# Data from the Johnson Laboratory (University of Arizona) Should Not Be Used as Evidence of Cardiac Teratogenicity of TCE

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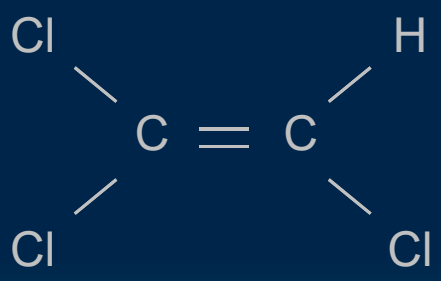
# Trichloroethylene

(Trichloroethene; TCE; TRI)




- **Colorless, halogenated DNAPL**
- **Aqueous solubility: ~1100 ppm (1.1 g/L)**
- **Odor threshold in water: ~ 28 ppm**
- **Vapors mildly addictive**
- **Current MCL is 5 µg/L (5 ppb)**

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# Epidemiological Studies

| Location (Reference) | Evaluated substance | Concentration |
|---|---|---|
| Tucson Valley, AZ (Goldberg et al., 1990) | TCE | 6-239 ppb |
| Northern NJ (Bove et al., 1995) | TCE | 55 ppb |
| Woburn, MA (Lagakos et al, 1986) | TCE (other chlorinated organics were also present) | 267 ppb |
| Milwaukee, WI (Yauck et al., 2004) | TCE | Not indicated |
| Santa Clara, CA (several investigators) | Trichloroethane | 8800 ppb at well head† |
| San Francisco, CA (Shaw et al., 1992) | Hydrocarbon solvents | Not indicated |
| Baltimore/ Washington (Wilson et al., 1998) | Solvents, degreasing agents | Not indicated |

† The conc. in the public in drinking water is likely to be significantly lower.

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## Tucson Valley, AZ

- 6-239 ppb TCE detected in the drinking water in 1981
- Contamination likely began in the 1950's
- Pediatricians noticed that children with a congenital heart defect (CHD) lived in region believed to be a contaminated water area (CWA).
- None of authors has epidemiology credentials

## Goldberg et al., 1990 study

- Interviewed 707 parents of children with a CHD
- Controls for % population in CWA obtained through random telephone interviews (N not indicated)
- Study results
  - 6.8/1000 CHDs in infants of exposed mothers
  - 2.6/1000 CHDs in infants of unexposed mothers
  - 10.8% of households included one person with regular exposure to CWA
  - ~35% of infants with CHD were born to mothers residing in the CWA
  - Following closure of contaminated wells, rate of CHD in former CWA dropped to 4.6/1000

# Limitations of Goldberg et al., 1990 Study

- Assumption of uniform distribution of childbearing families
- Socioeconomic differences not fully considered
- Imprecise delineation of CWA
- Pre- and post-closure CHD rates in CWA are not statistically different
- The background rate of CHDs is ~8/1000

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# Mammalian Studies Suggesting a Positive Correlation Between TCE and CHD

| Reference | Exposure route | TCE exposures | Conc @ ↑ CHD | Animal model and N |
|---|---|---|---|---|
| Dawson et al., 1990 | Intrauterine pump throughout pregnancy | 15 ppm<br>1500 ppm | 1500 ppm* | Sprague Dawley rats<br>N=10-17/group |
| Dawson et al., 1993† | Maternal exposure to drinking water during pregnancy | 1.5 ppm<br>1100 ppm | 1100 ppm* | Sprague Dawley rats<br>Litters/group not reported |
| Johnson et al., 2003† | Maternal exposure to drinking water during pregnancy | 2.5 ppb<br>250 ppb<br>1.5 ppm<br>1100 ppm | --<br>250 ppb§<br>--<br>1100 ppm§ | Sprague Dawley rats (treated)<br>N=9-13/group |

* Statistically significant on a per-fetus basis
§ Statistically significant on a per-litter basis
† Data presented in Dawson, 1993 were again presented in Johnson et al., 2003; Also, both studies use the Dawson method of dissection.

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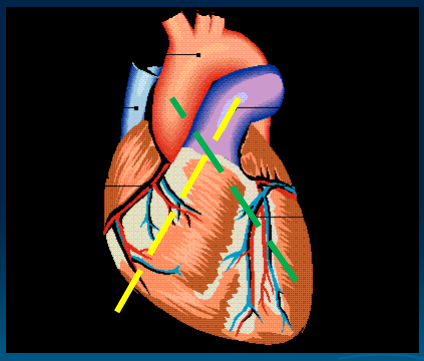
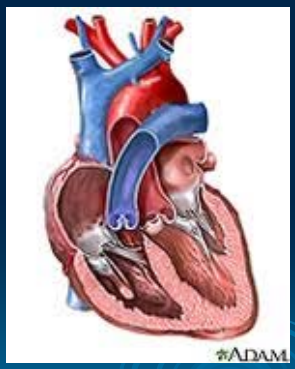
## Problems with Methodology

- None of authors have experience performing DART studies
- Earlier 2 studies used fetus as statistical unit
  - Number of litters not reported
- Unbalanced numbers of litters per group
  - Wanted ~100 fetuses per group (1993)
  - 55 control litters vs. 9-13 treated litters (2003)
- In Johnson (2003), dose range covered 6 orders of magnitude
- Created a new method for examining hearts

# Standard Method of Heart Evaluation



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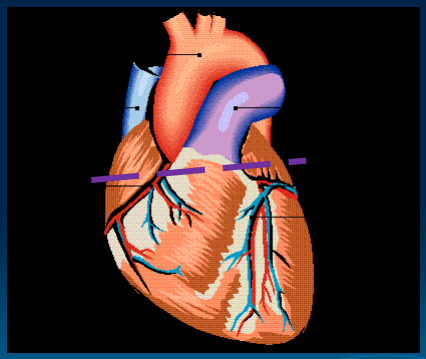
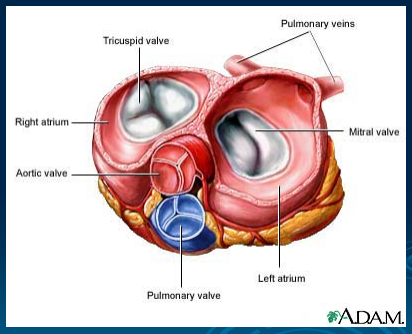
# Dawson Method of Heart Evaluation






Changed procedures with subsequent studies (e.g., flushing of hearts in situ vs. removal of hearts first; use of dye)

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# Problems with Results

- Heart valves are fragile tissues
  - Easily damaged when manipulated
- Findings are subjective
- Observers not blinded
- No historical data on findings by new method

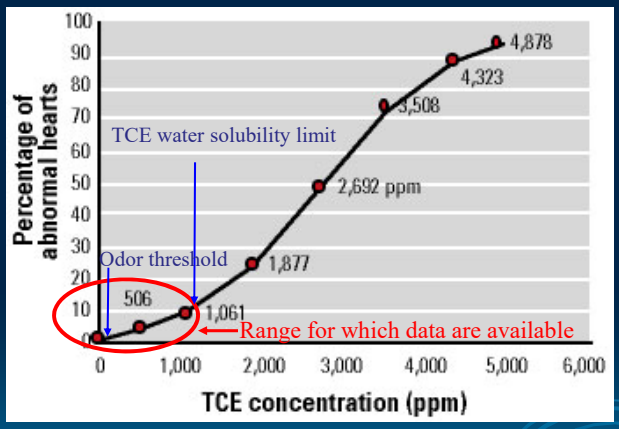
# Comparison of Dawson (1993) with Johnson (2003)

| | TCE dose | | | TCE dose | |
|---|---|---|---|---|---|
| Cardiac abnormalities (Dawson et al. 1993) | 1.5 ppm | 1,100 ppm | Heart malformations[b] (Johnson et al. 2003) | 1.5 ppm | 1,100 ppm |
| L-Transposition (left chest) | 1 | 0 | Abnormal looping | 2 | 0 |
| Great vessel defect | 1 | 0 | Aortic hypoplasia | 1 | 0 |
| Pulmonary valve defect | 1 | 0 | Pulmonary artery hypoplasia | 1 | 0 |
| Atrial septal defect | 4 | 7 | Atrial septal defect | 4 | 7 |
| Ventricular septal defects | | | Ventricular septal defects | | |
| Subaortic | 2 | 1 | Perimembranous (subaortic) | 3 | 3 |
| Muscular | 1 | 4 | Muscular | 1 | 1 |
| Endocardial cushion defect | 0 | 1 | Atrioventricular septal defect | 0 | 1 |
| Aortic valve defect | 0 | 2 | Aortic valve defect | 0 | 2 |
| No. with abnormal hearts | 9 | 11 | | 9 | 11 |
| No. fetuses examined | 181 | 105 | | 181 | 105 |

Modified from Hardin et al, 2004

# Predicted Dose-response Curve

Based on data from University of Arizona Goldberg/Johnson/Dawson laboratory with the exception of the Fisher et al., 2001 study




Johnson et al., 2003: "A probit analysis of the frequency of abnormal hearts in each group was done to identify the dose-response curve. Probit analysis was performed with logit transformation with the natural response rate calculated from the rate seen in the control group."

# All animal data reporting heart defects with TCE come from one laboratory

- Data from that lab were accumulated over ten years (Johnson et al 2003; Dawson et al 1993)
- Deficiencies in study design and reporting make the interpretation of data tentative at best
- Major effect was increased incidence of atrial septal defects (or the foramen ovale, which closes around the time of birth)
- May be related to examination procedure or possible delays in development, rather than actual heart defects.

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## Animal Studies Which Do Not Indicate a Positive Association Between TCE and CHD

TCE inhalation:

| Reference | Exposure route | Vapor TCE Conc | Animal Model/N |
|---|---|---|---|
| Schwetz et al., 1975 | Maternal inhalation of vapors 7 hrs/day during pregnancy | 300 ppm | SD rats, 20-35/group<br>Swiss Webster mice<br>N=30-40/group |
| Dorfmeuller et al., 1979 | Maternal inhalation before, during, or before and during pregnancy<br>(-14 days thru GD 20) | 1800 ± 200 ppm | Long Evans rats<br>N=30/group |
| Hardin et al., 1981 | Maternal inhalation of vapors for 6-7 hrs/day during pregnancy (GD 1-19 rat; 1-24 rabbit) | 500 ppm | SD and Wistar rats<br>N=20-35/group<br>New Zealand rabbits<br>N=15-20/group |
| Healy et al., 1982 | Maternal inhalation for 4 hrs/day during pregnancy (GD 8-21) | 100 ppm | Wistar rats/group<br>N=31-32/group |
| Carney et al, 2006 | Maternal inhalation 6 hrs/day during GD 6-20 | 50, 150, 600 ppm | SD rats, 20/group |

## Animal Studies Which Do Not Indicate a Positive Association Between TCE and CHD, Continued

TCE Oral exposure:

| Reference | Exposure route | TCE Conc. | Animal Model/N |
|---|---|---|---|
| National Toxicology Program, 1985 | Daily maternal oral gavage during pregnancy | 100, 300, and 700 mg/kg/day TCE† | ~20 Swiss CD-1 mice/group |
| National Toxicology Program, 1986 | Daily maternal oral gavage during pregnancy | 76, 156, and 289 mg/kg/day TCE† | ~20 Fisher 344 rats/group |
| Cosby and Dukelow, 1992 | Daily maternal oral gavage on GD 1-5, 6-10, or 11-15 | 24 and 240 mg/kg/day† | ~10 B6D2F1 mice/group |
| Fisher et al., 2001 | Daily maternal oral gavage GD 6-15 | 500 mg/kg/day TCE† | 20 Sprague Dawley rats per group |

† TCE doses exceed those in Dawson 1990,1993 and Johnson, 2003

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**Trichloroethylene, Trichloroacetic Acid, and Dichloroacetic Acid: Do They Affect Fetal Rat Heart Development?**

Jeffrey W. Fisher,[1] Stephen R. Channel,[1] Jeffrey S. Eggers,[1] Paula D. Johnson,[2] Kathleen L. MacMahon,[1] Chuck D. Goodyear,[3] Gregory L. Sudberry,[1] D. Alan Warren,[1] John R. Latendresse,[4] and Linda J. Graeter[4]

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# Dose Groups and Sizes from Fisher et al, (2001)

**TABLE 1**
Pregnant rat treatment groups and the number of dams and fetuses per dose group

| Treatment group | Dose (mg/kg/day) | Number of dams | Number of fetuses |
|---|---|---|---|
| Trichloroethylene | 500 | 20 | 292 |
| Trichloroacetic acid | 300 | 19 | 269 |
| Dichloroacetic acid | 300 | 20 | 303 |
| Retinoic acid | 15 | 12 | 160 |
| Soybean oil—control | — | 25 | 378 |
| Water—control | — | 19 | 275 |

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# Advantages of Fisher et al (2001)

- Exposures included TCE metabolites that may be developmentally toxic as well as a positive control
- Used Johnson dissection method (Paula Johnson performed dissections and was co-author)
- Investigators were blinded

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# How to Decide

(Watson et al, 2006)

## Does TCE cause a specific type of CHD?

| Reference | Concentration of evaluated substance | Study type | Predominant type of CHD reported |
|---|---|---|---|
| Tikkanen and Heinonen, 1988 | No specific concentrations of compounds indicated; study examines effect of hydrocarbon solvents | Epidemiological; Case-control | • Ventricular Septal Defect (VSD; specific type not indicated) |
| Smith et al., 1989 | 300-1,800 mg/kg/day trichloroacetic acid | Animal study: Long Evans rat | • Levocardia<br>• VSD (specific type not indicated) |
| Smith et al., 1992 | 14-2,400 mg/kg/day of dichloroacetic acid (DCA) | Animal study: Long Evans rat | • Levocardia<br>• Defects between ascending aorta and right ventricle<br>• VSD (specific type not indicated) |
| Epstein et al., 1992 | 1,900-3,500 mg/kg/day DCA | Animal study: Long Evans rat | • VSD type I<br>• VSD type II |

## Predominant types of developmental mechanisms

- Cellular migration
- Extracellular matrix formation
- Hemodynamics
- Targeted growth
- Cell death
- Visceral situs

## Distribution of CHD types and the Developmental Process Disturbed in the General Population

| Type of Congenital heart defect | % CHD patients with this defect | Developmental Process Disturbed |
|---|---|---|
| Ventricular septal defects | 15-50% | 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 |

Information from this table was obtained from statistics provided by the American Heart Association (2005b) and Hoffman and Kaplan, 2002.

## Does TCE Perturb a Particular Developmental Process?

| Reference | Concentration of evaluated substance | Type of Study | Predominant rype of CHD‡ reported | Developmental process likely to be disturbed |
|---|---|---|---|---|
| Tikkanen and Heinonen, 1988 | No specific concentrations of compounds indicated; study examines effect of hydrocarbon solvents | Epidemiological: Case-control | • Ventricular Septal Defect (VSD; specific type not indicated) | • Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics |
| Smith et al., 1989 | 300-1,800 mg/kg/day trichloroacetic acid | Animal study: Long Evans rat | • Levocardia<br>• VSD (specific type not indicated) | • Positional information and cardiac looping<br>• Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics |
| Smith et al., 1992 | 14-2,400 mg/kg/day of dichloroacetic acid (DCA) | Animal study: Long Evans rat | • Levocardia<br>• Defects between ascending aorta and right ventricle<br>• VSD (specific type not indicated) | • Positional information and/or cardiac looping<br>• Cell migration and/or extracellular matrix formation<br>• Hemodynamics, cell migration, extracellular matrix formation, cell death, and hemodynamics |
| Epstein et al., 1992 | 1,900-3,500 mg/kg/day DCA | Animal study: Long Evans rat | VSD type I<br>VSD type II | • Cell migration<br>• Hemodynamics |

# Conclusions

- The aggregate available data do not support the concept that TCE exposure increases the risk of CHDs
- Those data used to posit a positive correlation between TCE and CHDs are from flawed studies and/or were performed with exceptionally high concentrations of TCE that are not relevant to expected environmental exposures

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