A Review: Trichloroethylene Metabolites: Potential Cardiac Teratogens

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This review is of a series of the authors' studies designed to test the hypothesis that administration of trichloroethylene (TCE), dichloroethylene (DCE), their metabolites, and related compounds are responsible for fetal cardiac teratogenesis when given to pregnant rats during organogenesis. Identification of teratogenic compounds will allow more accurate assessment of environmental contaminants and public health risks. Epidemiologic studies and previous teratogenic studies using chick embryos and fetal rats have reported an increased number of congenital cardiac defects when exposed to TCE or DCE during fetal development. Metabolites of TCE and DCE studied in the drinking-water exposure study include trichloroacetic acid (TCAA), monochloroacetic acid, trichloroethanol, carboxymethylcysteine, trichloroacetaldehyde, dichloroacetaldehyde, and dichlorovinyl cysteine. Varying doses of each were given in drinking water to pregnant rats during the period of fetal heart development. Rats receiving 2730 ppm TCAA in drinking water were the only metabolite group demonstrating a significant increase in the number of cardiac defects in fetuses on a per-litter basis ($p = 0.0004$ Wilcoxon test and $p = 0.0015$ exact permutation test). Maternal and fetal variables showed no statistically significant differences between treated and untreated groups. When treated with TCAA the increased cardiac defects, as compared to controls, do not preclude the involvement of other metabolites as cardiac teratogens, but indicates TCAA as a specific cardiac teratogen. Further studies of drinking-water exposure and potential mechanisms of action on the developing heart are proceeding. — *Environ Health Perspect* 106(Suppl 4):995–999 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/995-999johnson/abstract.html*

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Introduction

Halogenated hydrocarbons such as trichloroethylene (TCE), and its metabolic products, are among the most common water supply contaminants in the United States and around the world (*1*). Also, in the presence of natural organic material, chlorination of municipal water to eliminate coliform bacteria produces a number of quantitatively important chlorinated organic compounds including the TCE metabolites chloral hydrate (TCAld), trichloroacetic acid (TCAA), and dichloroacetic acid (DCAA) (*2–4*). The U.S. population consumes chlorinated drinking water, which is a major source of ingestion of these chlorinated organic compounds (*5*). Thus, both contaminated water and municipal water are both potential sources of exposure to TCE and its metabolites.

A previous epidemiologic study in Santa Clara, California, by Swan et al. (*6*) also found an association between these halogenated hydrocarbons and increased incidence of major cardiac malformations in children born to mothers who lived in areas of water contamination. Shaw et al. (*7*) correlated the presence of birth defects in the San Francisco Bay area in California with the level of industrial pollutants by census tract and found an elevation in the risk that infants would be born with malformations of the heart and circulatory system (relative risk = 1.5), even though birth weight (often a reliable index of general fetal toxicity) was not related to pollution levels (*7*).

An epidemiologic study in the Southwestern United States found an association between the presence of TCE and dichloroethylene (DCE) in contaminated water and an increased incidence of major cardiac malformations in children born to mothers residing in contaminated areas (*8*). Although this case–control study demonstrated an association between the halogenated hydrocarbon contaminants and congenital heart defects, it was not designed to establish a cause and effect relationship. As a result, *in vivo* studies were undertaken to determine the possible teratogenicity of TCE and DCE in both avian and rat models.

Avian studies (*9,10*) demonstrated that TCE and DCE had both general and cardiac teratogenic effects on the developing chick. Subsequent controlled studies in the pregnant rat model (*11,12*) determined a significantly increased frequency of cardiac defects in fetuses, as compared to controls, after direct intrauterine exposure to TCE and DCE. Compounds were then administered to the dam in drinking water during the critical days of fetal organogenesis to more closely simulate human exposure conditions. Similar significant increases in cardiac defects were observed. Other researchers have studied chlorinated hydrocarbon effects and those that studied the heart also demonstrated a significant increased frequency of congenital cardiac malformations (*13–21*). A recent study of whole-embryo exposure to TCE and other related compounds (including metabolites) demonstrated embryotoxicity and various developmental abnormalities (*22*).

Our objective was to determine whether the parent compounds (TCE or DCE) or

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Abbreviations used: CMC, carboxymethylcysteine; DCAld, dichloroacetaldehyde; DCAA, dichloroacetic acid; DCE, dichloroethylene; DCVC, dichlorovinyl cysteine; MCAA, monochloroacetic acid; p, pregnancy; p + p, prepregnancy and pregnancy; TCAld, trichloroacetaldehyde; TCAA, trichloroacetic acid; TCEth, trichloroethanol; TCE, trichloroethylene.

one or more of their metabolites showed increased numbers of cardiac defects in fetal rats, as compared to controls, when the pregnant rats were exposed via drinking water. Metabolites tested included TCAA, monochloroacetic acid (MCAA), trichloroethanol (TCEth), carboxymethylcysteine (CMC), TCAld, dichloroacetaldehyde (DCAld), and dichlorovinyl cysteine (DCVC). Based on work by others, the most suspect metabolites were weak acids such as TCAA or MCAA or an alcohol such as TCEth (*4,13–22*).

The initial concentration selection was based on the level of the maximum solubility of TCE (1100 ppm). This was considered the high level, and a concentration level of approximately 1000-fold less was used as a low exposure level (1.5 ppm TCE). The animals were initially given the drinking water during pregnancy and prepregnancy and pregnancy alone to determine if the timing of the ingestion was related to an effect on the heart. The high concentration of DCE (110 ppm DCE) was given in proportion to the levels found in the closed Tucson, Arizona, wells (100-fold less than TCE) (*23*). Again, an approximately 1000-fold lower dose was used as the low dose (0.15 ppm DCE). The initial metabolite concentration selection was based on the maximum level of the metabolite that would be expected to be produced from breakdown of the maximum solubility level of TCE tested (1100 ppm TCE). These concentrations were chosen as initial provocative screening levels, and depending on the results other concentrations may be tested. The concentration (the amount of compound in the drinking water solution) was initially used for reporting because of its ease in comparing to levels found in the contaminated drinking water wells. For comparison with other studies, we converted the levels of concentration into a dose level using the amount consumed per day, the number of days consumed, and the average weight of the rat during the treatment period. Table 1 contains concentration and corresponding dose levels for TCE, DCE, and the metabolites studied.

All studies using animals were conducted in accordance with guidelines established by the Animal Welfare Act (*24*) and in the University of Arizona's approved Association for Assessment and Accreditation of Laboratory Animal Care-accredited facilities.

Review of Study Results

The following review is a compilation of the drinking water studies performed by this laboratory (*12,25*). Data were compiled for treatment groups of all compounds and included maternal observations, *in situ* uterine abnormalities, fetal observations, types of cardiac anomalies, and percent of congenital fetal cardiac anomalies calculated both on a per-fetus basis and a per-litter basis. The results are given in Figure 1 and Table 2.

Table 1. Concentration versus average dosage consumed.

Drinking water dosage	Concentration, ppm	Equivalent dosage, mg/kg/day	Average ml/day/rat
Normal	Water	N/A	46
TCE	1100	129	45
TCE	1.5	0.218	57
DCE	110	10.64	38
DCE	0.15	0.015	38
TCAA	2730	291	38
MCAA	1570	193	21
TCEth	1249	153	53
TCAld	1232	151	42
DCAld	174	21	55
CMC	473	58	55
DCVC	50	6	40

Maternal Observations

Maternal observations included weight gain, pregnancy complications, and uterine examination. All maternal rats in the TCE, DCE, and metabolite studies were healthy, with steady weight gain, throughout the study and without evidence of toxicity. There were no pregnancy complications, and at termination, all uterine and ovarian morphologic examinations were normal.

In Situ Uterine Abnormalities

Uterine examination included inspection for implantation sites (sites of initial attachment of a fertilized ovum, but with no further growth, leaving a yellowish tissue site), resorption sites (sites of implantation with embryonic growth but arrest and necrosis during development as evidenced by the necrotic embryonic tissue that remains), and external inspection of live and dead fetuses. Using Fisher exact analysis, no differences were found between treated groups and controls for the mean number of implantation sites and resorption sites except for the TCAA group, in which a significant increase occurred (1.1 average implantation sites per litter [$p = 0.0006$] for TCAA exposure, as compared to 0.20 average implantation sites/litter for controls and 2.7 average resorption sites/litter [$p = 0.0001$] TCAA, as compared to 0.70 average resorption sites per litter for controls).

Fetal Observations

Fetal observations and measurements included numbers of live or dead fetuses, fetal weight, placental weight, crown–rump length, and external morphology. No significant difference was found when comparing treated and control fetal groups for these observations. There were no gross external or noncardiac internal organ congenital abnormalities found in any treated or control groups.

Cardiac Anomalies

Although several different cardiac malformations were found in the metabolite exposures, no one lesion or grouping predominated. Fetuses exposed to TCE and DCE had cardiac defects that were also diverse and demonstrated no grouping. Cardiac defects in the metabolite study were grouped in general categories and listed by treatment in Table 2. Variations of normal morphology similar to those found in humans were not classified as defects (for example, tricuspid valve leaflet contribution to complete coverage of a membranous ventricular defect). Defects in the metabolite study group were as follows: secundum type atrial septal defects ($n = 16$); hypoplasia of the aorta or pulmonary artery ($n = 9$); aortic valve defects with fused leaflets (bicuspid or tricuspid) creating aortic valvular stenosis ($n = 2$); pulmonary valve stenosis ($n = 6$), including hypoplastic annulus and leaflet adhesions; hypoplastic mitral valve annulus ($n = 6$); tricuspid valve defects ($n = 1$); abnormal looping ($n = 2$); atrioventricular septal defect ($n = 1$); and both perimembranous ($n = 10$) and muscular ($n = 8$) ventricular septal defects. A nearly equal proportion of defects was demonstrated when the defects were divided into left and right heart abnormalities: 11 left-sided versus 13 right-sided defects. Septation defects appear to be more represented than a

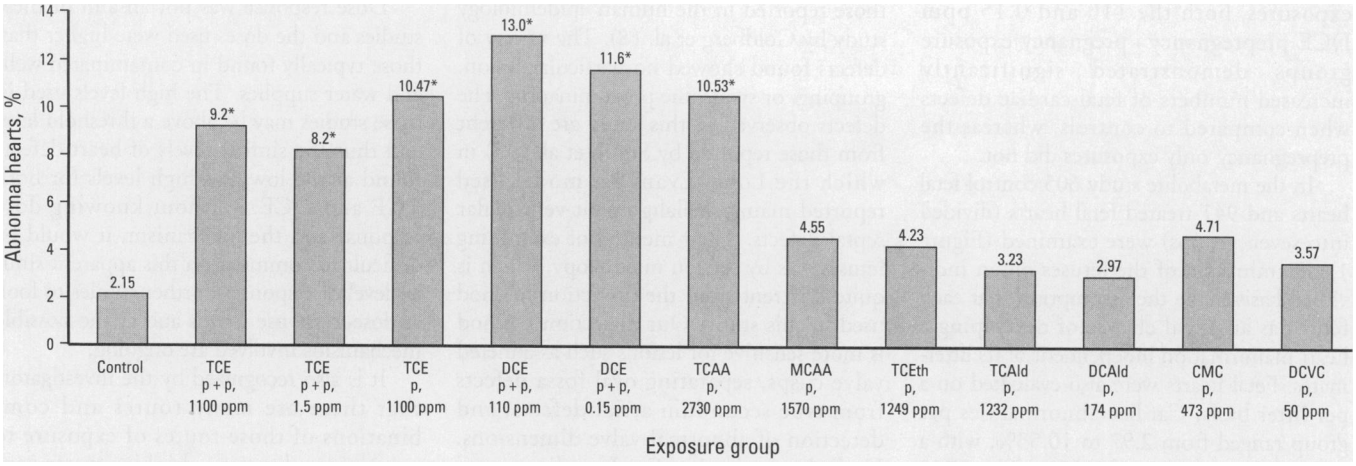

Figure 1. Percent of abnormal hearts (fetal basis). Abbreviations: p + p, prepregnancy and pregnancy exposure; p, pregnancy-only exposure. *Statistical significance between the treated and control group.

Table 2. Congenital cardiac malformations.

Heart abnormalities	Group												
	Normal water	TCE p + p, 1100 ppm	TCE p + p, 1.5 ppm	TCE p, 1100 ppm	DCE p + p, 110 ppm	DCE p + p, 0.15 ppm	TCAA p, 2730 ppm	MCAA p, 1570 ppm	TCEth p, 1249 ppm	TCAld p, 1232 ppm	DCAld p, 174 ppm	CMC p, 473 ppm	DCVC p, 50 ppm
Abnormal looping	2	–	2	–	–	–	–	–	–	–	–	–	–
Aortic hypoplasia	–	1	1	–	1	–	1	–	1	–	1	–	1
Pulmonary artery hypoplasia	–	–	1	–	–	–	2	1	–	–	2	–	–
Atrial septal defects	7	19	5	7	11	7	3	3	–	2	–	–	1
Mitral valve defects, hypoplasia or ectasia	1	5	8	–	4	3	1	–	1	2	–	–	1
Tricuspid valve defects, hypoplasia or ectasia	–	1	1	–	1	–	–	–	1	–	–	–	–
Ventricular septal defects													
Perimembranous[a]	2	6	2	1	4	1	4	–	–	3	–	1	–
Muscular	2	4	–	4	2	1	1	–	1	–	–	2	2
Atrioventricular septal defects	1	–	–	1	1	–	–	–	–	–	–	–	–
Pulmonary valve defects	–	2	1	–	1	–	1	3	1	1	–	–	–
Aortic valve defects	–	2	2	2	2	3	–	–	1	–	–	1	–
Situs inversus	–	1	–	–	–	–	–	–	–	–	–	–	–
Total													
Abnormal hearts	15	41	23	15	25	15	13	7	6	8	3	4	5
Fetuses with abnormal hearts	13	40*	22*	11*	24*	14*	12*	6	5	8	3	4	5
Fetuses	605	434	255	105	184	121	114	132	121	248	101	85	140

[a]Subaortic. *Per-fetus statistical significance (Fisher exact analysis).

difference between lesions of the left and right sides of the heart.

Percent of Congenital Fetal Cardiac Anomalies

In our initial studies, which involved administering TCE and other compounds *in utero* via an intrauterine osmotic mini-pump (Alzet Corp., Inc., Palo Alto, CA), the members of a litter were not necessarily exposed equally to the compounds, as delivery was not related to maternal metabolism. On the recommendations of a biostatistician, these cases were statistically analyzed on a per-litter and per-fetus basis.

The previous study on TCE and DCE in drinking water examined over 1800 fetal rat hearts with a variety of exposure times during prepregnancy and pregnancy. The 1100 and 1.5 ppm TCE prepregnancy + pregnancy exposure groups and the 1100 ppm TCE pregnancy-only group had a significantly increased number of fetal cardiac defects when compared to controls (Figure 1) on a per-fetus and per-litter basis. The groups tested under prepregnancy only showed no statistical significance when compared to controls. Because the initial studies of TCE exposure during prepregnancy + pregnancy and pregnancy only demonstrated similar outcomes, it was decided to test DCE only in the prepregnancy and prepregnancy + pregnancy exposures to determine if pregnancy was the vulnerable stage and prepregnancy exposure had no effect. Similar to the TCE

exposures, both the 110 and 0.15 ppm DCE prepregnancy + pregnancy exposure groups demonstrated significantly increased numbers of fetal cardiac defects when compared to controls, whereas the prepregnancy-only exposures did not.

In the metabolite study 605 control fetal hearts and 941 treated fetal hearts (divided into seven groups) were examined (Figure 1). Examination of the fetuses on an individual basis made the assumption that each fetus has an equal chance of developing a heart malformation independent of its littermates. Fetal hearts were also evaluated on a per-litter basis. Cardiac abnormalities per group ranged from 2.97 to 10.53%, with a control group value of 2.15%. The 2730 ppm TCAA (291 mg/kg/day) during pregnancy group had 10.53% abnormal hearts from a total of 114 fetuses. The malformation rate for the TCAA group was significantly greater than controls on a per-fetus basis ($p = 0.0001$, Fisher exact test) and a per-litter basis [Wilcoxon and exact permutation tests, $p = 0.0004$ and $p = 0.0015$, respectively (*26*)]. As compared to controls, no other group demonstrated a significant increase in cardiac malformations.

Discussion

As demonstrated in previous studies by Dawson et al. (*12*), a significantly increased incidence of cardiac defects as compared to controls occurred when the parent compounds TCE and DCE were administered in a similar rat model. This study also demonstrated that exposure to TCAA, a major metabolite of TCE, administered in drinking water resulted in increased numbers of implantation and resorption sites and selective cardiac teratogenicity.

The logic for our initial dose selection was based on the highest possible level of the metabolite that would be expected to be produced from the breakdown of the maximum TCE dose tested (1100 ppm) (*11*). The drinking-water dose of TCAA given in this study (291 mg/kg/day) was similar to that administered by gavage (330 mg/kg/day TCAA) by Smith et al. (*13*). At levels of 330 mg/kg/day and above in the Smith et al. (*13*) study, a dose-dependent increase in implantation sites and resorption sites and an increase in frequency of soft-tissue malformations (mainly cardiovascular) occurred. Similar effects were observed for the dosage of TCAA used in this study.

The types of cardiac defects produced in this study (Table 2) are consistent with those in our previous reports (*9–11*) and those reported in the human epidemiology study by Goldberg et al. (*8*). The variety of defects found showed no particular lesion, grouping, or syndrome predominating. The defects observed in this study are different from those reported by Smith et al. (*13*) in which the Long–Evans rat model used reported mainly malalignment ventricular septal defects. Their method of examining fetuses was by section microscopy, which is quite different from the dissection method used in this study. Our dissection method is more sensitive for lesions such as adhered valve cusps, separating oval fossa defects from true secundum atrial defects, and detection of abnormal valve dimensions. Until the mechanism for these diverse cardiac defects is determined, it is difficult to speculate on why there is no predominance of one type of lesion. The authors are currently studying these mechanisms.

Although it is accepted that "no animal test and battery of tests will provide complete assurance in the prediction of human teratologic risk" (*27*), the similarities in the timing and sequencing of events during crucial periods of embryogenesis and organogenesis (particularly cardiogenesis) between humans and rats suggest that the rat is a suitable nonprimate choice for studying teratogenic effects on developing fetuses (*27–34*). The Sprague–Dawley rat model was specifically selected because of the very low incidence of spontaneous cardiovascular anomalies (*35,36*) and the general similarity to the human incidence and types of spontaneous cardiac abnormalities occurring (*27–30*). All these factors contribute to the potential importance of these findings and their possible application to the human situation.

It was not within the scope of these studies to determine the mechanisms responsible for these defects, but rather the incidence and types of cardiac defects that occur. Other limitations of this project include certain cardiac and great vessel abnormalities that may have gone undetected, such as coarctation of the aorta, regurgitant valves, and abnormal coronary artery distribution beyond the ostium. It was also not possible to substantiate delivery of compounds to the fetal tissue because of sensitivity limitations of our gas chromatography methods. Failure to demonstrate cardiac defects associated with other metabolites in this study may be due to their lack of teratogenesis or their inability to cross the fetal membranes and/or the rapid clearance of those compounds from the maternal body.

Dose response was not an aim of these studies and the doses used were higher than those typically found in contaminated wells and water supplies. The high levels used in these studies may be above a threshold level and thus the similar levels of heart defects found at the low and high levels for both TCE and DCE. Without knowing dose response and the mechanism, it would be difficult to comment on this apparent similar level of response. Further studies to look at dose–response trends and at the possible mechanisms involved are ongoing.

It is also recognized by the investigators that there are many routes and combinations of those routes of exposure to contaminated water—drinking water consumption, inhalation, and dermal exposure—but because of the constraints of the research study, only drinking water consumption was studied at this time. When all possible routes of exposure are considered the actual dose or concentration of exposure may in fact be higher than that with drinking-water exposure alone. A dose–response study is currently being completed.

The low number of cardiac defects found in non-TCAA, TCE, or DCE groups does not preclude the cardiac teratogenicity of these metabolites, but rather demonstrates that an increase could not be detected at the power level we used. The findings of the study do not prove that human cardiac defects are caused by TCE, DCE, or TCAA; however, these studies raise awareness of the potential cardiac teratogenicity of some halogenated compounds.

These data demonstrate that when TCE, DCE, or TCAA in the doses studied are administered in drinking water to a pregnant rat, specific fetal cardiac teratogenicity occurs. TCAA also results in an increased number of implantation and resorption sites as compared to the control animals. These data therefore support the hypothesis that not only TCE and DCE, the parent compounds, but also a metabolic breakdown product may cause selective cardiac teratogenesis in a mammalian model. Detailed reports have been or will be published (*11,12,25*).

Experimental studies discussed here cannot be extrapolated directly to humans, although many processes of cell division, migration, and differentiation are common to all mammals during gestation. Thus it is anticipated that further investigation may elucidate mechanisms relating gestational exposure to specific halogenated hydrocarbons and congenital cardiac defects.

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