129</td>
<td>9</td>
<td>105</td>
</tr>
<tr>
<td>1.5 ppm</td>
<td>1,175</td>
<td>0.218</td>
<td>13</td>
<td>181</td>
</tr>
<tr>
<td>250 ppb</td>
<td>208.75</td>
<td>0.048</td>
<td>9</td>
<td>110</td>
</tr>
<tr>
<td>2.5 ppb</td>
<td>2.09</td>
<td>0.00045</td>
<td>12</td>
<td>144</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>55</td>
<td>606</td>
</tr>
</tbody>
</table>

### Table 2. Types of heart malformations per 100 fetuses.

<table>
<thead>
<tr>
<th>Type of defect/100 fetuses</th>
<th>TCE dose group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.100 ppm</td>
</tr>
<tr>
<td>Abnormal looping</td>
<td>0.33</td>
</tr>
<tr>
<td>Coronal artery/sinus</td>
<td>0.55</td>
</tr>
<tr>
<td>Aortic hypoplasia</td>
<td>1.16</td>
</tr>
<tr>
<td>Pulmonary artery hypoplasia</td>
<td>0.17</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>0.33</td>
</tr>
<tr>
<td>Mitral valve defect</td>
<td>0.33</td>
</tr>
<tr>
<td>Tricuspid valve defect</td>
<td>0.17</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>1.9</td>
</tr>
<tr>
<td>Perimembranous (subaortic)</td>
<td>9/55</td>
</tr>
<tr>
<td>Muscular</td>
<td>11</td>
</tr>
<tr>
<td>Atrioventricular septal defect</td>
<td>606</td>
</tr>
<tr>
<td>Pulmonary valve defect</td>
<td>9/55</td>
</tr>
<tr>
<td>Aortic valve defects</td>
<td>16.4</td>
</tr>
</tbody>
</table>

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 and lipid solubility permit TCE to easily control litters.

Environmental Health Perspectives • Dose–response pattern for treated and

Figure 2. 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).

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 3. Results of probit analysis.

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 teratogenicity 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.

REFERENCES AND NOTES
3. Swan SH, Deane M, Harris J, Neutra R. Cardiac defects in our previous studies regarding the 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).
TCDD and Puberty: Warner and Eskenazi Respond

As Wolff et al. note, in data from the Seveso Women’s Health Study (SWHS) we found no change in age of onset of menarche associated with TCDD exposure in all women in the cohort or in women exposed before 8 years of age (Warner et al. 2004). However, Wolff et al. comment that hormonal exposures before 5 years of age might be the more relevant time period, given that the pubertal transition occurs around 5–7 years of age. Recognizing that our data may be limited by small numbers, Wolff et al. are interested in knowing whether risk of earlier (or later) puberty was seen among girls who were exposed before 5 years of age.

Of the 282 women in the SWHS cohort who were premenarcheal at the time of the explosion on 10 July 1976, 84 women were < 5 years of age. The mean age of menarche reported for the 84 women was 12.6 ± 1.5 years, and the median lipid-adjusted serum TCDD level was 233 ppt (range, 3.6–56,000 ppt). In Cox proportional hazards models, when log10TCDD was entered as the exposure variable, the hazard ratio associated with a 10-fold increase in TCDD was 1.2 (95% confidence interval, 0.98–1.6; p for trend = 0.07). That is, the risk of early menarche was increased with the presence of a 10-fold increase in serum TCDD level (e.g., from 10 to 100 ppt), but not significantly. The data were too sparse in the lower exposure groups to perform categorical analyses. The observed increase was limited to the subset of women who were < 5 years of age at exposure, as the effect was diminished when we considered including older ages (< 6 years, < 7 years).

In summary, the sample size is too small to state with certainty, but it seems that the women who received higher exposure and were < 5 years of age at the time of the explosion may have been at somewhat increased risk for earlier menarche. As we stated in our article (Warner et al. 2004), the women in this study experienced significant TCDD exposure during the postnatal but prepubertal developmental period. Given that animal evidence suggests in utero exposure can affect onset of puberty, continued follow-up of the offspring of the SWHS cohort is important.

The authors declare they have no competing financial interests.

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REFERENCES


Comment on “Breast Milk: An Optimal Food”

In their editorial “Breast Milk: An Optimal Food,” Pronczuk et al. (2004) stated that “in most cases, mothers can and should be reassured that breast milk is by far the best food to give to their babies,” despite the evidence that “a myriad of potential chemical contaminants … can be detected in breast milk,” mainly because a) levels of environmental contaminants, as determined by subsequent surveys, continue to decrease; b) exposure through