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

John M. DeSesso[1], Christopher Bevan[2], James S. Bus[1]

[1]Exponent, [2]Halogenated Solvents Industry Alliance

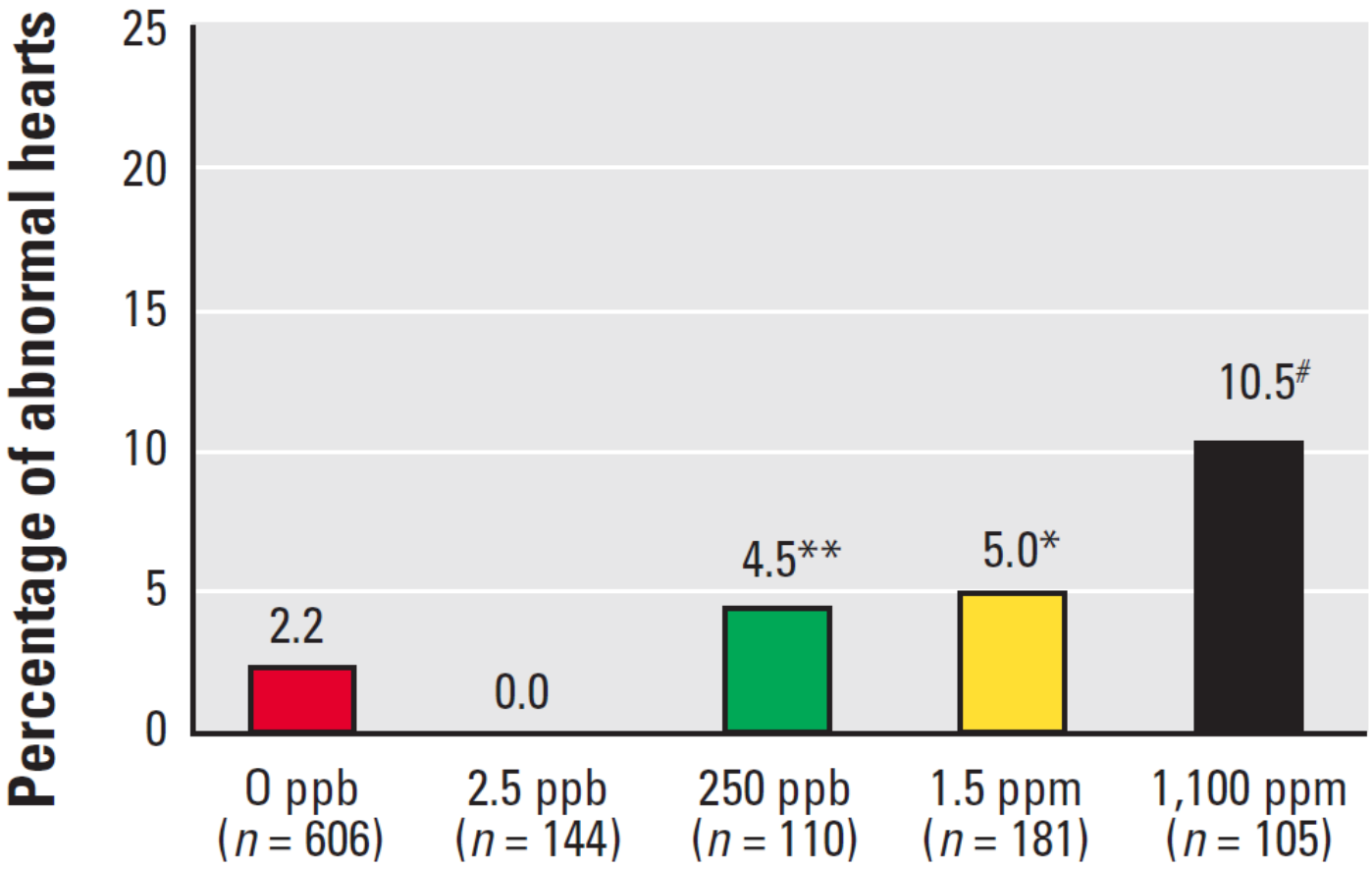
Presented to:

United States Environmental Protection Agency

Washington, DC

30 September 2019

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

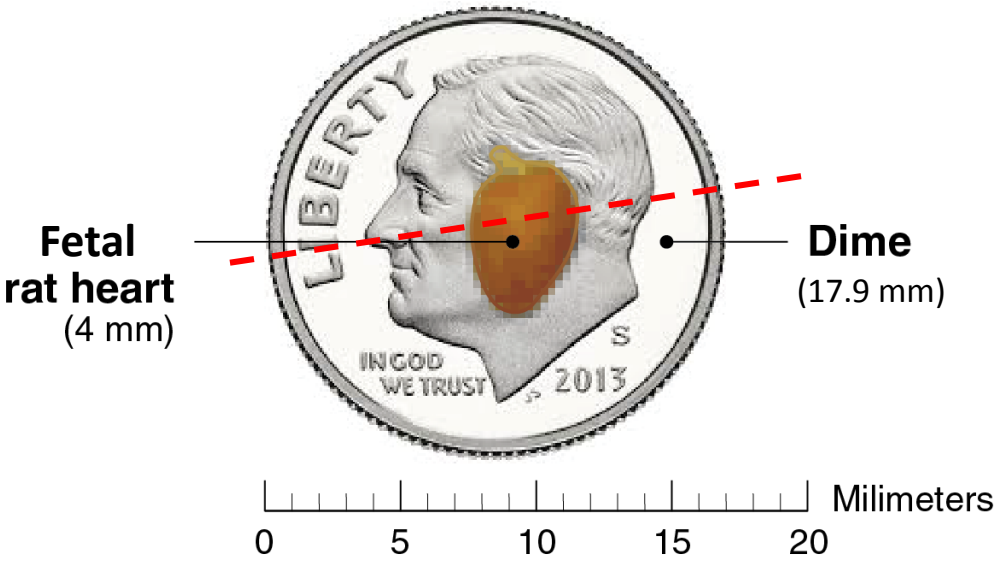


From: Johnson *et al., Env. Health Perspect.* 111: 289-292 (2003)

Concerns with Johnson *et al.* (2003)

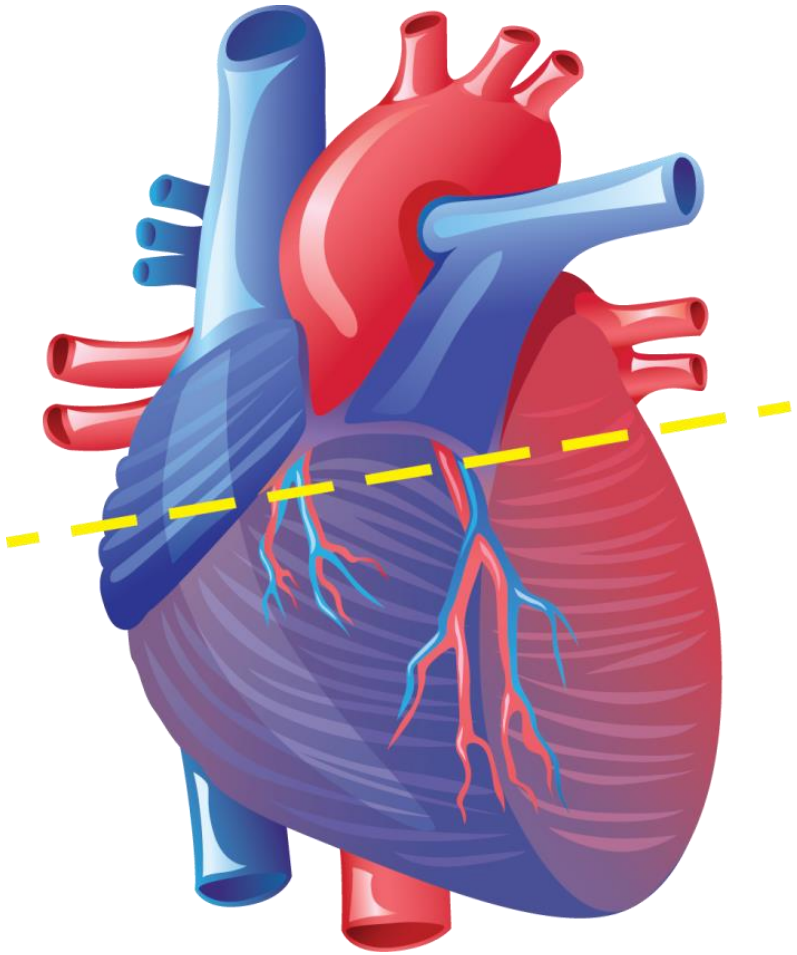
- Small study size:
  - 9-13 treated dams per dose; 55 control dams.
- 1.5 and 1,100 ppm data published earlier (Dawson *et al.,* 1993).
  - 1.5 ppm was reported as not statistically different from control.
  - Statistics used fetus as statistical unit.
- Likely lack of concurrent controls preventing matching of per litter incidence treated responses with concurrent control incidence data.
- Highly unusual dose-response:
  - Positive responses claimed over 4,400X dose range.
- 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.
- Subject to two *errata* (Johnson *et al.,* 2005, 2014) and one letter-to-the-editor correction (Johnson *et al.*, 2004).

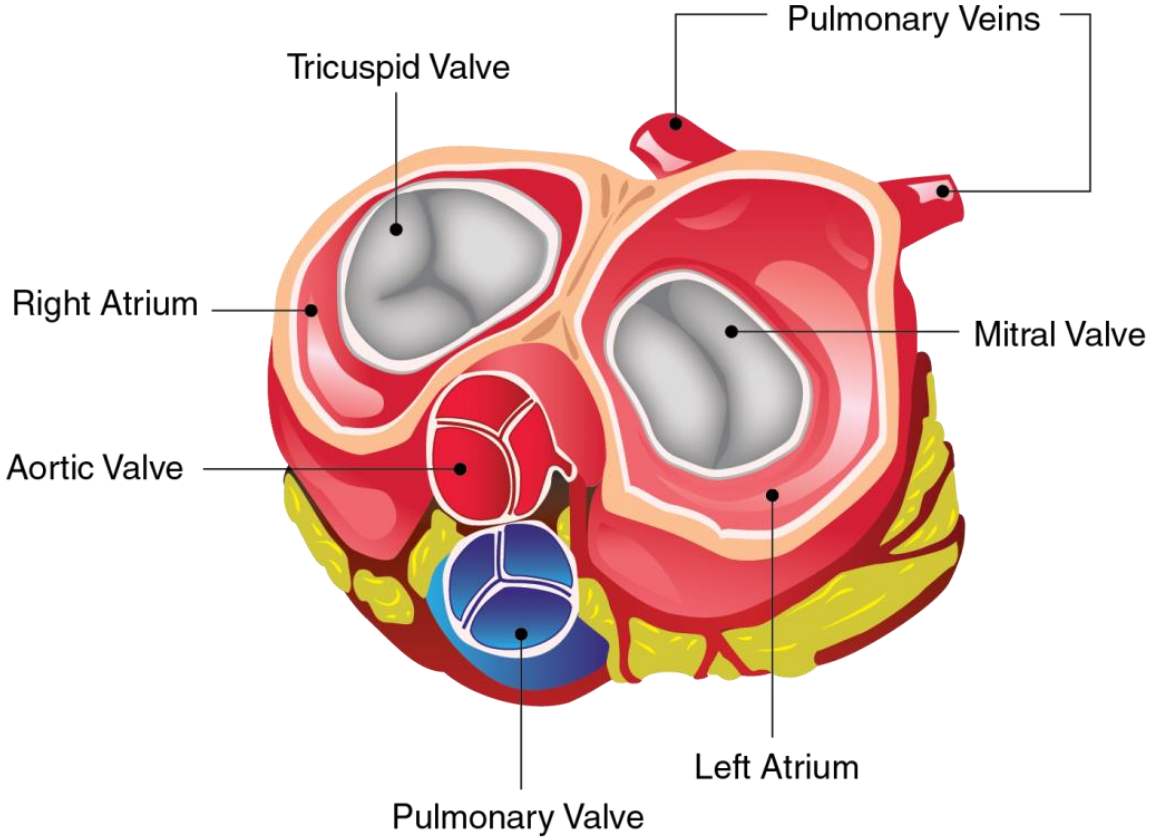
Perspective on Size of Fetal Rat Heart

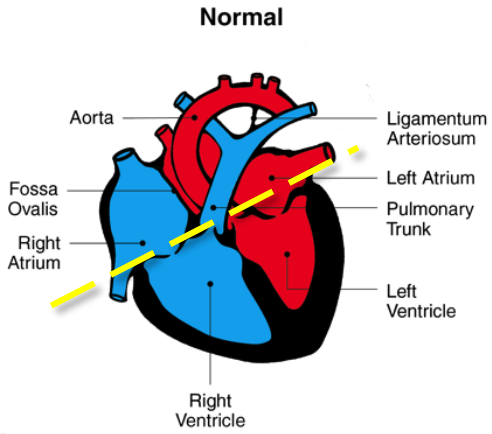


- Dotted line denotes the approximate level where atria were removed using the Johnson method

Johnson Heart Evaluation Method

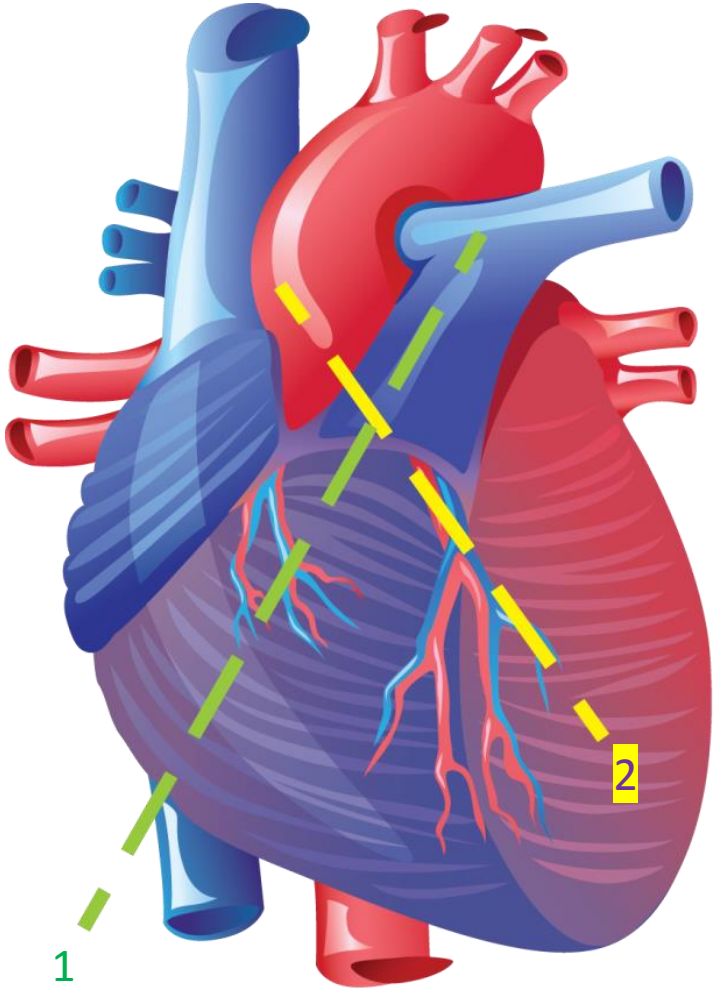


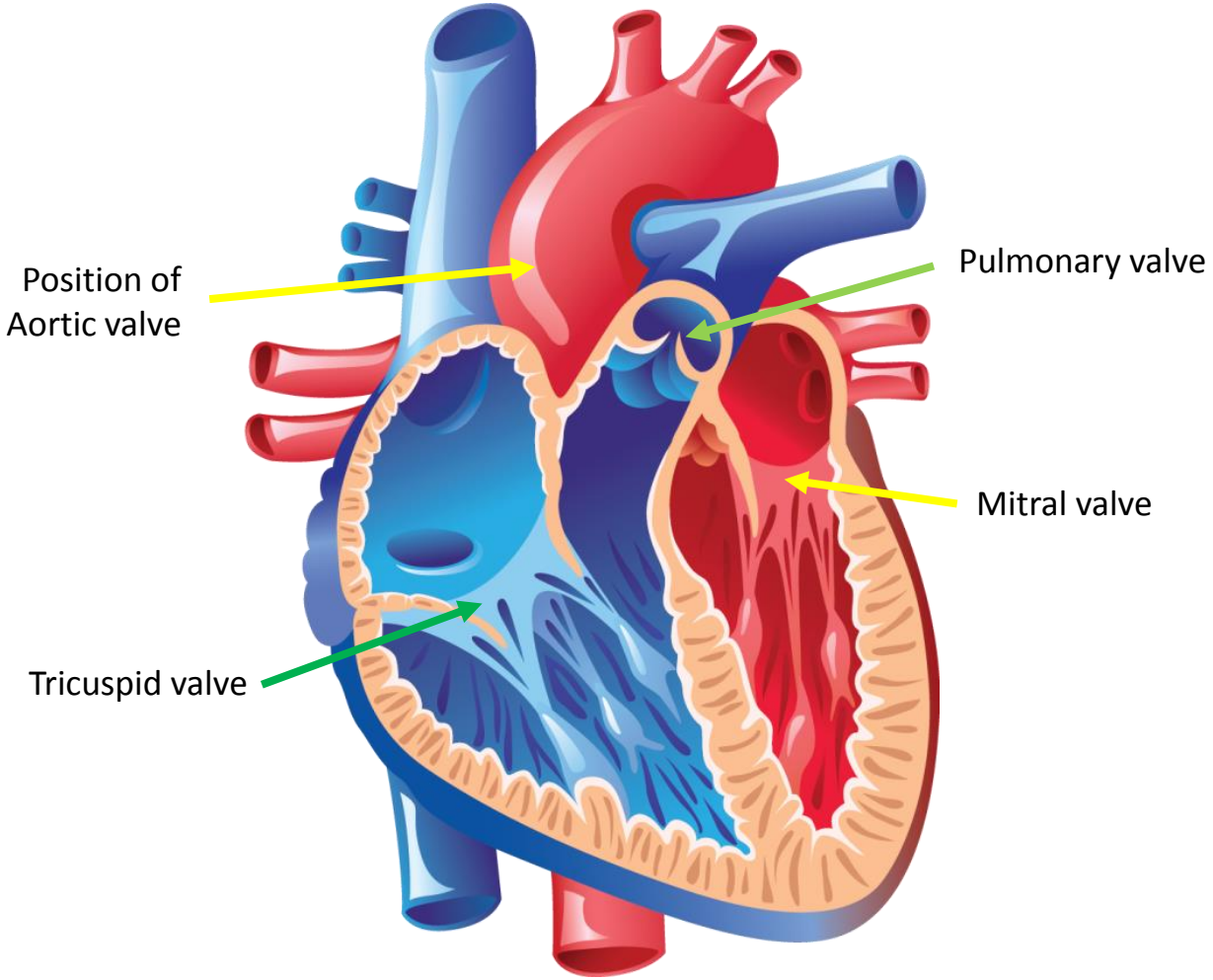




- Dotted line denotes position at which atria were removed
- Right image shows view of valves from above with atria removed

Standard Method of Heart Evaluation





- Green dotted line denotes position of first incision revealing right ventricle, tricuspid and pulmonary valves
- Yellow dotted line shows position of second cut revealing left ventricle, mitral and aortic valves
- Right image shows interior of heart and relative position of atria, ventricles, and all valves

Interpretation Challenges

- Hearts were irrigated with fixative prior to dissection
- Observers not blinded
- “Abnormal heart” scored if a septum, valve, or great vessel had any anomaly
- Heart valves are fragile tissues
  - Fixative in solutions dehydrate and stiffen valves
  - Easily damaged when manipulated
- Findings are subjective
- No historical data on findings by new method

Distribution of Cardiovascular Anomalies Reported in Johnson et al., 2003

TCE (ppm)	No Dams	No Fetuses	No Hearts	Abnormal Hearts	ASD	VSD	Valves				Transp Grt Ves	Large Coron Sinus
							Mitral	Tricusp	Aortic	Pulmon		
1,100	9	105	94	11	7	5	-	-	2 (1 w/hole)	-	-	-
1.5	13	181	172	9	4	3	-	-	-	-	2 (1=partial)	-
0.25	10	110	105	5	-	-	1	2	1	-	-	1
0.025	12	144	144	0	-	-	-	-	-	-	-	-
0	55	606	593	13	7	5					-	-

Septa

Atrioventricular valves

Great vessel valves

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.

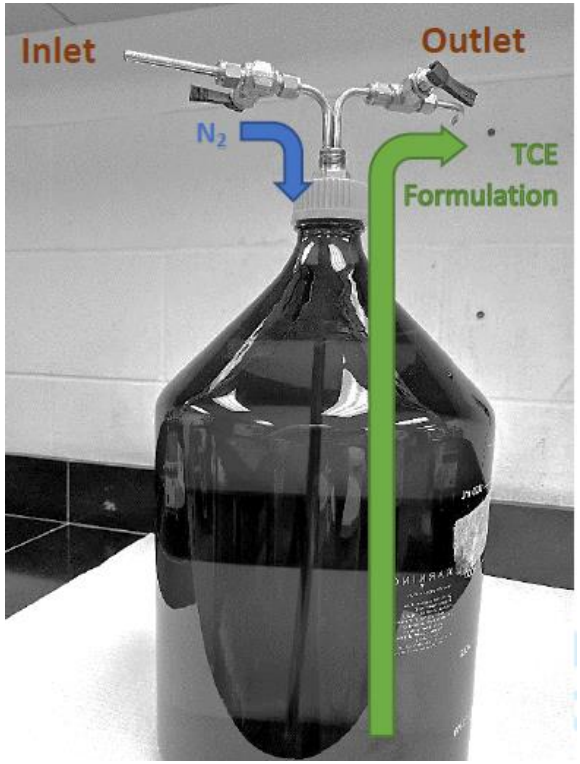
Reproducibility Implications – Other GLP-Quality Studies

- 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 concentrations similar to Johnson *et al.* (2003):
  - 0.25, 1.5, 500 and 1,000 ppm TCE (1,000 ppm just below TCE water solubility limit).
- 25 mated Sprague-Dawley rats per dose group, exposed to TCE in drinking water on Gestation Days 1-21.
- Detailed attention to TCE drinking water concentrations due to volatility concerns.
  - Focused attention on fetal cardiac evaluations (fresh dissection).
- TCE and TCA determined at various times in maternal and fetal blood.
- Retinoic acid used as positive control.

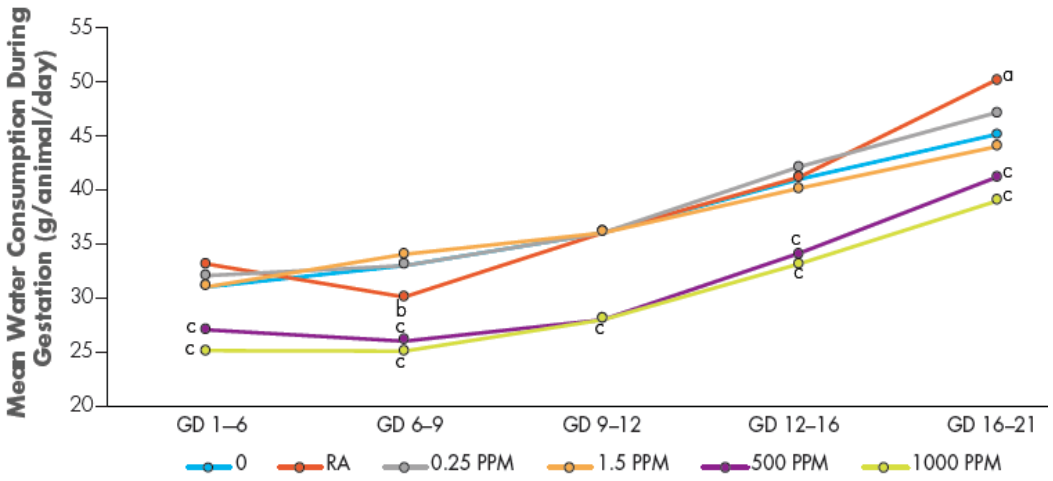
Drinking Water Dose Preparation and Confirmation

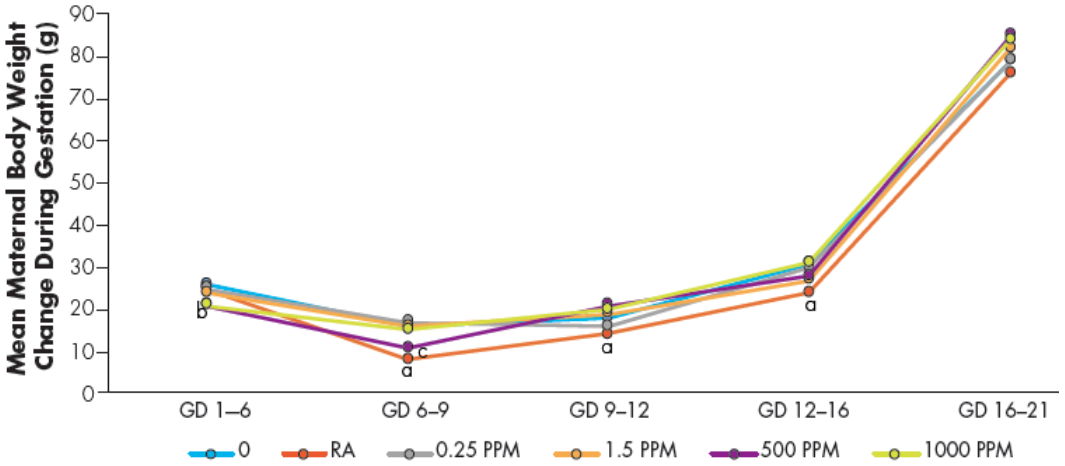


TCE concentrations (% target) in drinking water at:

- 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))

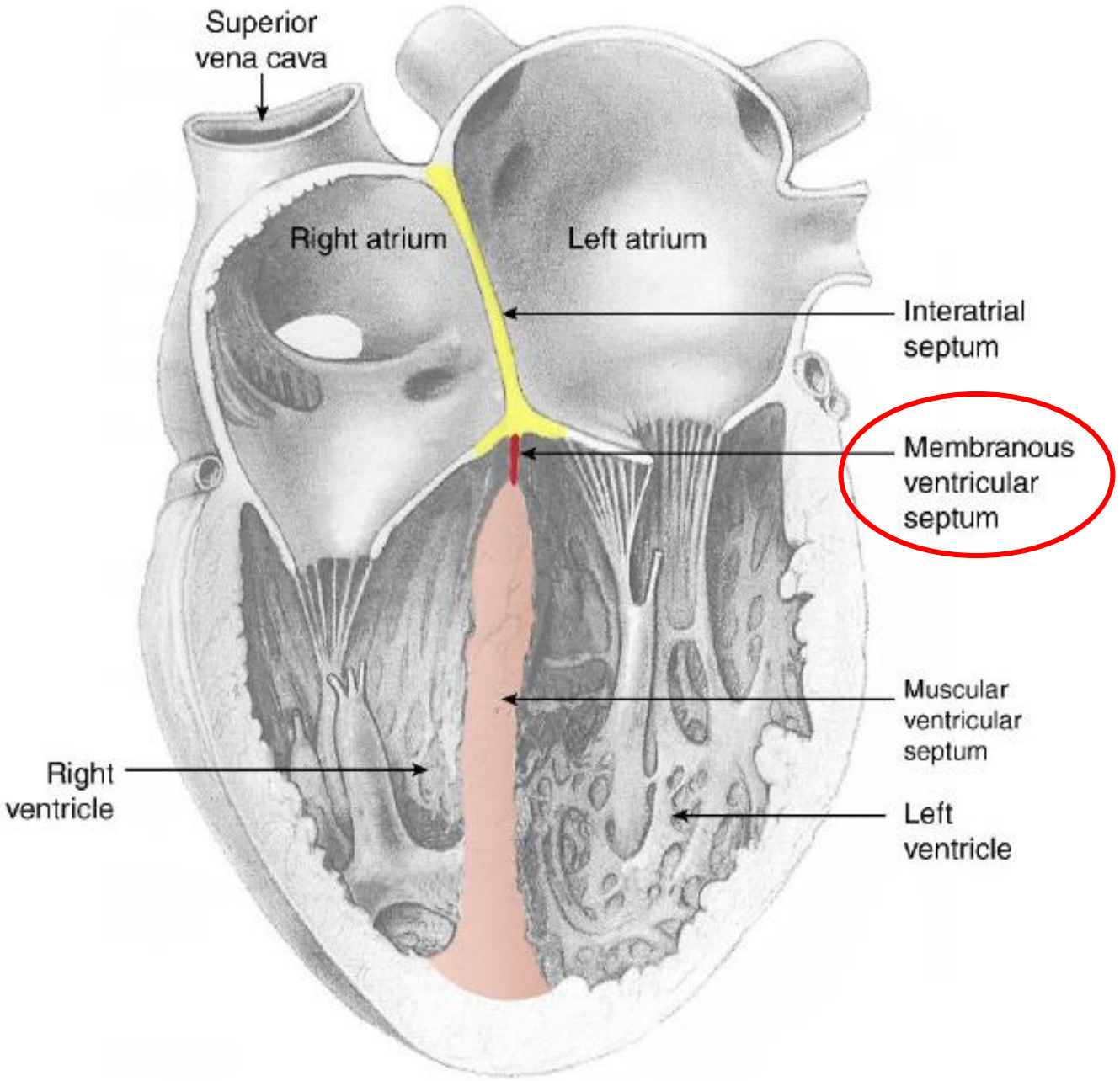
[a] One female had a very early total litter resorption and therefore had no available fetuses

** Significantly different from vehicle control group at $p \leq 0.01$

Mem = Membranous portion of ventricular septum

Mus/Mem = Muscular and membranous portions of ventricular septum

Location of Ventricular Septal Defects



VSD Incidence in Control S-D Rats Using Enhanced Cardiac Evaluations Similar to Current Study

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

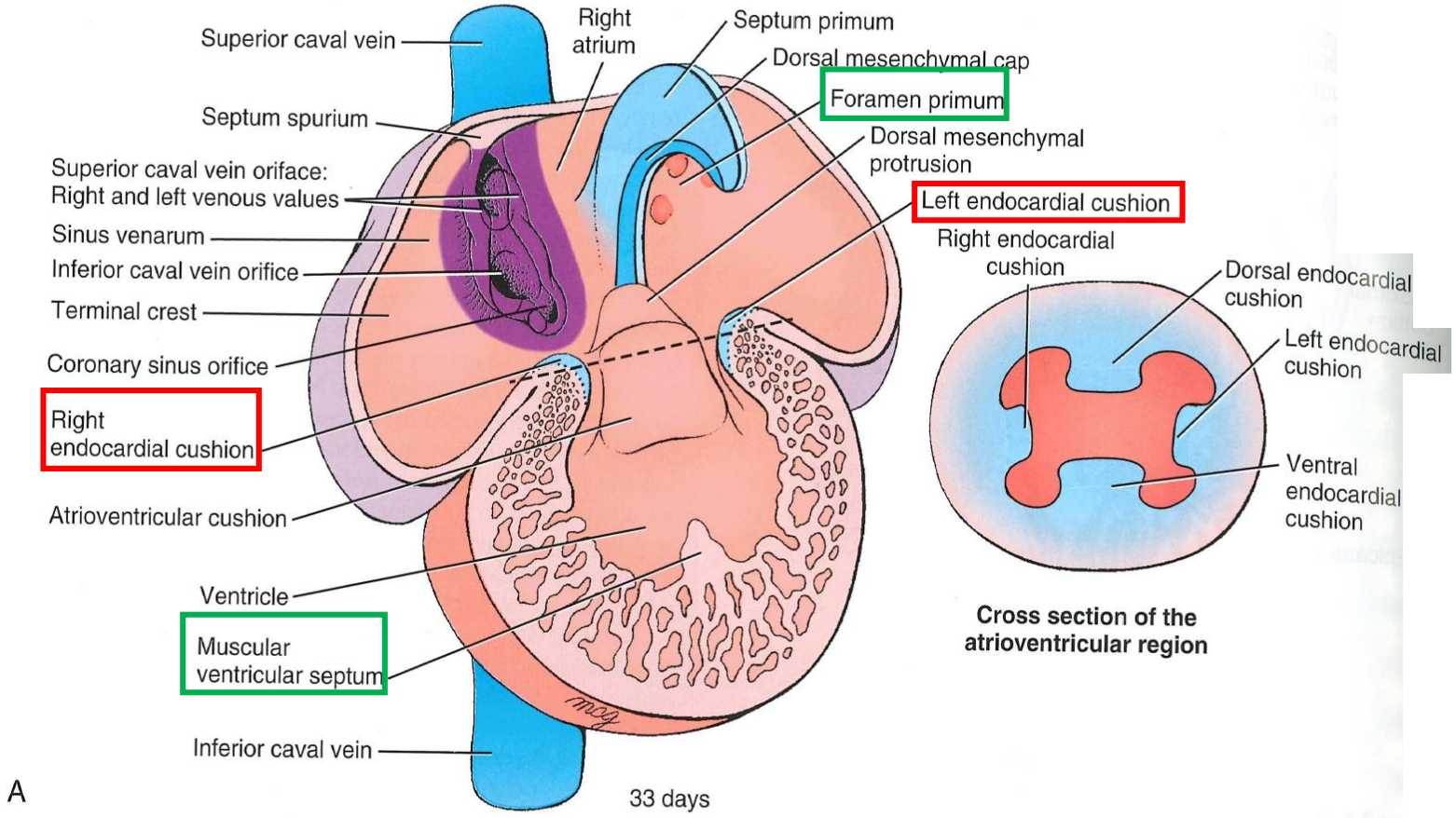
Health Risk Implications of Small (< 1 mm) VSDs in Rats and Humans

- VSDs in control and TCE-treated rats were all < 1 mm in size.
  - Solomon *et al.* (1997) found 2.4% incidence of < 1 mm VSDs in untreated near-term SD rat fetuses.
    - VSDs were completely resolved at weaning.
  - Fleeman *et al.*, (2004) used trimethadione to induce VSDs in rat fetuses.
    - Small VSDs were resolved at weaning; large VSDs were not.
    - Concluded that closure of VSDs depends on the size of the lesion at term.
- Non-statistically significant formation of VSDs of < 1 mm in TCE drinking water treated rats is not an adverse health risk.

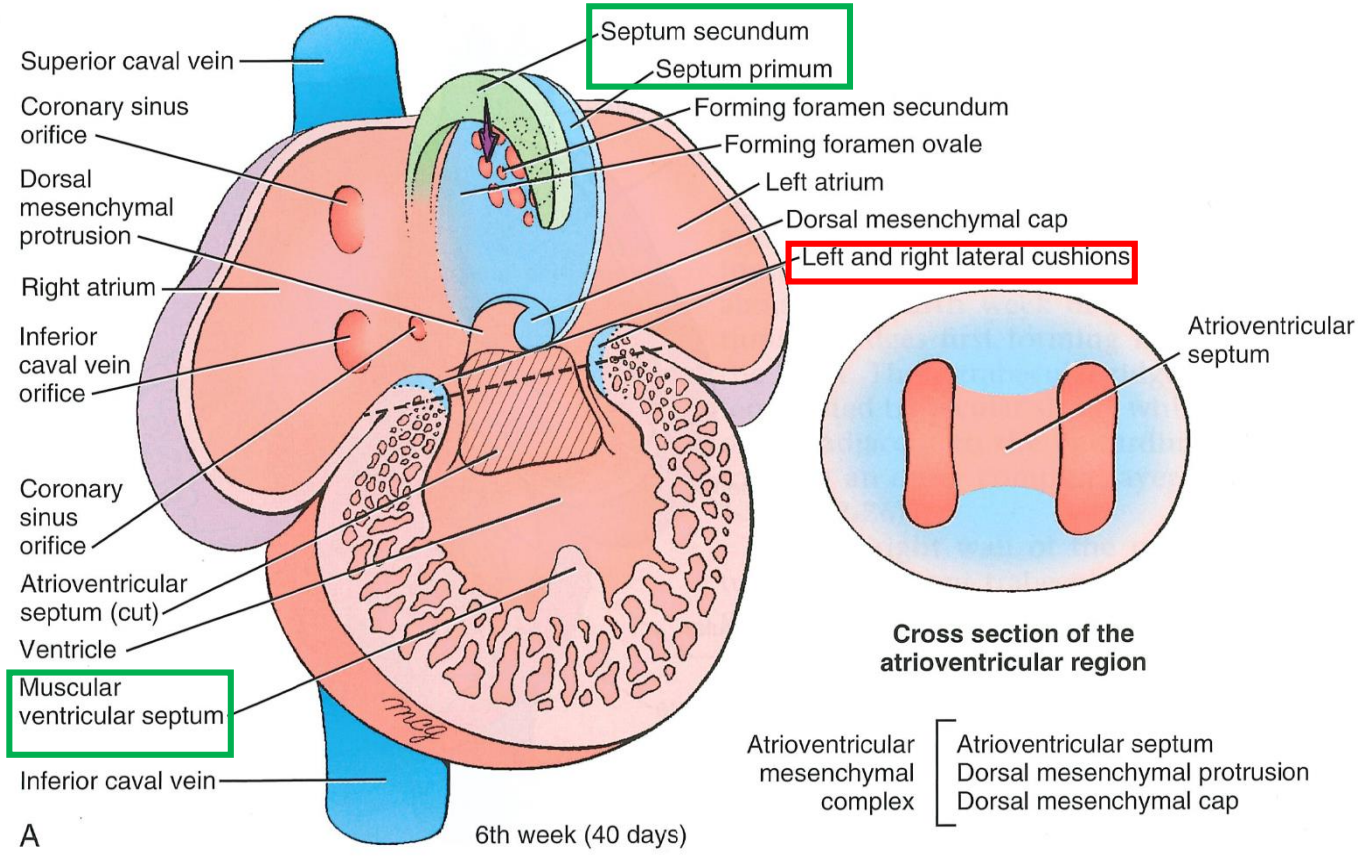
Endocardial cushions contribute little to the septa

Endocardial cushions and septa

- Right and left endocardial cushions outlined in red boxes
- Primordia of atrial (septum primum) and muscular ventricular septa outlined in green boxes



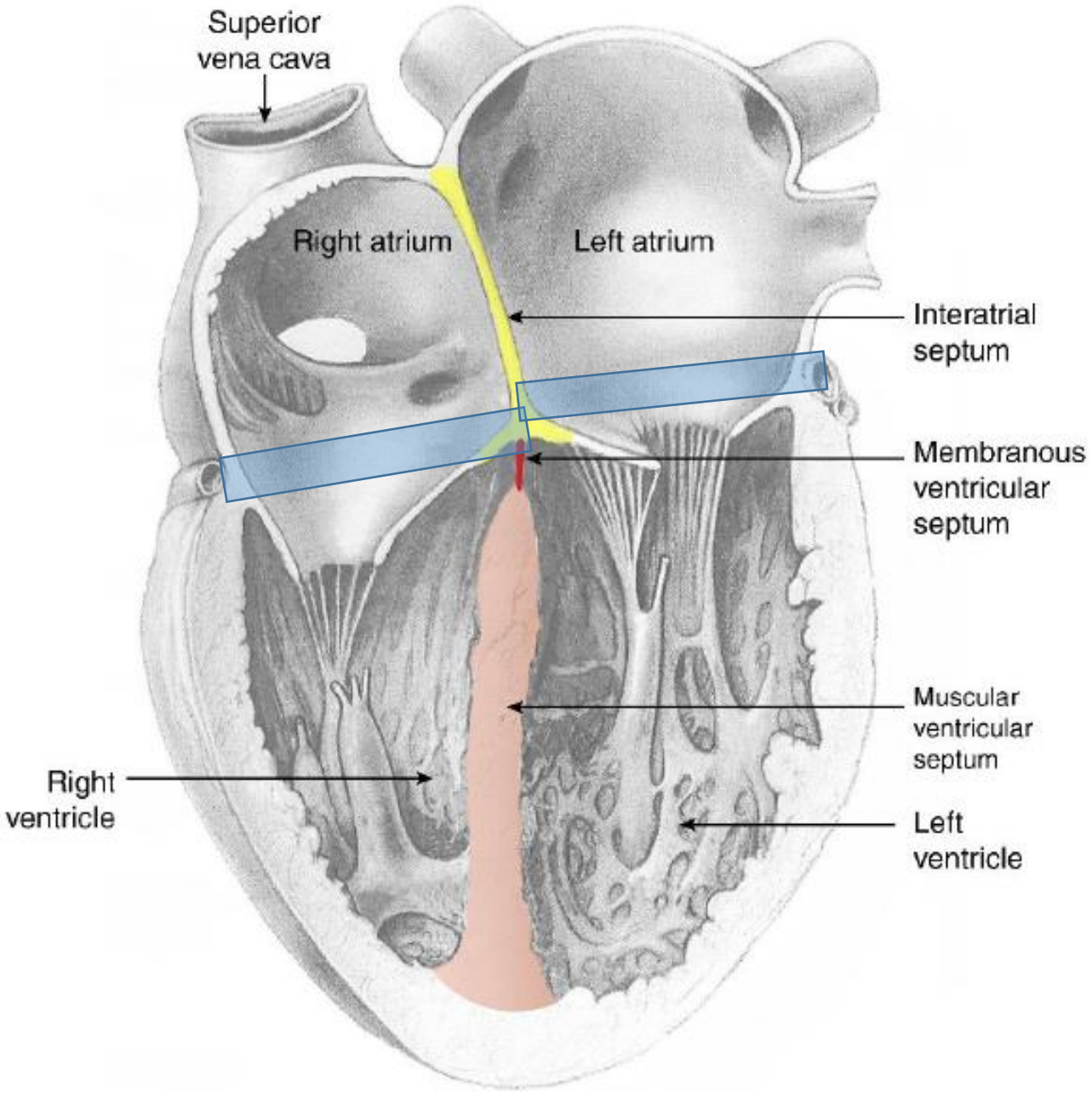
Endocardial cushions and septa - 2



- Right and left endocardial cushions outlined by red box
- Primordia of atrial (septum primum & septum secundum) and muscular ventricular septa outlined in green boxes
- Note geographic distances from endocardial cushions

Major endocardial cushion derivatives

- Cardiac skeleton
  - Floor of atria/roof of ventricles
- Attachments of atrio-ventricular valves
  - Blue rectangles



TCE & TCA Plasma Levels After Inhalation or Oral Treatments

Study	DeSesso et al. (2019)				Fisher et al. (1989)			Larson and Bull (1992)
Route	Drinking water				Drinking water	Inhalation	Oral gavage	Oral gavage
TCE exposure	0.25 ppm	1.5 ppm	500 ppm	1,000 ppm	350 ppm	600 ppm (4 hr)	2.3 mg/kg	591 mg/kg
TCE in maternal blood	ND (0.006 ppb)[a] [ng/ml]	ND (0.04 ppb) [ng/ml]	ND (12.5 ppb) [ng/ml]	ND (25 ppb) [ng/ml]	ND (15 ng/ml)[b]	24 µg/ml	0.26 µg/ml	33 µg/ml
TCA in maternal plasma	ND	ND	1.1 µg/ml	1.2 µg/ml	2.7 µg/ml	13 µg/ml	0.7 µg/ml	25 µg/ml

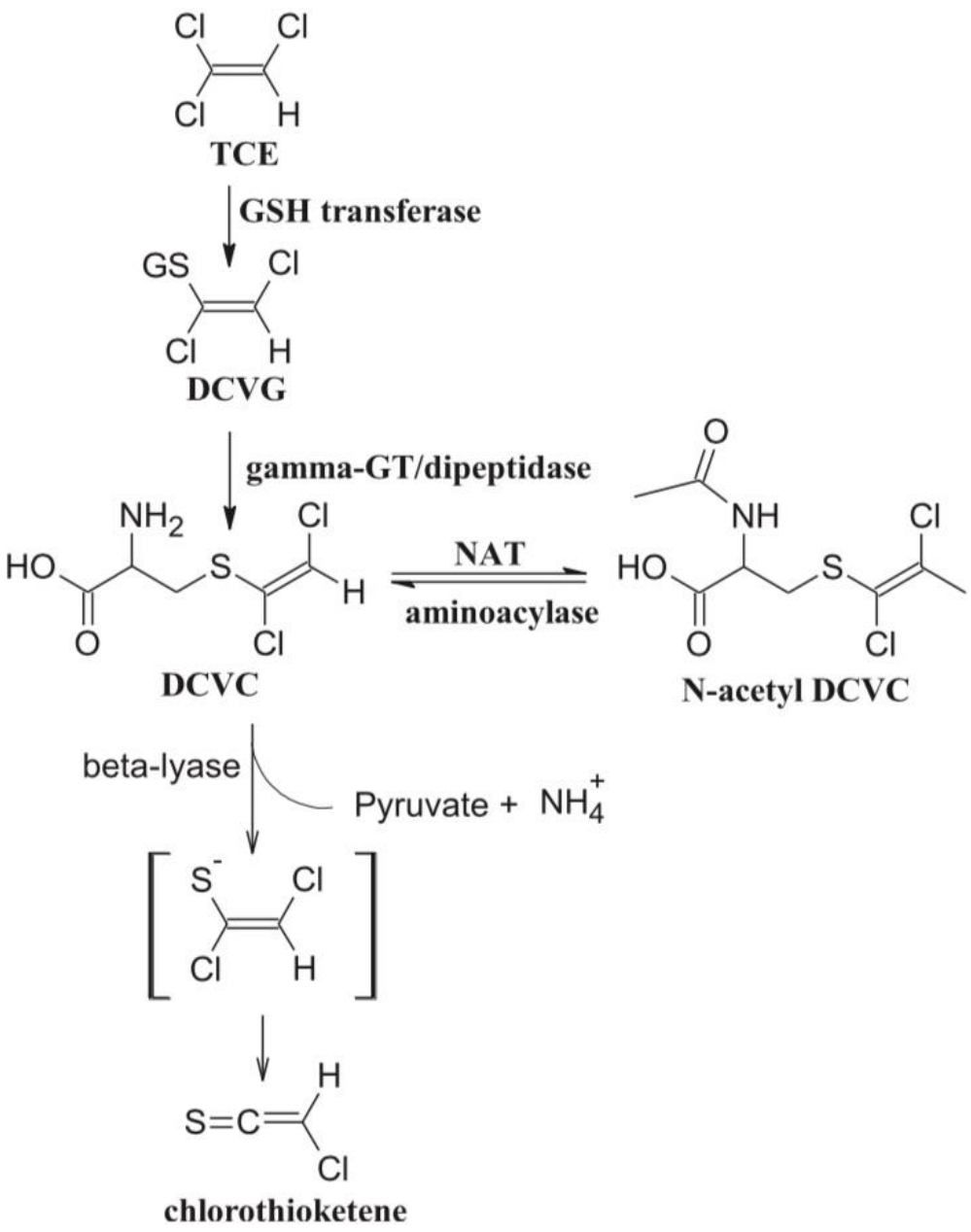
Abbreviations: TCA, trichloroacetic acid; TCE, trichloroethylene; ND, not detected.
[a]Extrapolated assuming concentration of ½ LOQ (50 ppb) at highest dose and linear toxicokinetics across dose range.
[b]Extrapolated assuming concentration of ½ LOQ (30 ng/ml).

From: DeSesso et al., Birth Def Res, 2019

- TCE Non-Detects in drinking water dosing indicate 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.

TCE Metabolism via Glutathione (GSH) Conjugation Pathway

HSIA Research Projects


TCE
GSH transferase
DCVG
gamma-GT/dipeptidase
DCVC
NAT
aminoacylase
N-acetyl DCVC
beta-lyase
Pyruvate + NH_4^+
chlorothioketene

TCE GSH Conjugation Pathway – EPA IRIS Assessment

- PBPK model-derived estimated levels of kidney DCVC-bioactivated "reactive metabolites" were used in IRIS assessment for RfD/RfC values (kidney toxicity) and kidney cancer risks.
- EPA noted the controversy over the analytical methods used to measure these TCE GSH conjugate metabolites in biological tissues.
  - HPLC/UV method (involving derivatization) showed considerably higher levels of DCVG/DCVC formed from TCE than the more specific HPLC/MS methods.
- Yet EPA's PBPK model utilized in vitro kinetic TCE GSH conjugate data and human DCVG blood data quantitated using the HPLC/UV analytical method.

TCE GSH Conjugation Pathway – HSIA Projects

- HSIA study (Zhang et al. 2018) showed endogenous glutamate in cells interferes with HPLC/UV measurements of DCVG
  - DCVG and glutamate are derivatized (with difluorodinitrobenzene) in the sample preparation steps prior to the use of HPLC/UV.
  - Both co-elute together as one peak with majority being DNP-glutamate.
  - Consequently, DCVG levels are considerably overestimated.
- Ramboll (Harvey Clewell) re-analyzed the GSH conjugation pathway in the TCE harmonized PBPK model with data not generated by the HPLC/UV method.
  - Verification to be conducted by another PBPK modeling group.