Fetal Cardiac Findings in Rats Exposed to Trichloroethylene (TCE) in Drinking Water

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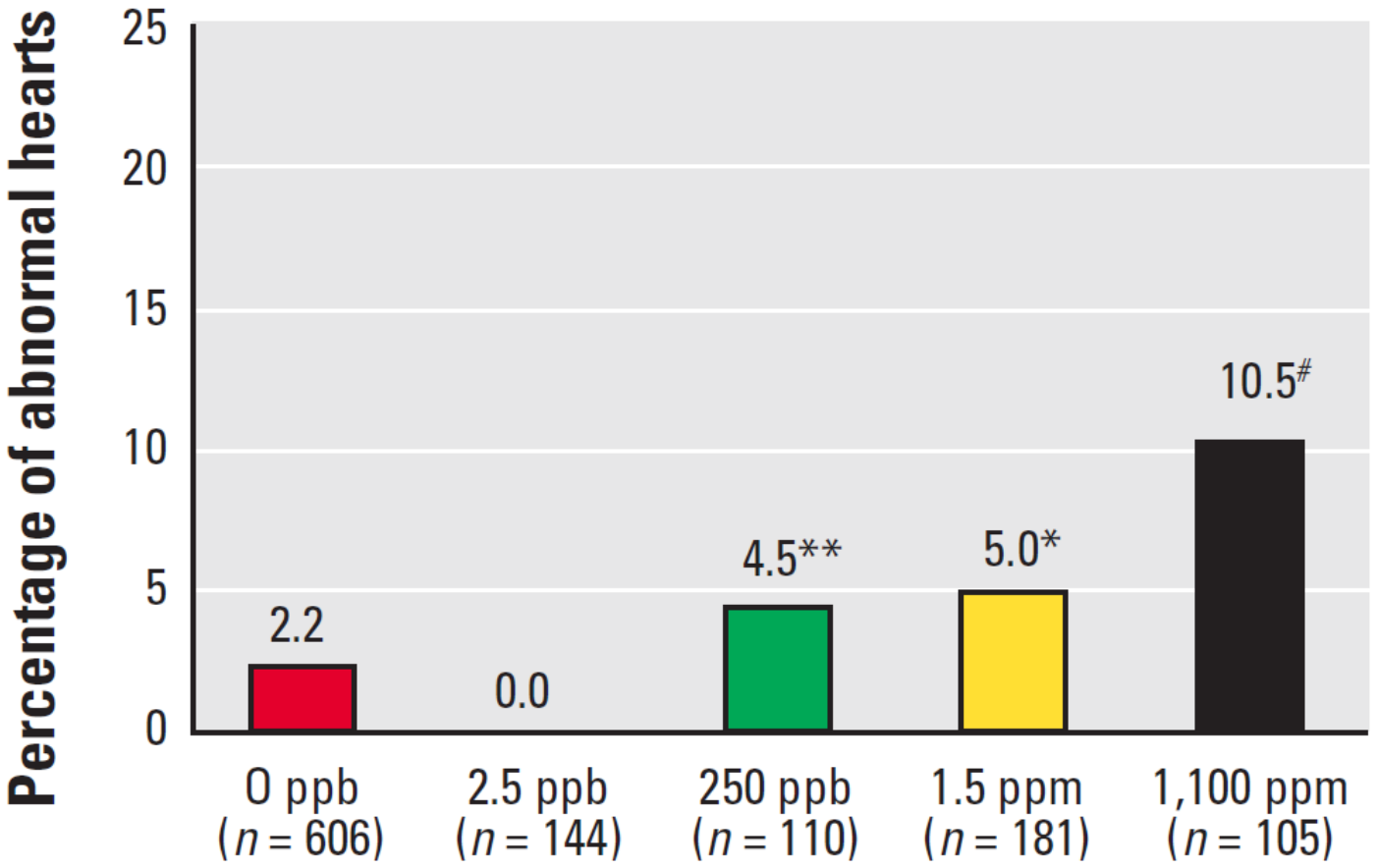
Issue: Increased Cardiac Malformations in Rats Treated with TCE in Drinking Water (Johnson *et al.*, 2003)



From: Johnson *et al., Env. Health Perspect.* 111: 289-292 (2003)

Concerns with Johnson *et al.* (2003)

- 1.5 and 1,100 ppm data published earlier (Dawson *et al.,* 1993).
- Highly unusual dose-response: positive responses over 4,400X dose range.
- Subject to two *errata* (Johnson *et al.,* 2005, 2014) and one letter-to-the-editor correction (Johnson *et al.*, 2004).
- Small study size: 9-13 treated dams per dose.
- Likely lack of concurrent controls preventing matching of per litter incidence treated responses with concurrent control incidence data.
- Non-standard cardiac evaluation: fixation/dissection with manipulation of heart to assess valvular function; technique changed with time.
- Raw data not available for regulatory or public review.

Implications of Use of Johnson *et al.* (2003) for Regulatory Evaluation, e.g., RfD, RfC development

Regulatory Implications – EPA IRIS (2014)

- Based on Johnson study, EPA TCE IRIS set RfC = 0.4 ppb and RfD = 0.0005 mg/kg/day.
- Indoor air exposure exceedances are primarily due to vapor intrusion.

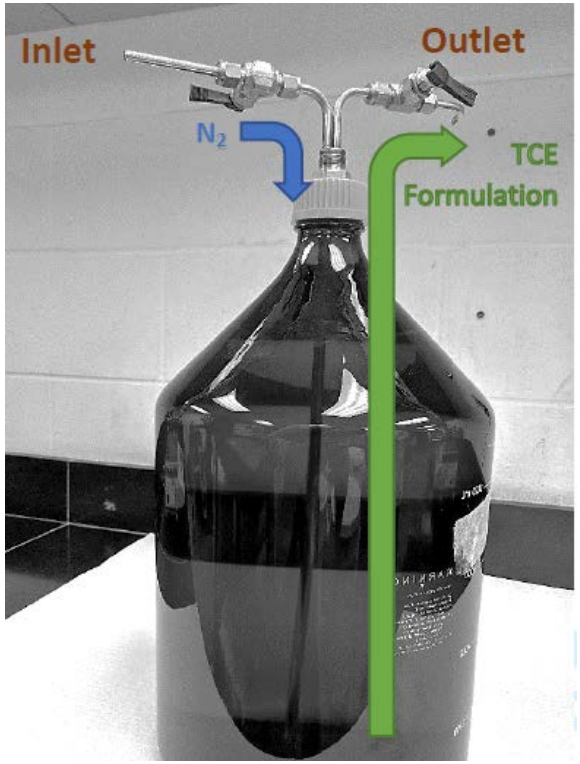
Reproducibility Implications – Other GLP-Quality Studies

- Negative findings with oral gavage TCE (500 mg/kg/day) or TCE oxidative metabolite, TCA (300 mg/kg/day (Fisher *et al.*, 2001)
  - Included Johnson as co-investigator, using Johnson exam techniques
- Negative findings with inhalation TCE (500 ppm) (Carney *et al.*, 2006)

HSIA Drinking Water Repeat Study

- Drinking water doses similar to Johnson *et al.* (2003): 250, 1,500, 500 and 1,000 ppm TCE (1,000 ppm just below TCE water solubility limit)
- 24 pregnant Sprague-Dawley rats per dose group, exposed to TCE in drinking on Gestation Days 1-21.
- Detailed attention to TCE drinking water concentrations due to volatility concerns.
- Focused attention on cardiac evaluations (fresh dissection).
- Retinoic acid used as positive control.
- TCE and TCA determined at various times in maternal and fetal blood.

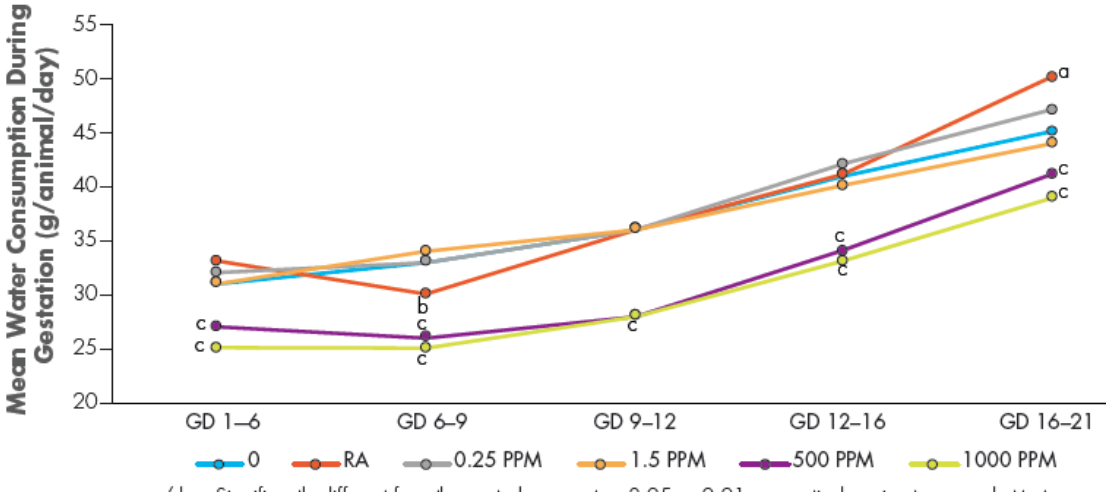
Drinking Water Dose Preparation and Confirmation

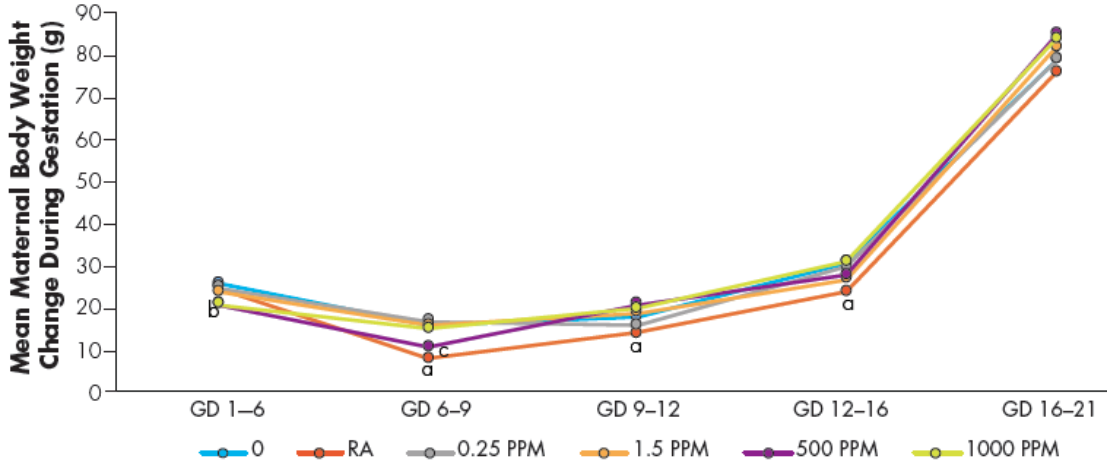


TCE concentrations (% target) in drinking water at:

- Dose preparation: 90 – 130%
- Cage bottle (initial): 94 -- 166%
- Cage bottle (24 hr): 32 – 49%

TCE Decreased Dam Drinking Water Consumption but Body Weights are Similar: Non-Adverse Finding





TCE Drinking Water Treatment did not Affect Ovarian and Uterine Parameters

TCE (ppm)	No. Pregnant Females	Mean No. Corpora Lutea	Mean No. Implantation sites	Pre-Implantation Loss (%)	Resorptions		Dead Fetuses	Post Implantation Loss (%)	Mean No. Viable Fetuses	Mean Fetal Weights (g)
					Early	Late				
0	24	15.0 ± 2.7	13.7 ± 4.4	1.3 ± 2.2	0.8 ± 1.2	0.0 ± 0.2	0	0.9 ± 1.4	12.8 ± 4.3	6.0 ± 0.3
0.25	23	15.6 ± 2.6	13.2 ± 3.4	2.4 ± 4.7	1.2 ± 2.5	0.0 ± 0.0	0	1.2 ± 2.5	12.0 ± 4.1	6.2 ± 0.3
1.5	24	15.1 ± 2.1	13.8 ± 3.2	1.3 ± 1.8	0.5 ± 0.9	0.0 ± 0.0	0	0.5 ± 0.9	13.4 ± 3.1	5.9 ± 0.4
500	24	15.4 ± 1.9	14.4 ± 1.8	1.0 ± 1.2	0.7 ± 1.1	0.0 ± 0.0	0	0.7 ± 1.1	13.8 ± 2.2	6.0 ± 0.3
1,000	24	16.3 ± 2.2	14.9 ± 2.5	1.3 ± 2.2	0.6 ± 0.7	0.0 ± 0.2	0	0.7 ± 0.8	14.3 ± 2.4	5.9 ± 0.3

Data are presented as mean + standard deviation, where appropriate.

TCE in Drinking Water did not Increase Cardiac Ventricular Septal Defects (VSDs)

Fetal Parameter	TCE Concentration					Positive Control
	0 ppm	0.25 ppm	1.5 ppm	500 ppm	1000 ppm	RA 15 mg/kg/day
Available Fetuses (Litters)	308 (24)	275 (22)[a]	321 (24)	330 (24)	342 (24)	269 (25)
Affected Fetuses (Litters)	7 (5)	4 (4)	5 (3)	13 (8)	12 (6)	112 (23)
Mean Litter Proportion (% per Litter)	2.4	1.4	1.5	3.8	3.7	42.2**
No. Fetuses (Size of Opening)	6 (<1mm) 1 (1–2mm)	All (<1mm)	All (<1mm)	All (<1mm)	All (<1mm)	103 (<1 mm) 8 (1–2 mm) 1 (>2 mm)
Location of Opening	Mem	Mem	Mem	Mem	Mem	Mem (111 fetuses) Mus/Mem (1 fetus))

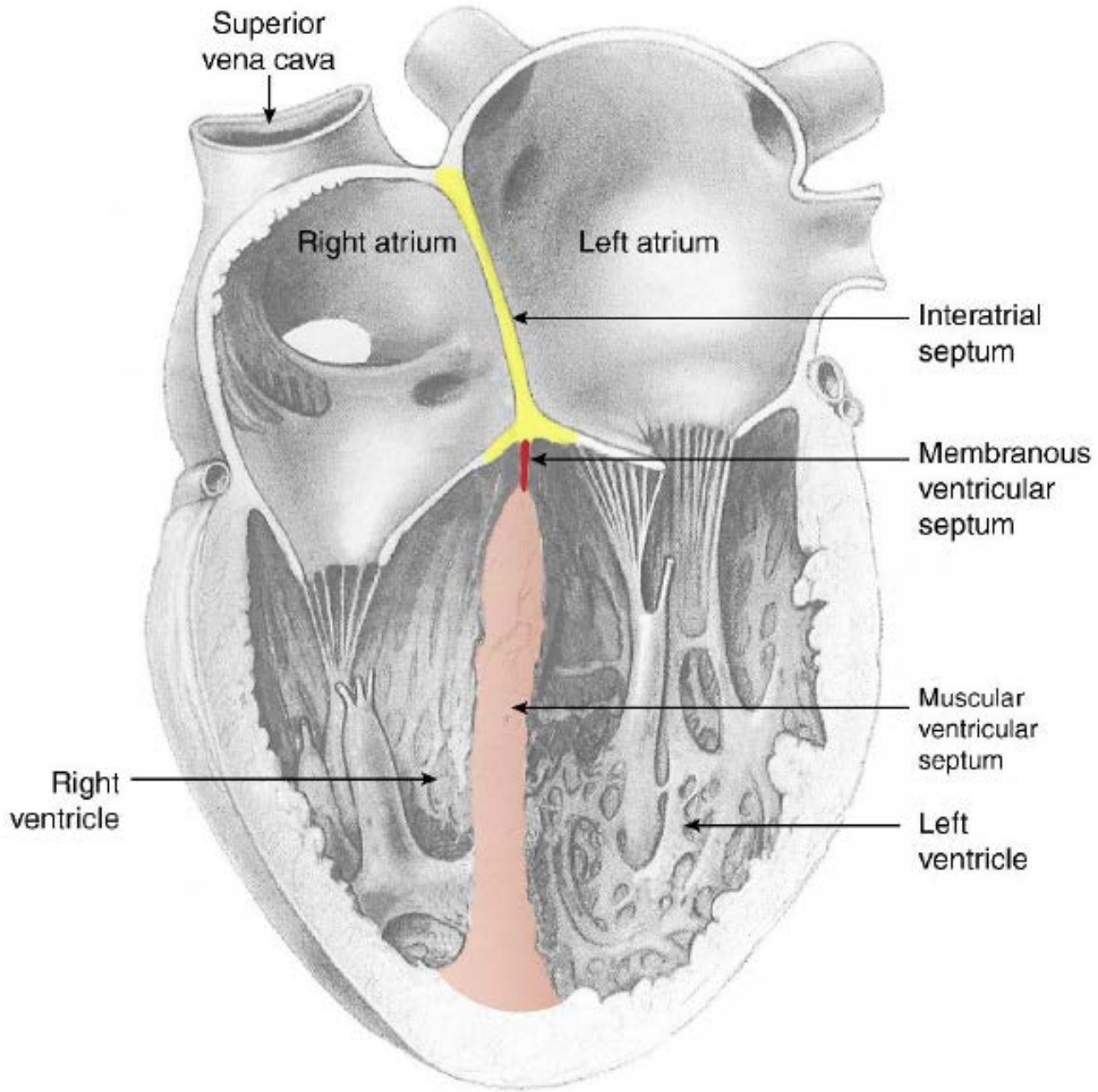
[a] One female had a very early total litter resorption and therefore had no available fetuses

** Significantly different from vehicle control group at p≤0.01

Mem = Membranous portion of ventricular septum

Mus/Mem = Muscular and membranous portions of ventricular septum

Location of Ventral Septal Defects



VSD Incidence in Control S-D Rats Using Enhanced Cardiac Evaluations

Study	Ventricular Septal Defects (%)
Haring, 1964[a]	3.2
Haring, 1966[a]	2.8
Inomata & Yasuda, 1971[b]	5.2
Inomata & Yasuda, 1971[b]	3.6
Solomon et al., 1997	2.4
Current Study[c]	2.4

[a] Hearts were embedded in paraffin, serially sectioned and examined by light microscopy

[b] Examined by a combination of Wilson freehand razor sections plus microdissection of the cardiac outflow tract

[c] Examined by fresh dissection

Health Risk Implications of Small (< 1 mm) VSDs in Rats and Humans

- VSDs in control and TCE-treated rats were all < 1 mm in size.
- A 2.4% incidence of < 1 mm VSDs present in untreated near-term SD rat fetuses was completely resolved at weaning (Solomon *et al.*, 1997).
- Trimethadione increased small VSDs in rat fetuses that were resolved at weaning while large VSDs were not, indicating closure of VSDs depends on the severity of the lesion at term (Fleeman *et al.*, 2004).
- Non-statistically significant formation of VSDs of < 1 mm in TCE drinking water treated rats is not an adverse health risk.

Comparison of TCE & TCA Drinking Water Plasma Levels to Other Inhalation or Oral Treatments

- TCE Non-Detects in drinking water dosing indicates parent TCE is not a dosimetrically plausible teratogen as postulated by Johnson *et al.* (2003).
- Higher TCE and TCA levels after inhalation and gavage doses indicates that an absence of cardiac malformations by these routes was not due to insufficient systemic TCE/TCA dosing.

Plasma TCE/TCA	Peak Plasma Concentration (µg/ml)				
	TCE				TCA
	Drinking Water[a] (0.25 & 1,000 ppm)	Drinking Water[b] (350 ppm)	Inhalation[b] (600 ppm, 4 hr/day)	Gavage[b] (2.3 mg/kg)	Gavage[c] (98 mg/kg)
TCE	ND	ND	24	0.26	----
TCA	1.1-1.2	2.8	13	25	201

[a] Current study, ND = Below LOD (50 ng/ml); TCA detected only 500 & 1000 ppm, not at 0.25 & 1.5 ppm.

[b] Fisher *et al.* (1989); TCE & TCA measured in pregnant GD21 SD rats.

[c] Larson & Bull (1992); TCA measured in adult male rats.

Conclusions: Risk Assessment Implications

- TCE ingestion in drinking water at high concentrations of 1000 ppm (close to limit of water solubility) does not cause cardiac defects in rats.
- The findings of Johnson *et al.* (2003) are not reproduced in a high-quality GLP and regulatory guideline-consistent study.
- The drinking water plasma toxicokinetic data further enhance the conclusion that TCE is not a cardiac teratogen even under conditions of high-dose inhalation or gavage dosing resulting in substantially higher plasma TCE and TCE concentrations.

RELIABLE EVIDENCE INDICATES TCE IS NOT A CARDIAC TERATOGEN AFTER DRINKING WATER, INHALATION OR GAVAGE EXPOSURES