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Low-Dose Trichloroethylene Alters Cytochrome P450-2C Subfamily Expression in the Developing Chick Heart

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Abstract Trichloroethylene (TCE) is an organic solvent and common environmental contaminant. TCE exposure is associated with heart defects in humans and animal models. Primary metabolism of TCE in adult rodent models is by specific hepatic cytochrome P450 enzymes (Lash et al. in Environ Health Perspect 108:177–200, 2000). As association of TCE exposure with cardiac defects is in exposed embryos prior to normal liver development, we investigated metabolism of TCE in the early embryo. Developing chick embryos were dosed in ovo with environmentally relevant doses of TCE (8 and 800 ppb) and RNA was extracted from cardiac and extra-cardiac tissue (whole embryo without heart). Real-time PCR showed upregulation of CYP2H1 transcripts in response to TCE exposure in the heart. No detectable cytochrome expression was found in extra-cardiac tissue. As seen previously, the dose response was non-monotonic and 8 ppb elicited stronger upregulation than 800 ppb. Immunostaining for CYP2C subfamily expression confirmed protein expression and showed localization in both myocardium and endothelium. TCE exposure increased protein expression in both tissues. These data demonstrate that the earliest embryonic expression of phase I detoxification enzymes is in the developing heart. Expression of these CYPs is likely to be relevant to the susceptibility of the developing heart to environmental teratogens.

Keywords TCE · Congenital heart defects · CYP2H1 · CYP2C45 · CYP1A4 · Xenobiotic metabolism

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Introduction

Trichloroethylene (TCE; TRI; C_2HCl_3) is an organic solvent used primarily as an industrial degreasing agent but also found in consumer products [41]. Though its use has been phased out for many applications, TCE remains a common environmental contaminant. According to an EPA national groundwater survey, TCE is the most frequently detected organic solvent in groundwater supplies and is estimated to be in up to 34 % of the nation's drinking water supplies [41]. TCE is found in at least 852 of the 1,430 EPA Superfund sites around the country [1].

TCE was first linked to altered heart development in an epidemiological study that found an odds ratio of 3 for congenital heart disease in children living in an area of the Tucson Valley with TCE groundwater contamination in the range of 100–270 ppb [14]. Defects found included both myocardial and valvular structures. Although this study was controversial for methodological reasons based upon the appropriateness of the selected controls, an independent reevaluation of the data validated the conclusion that TCE is cardio-teratogenic [2]. Further, a Wisconsin epidemiological study shows a correlation between the proximity of maternal residence to TCE emitting sites and increased heart defects in their children [40].

The connection between congenital heart disease and TCE has been a controversial issue for many years (see [3, 11]). A review by Hardin et al. [16] challenged the idea that TCE is a teratogen based largely upon inconsistent dose response data to various exposure protocols. However, an independent evaluation produced by the National Academy of Sciences [33] concluded that TCE or one or more of its metabolites could cause cardiac teratogenesis. They suggested that additional studies of lowest-observed adverse effect level and mode of action are necessary [33].

Prevalence of TCE as a soil and water contaminant argues for a better understanding of the consequences of low-level exposures. For yet poorly understood reasons, TCE does not have a consistent dose–response curve in the cardiovascular system. Early studies focused on moderate to high levels of exposure. However, recent studies of gene expression in H9C2 cells suggest that there are biphasic curves with maximum effects between 10–100 ppb and again at 10–100 ppm [6, 35]. As described by Drake et al. [10], concentrations of TCE (8 ppb) close to the EPA maximum contaminant levels (5 ppb) are sufficient to alter cardiac hemodynamics. While non-monotonic dose–response curves could reflect issues with metabolism, transport, compartmentalization or a combination of factors, we began to explore this issue by focusing on metabolism. Lash et al. [22] demonstrated that in the murine model system, as well as in human liver microsomes, trichloroethylene undergoes cytochrome P450-mediated oxidative metabolism. As seen by Lash et al. [22], the CYP2 family demonstrates both affinity and substrate specificity for TCE. CYP2E1 was implicated as the major TCE metabolizing CYP in both human liver microsomes and the murine model systems, while CYP2E1 is present in low levels in human fetal liver and brain [4, 7]. We, and others, found that TCE affects cardiovascular development prior to both liver and brain development [10, 25, 29]. If metabolic activation of TCE is relevant to teratogenesis, then the existence and localization of cytochrome P450 enzymes in the early embryo is important to examine.

In this study, avian embryos were injected in ovo [10] with low levels (8 and 800 ppb) of TCE, and cardiac and extra-cardiac tissues were analyzed for the expression of avian homolog of TCE-metabolizing cytochromes. The data show that cytochromes 1A4, 2C45, and 2H1 are present in the early embryonic heart and that expression of mRNA and protein for CYP2H1 is increased by TCE exposure. These enzymes are unique to the heart at an early stage of development as little signal is found in the rest of the embryo (extra-cardiac tissue). The expression data recapitulate the non-monotonic dose response seen previously [6, 10, 25, 34]. These data suggest that the developing heart, a target of TCE toxicity, has a unique ability to metabolize TCE at an early stage in the development. The localized expression of cytochromes at this early time may be relevant to the frequency of environmentally caused defects found in this organ.

Materials and Methods

Trichloroethylene ACS reagent ≥99.5 % (TCE) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) Catalog #251402.

Dosing

TCE was dosed at 8 ppb (60 nM) and 800 ppb (6,000 nM) through injection into stage Hamburger Hamilton (HH)13 eggs using Hamilton Co. (Reno, NV, USA) 800 Series Syringes (Part #7646-01) paired with custom needles (Part #7806-02) with the following specifications: RN NDL 6/PK (22 s/1″/4)L. Methods were as described by Drake et al. [10] and Makwana et al. [25].

Quantitative Real-Time PCR

After dosing embryos were allowed to develop for approximately 24 h until reaching stage HH17. Approximately two to three HH17 whole embryos with hearts removed, defined henceforth as "extra-cardiac tissue," were pooled for homogenization in Trizol (# 15596-018, Invitrogen) and processed for RNA isolation using PureLink™ Micro-to-Midi™ Total RNA Purification System (# 12183-018, Invitrogen). Additionally, this protocol was repeated for pooled samples of approximately twenty Hamburger Hamilton (HH) 17 hearts. Data shown are a representation of three experimental replicates representing a total of 9 extracardiac tissues and 60 hearts per treatment for a 24-h exposure period. Concentration of cDNA was measured using fluorometry (Turner Biosystems) after staining with Quant-iT™ OliGreen® ssDNA Assay Kit (# O11492, Molecular Probes), and equal aliquots of control and experimental cDNA samples were added to triplicate reaction mixtures. Real-time PCR reactions were carried out using primers in Table 1, FastStart SYBR Green Master (Roche) and the Rotor Gene 3000 System from Corbett Research. Analysis of the data was carried out using the Rotor Gene 6 software. All real-time PCR results were then normalized to the housekeeping gene GAPDH.

Statistical Analysis

One-way ANOVA was performed using GraphPad Prism version 5.0b for Mac OS X, GraphPad Software, San Diego California USA, www.graphpad.com.

Immunofluorescent Microscopy

Embryos were collected in Tyrode's solution and the thorax region dissected from the embryo. Thoraxes were fixed in a solution of 20 °C 80 % methanol/20 % DMSO and cryosubstituted in 100 % ethanol at 20 °C for 1 week. The tissues were then embedded in paraffin and sectioned. After deparaffinization, sections were rinsed in phosphate-buffered saline (PBS) for 10 min, blocked for 1 h at room temperature with a blocking solution containing 1 % bovine serum albumin and 0.1 % Tween 20 in PBS.

Table 1 Primers used for detection of avian cytochromes

Primers	Forward sequence	Reverse sequence
CYP1A4	5′-GTCAATGCTCGTTTCAGTGCCT-3′	5′-ATCCTCCCCTGTCCTTTTCTCC-3′
CYP2C45	5′-GGTTTGTGTTGCTTGCCTGC-3′	5′-TTCACCTCCAGTATGTTCCCTACG-3′
CYP2H1	5′-TGGCTTGAAAGGCAACCTACG-3′	5′-TTGTCTGCTCAGTATGGAGGAAGG-3′
CYP3A37	5′-CCTGGAATACCGCAAAGGCTT-3′	5′-CCACTGGTGAAGGTTGGAGAGA-3′
UGT1A1	5′-ACTCAATGTCCCAATCCCCCT-3′	5′-TCGGTATGGTCTGTAAATGCCCT-3′
GAPDH	5′-GTGTGCCAACCCCCAATGTCT-3′	5′-CCCATCAGCAGCCTTCA-3′

Sections containing the heart were processed using indirect immunofluorescence. Sections were incubated overnight with primary antibody (goat anti-CYP2C8/9/18/19K-21, Santa Cruz Biotechnology, no. sc-23435) at 4 °C in a moist chamber at 1:50 dilution. The immunogen used to make this antibody is an epitope from the C-terminus of human CYP2C9. This region is highly conserved with the C-terminus of chick CYP2H1. After several rinses in PBS, Alexa fluor 488- or 546-conjugated rabbit anti-goat secondary antibody (Molecular Probes, Eugene, OR; A-11008, A-11010) was incubated for 1 h at room temperature in a moist chamber at 1:200 dilution. After rinsing in PBS, the nuclei were stained with TO-PRO-3 or DAPI (Molecular Probes) and the sections mounted using Prolong Gold mounting media (Molecular Probes). Sections were analyzed using a Zeiss 510 Meta confocal microscope or with a Deltavision deconvolution microscope.

Results

Avian Cytochrome P450s

We identified the avian homolog of mouse and human cytochromes associated with TCE metabolism using NCBI homologene (http://www.ncbi.nlm.nih.gov/homologene; Table 2a) [22]. The major TCE metabolizing enzyme in adult organisms (mice and rats) and adult human liver microsomes indicated by Lash et al. [22] was CYP2E1. Other minor enzymes indentified by Lash et al. [22] were CYP1A1, CYP2C9, and CYP2B6. While the chicken does not express either CYP2E1 or CYP2B6, it does express CYP2 family members: CYP2H1 and CYP2C45 whose respective human homologs are CYP2C18 and CYP2C9 (Table 2). Of these two CYPs, Thum and Borlak [38] report CYP2C18 expression in adult human heart. Additionally, the avian homolog of CYP1A1 is CYP1A4 (Table 2a). CYP2H1 was characterized as being closely related to the human CYP2C subfamily (63.4 % DNA Identity with human) [26]. We designed PCR primers for CYP1A4, CYP2C45, and CYP2H1 (Table 1) and evaluated the expression in both cardiac and extra-cardiac tissue during development with or without TCE exposure.

Table 2 Cytochrome p450 homolog corresponding to TCE metabolizing enzymes identified by Lash et al. [23]

P450	Human	Murine	Chick (% DNA identity)
Major	CYP2E1	CYP2E1	–
Minor	CYP1A1	CYP1A1	CYP1A4 (66.9)
Minor	CYP2C9	CYP2C	CYP2C45 (61.4)
Minor	CYP2B6	CYP2B2	–
Cardiac	CYP2C18	CYP2C80	CYP2H1 (63.4)

No CYP2E1 or CYP2B6 homologs have been identified in the chick. CYP2C18 was identified in the human heart its avian homolog is CYP2H1

CYP Gene Expression After TCE Exposure

The developing heart of the embryo is seen as a looped structure on the anterior thorax during the organogenesis stage of the development. While the heart initially forms as a linear heart tube, developmental movements during morphogenesis produce a loop with concomitant internal processes of septation to produce a 4-chambered heart. Previous studies in animal models have shown that the window of sensitivity to TCE in chick and mouse models coincides with looping and septation [3, 8, 10, 19, 25, 29, 34]. In the interval between HH13 (exposure) and HH17 (collection), the heart is comprised of endothelial and myocardial cell layers with an intervening extracellular matrix. At HH17, some mesenchymal cells of endothelial origin arise in the atrioventricular (AV) canal between the single atrial and ventricular chambers [3]. These cells are the earliest precursors of the mitral and tricuspid valves. Embryos were collected at this stage of development for the analyses described here.

As the major cytochrome for TCE in mouse and humans is not expressed in the chick, we examined the minor cytochromes. CYP1A4 was expressed in isolated chick heart myocytes after TCDD exposure [13]. Here, real-time PCR was used to examine CYP1A4 expression after TCE exposure in ovo. Incubated avian embryos were exposed to TCE by injection into the yolk at 48 h of development (HH Stage 13), and embryos were allowed to develop for another 24 h (to reach HH 17) at which time extra-cardiac tissue and heart tissue were extracted for RNA isolation.

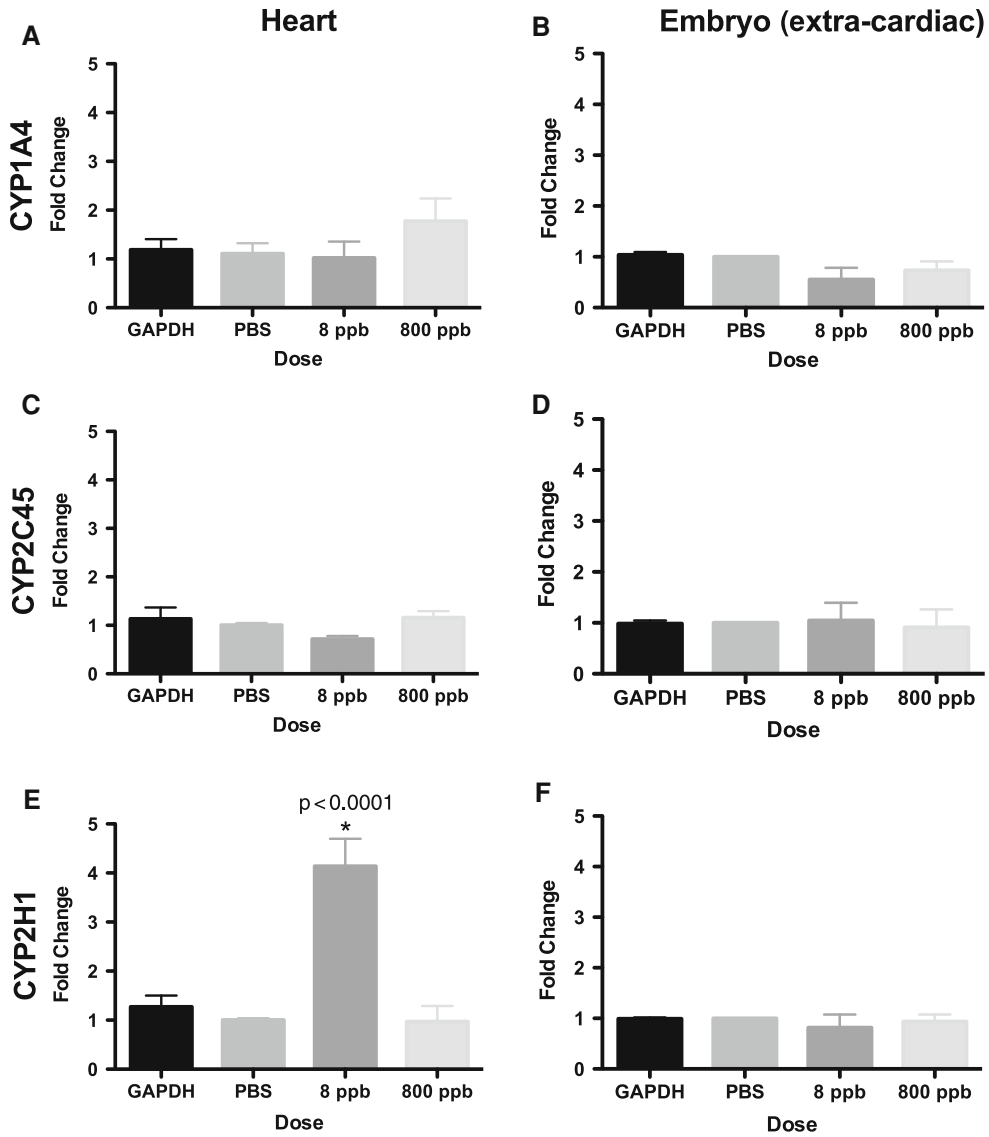

Fig. 1 Real-time PCR measurement of CYP expression. Data are normalized to GAPDH and PBS injection into the yolk. **a** CYP1A4 expression in HH17 hearts. **b** CYP1A4 expression in HH17 extra-cardiac tissue. CYP1A4 expression was not significantly altered relative to total RNA (GAPDH) in either HH17 hearts or extra-cardiac tissue at PBS, 8 or 800 ppb TCE exposure. **c** CYP2C45 expression in HH17 hearts. **d** CYP2C45 expression in HH17 extra-cardiac tissue. CYP2C45 expression was not significantly altered relative to total RNA (GAPDH) in either HH17 hearts or extra-cardiac tissue after PBS, 8 or 800 ppb TCE exposure. **e** CYP2H1 expression in HH17 hearts. **f** CYP2H1 expression in HH17 extra-cardiac tissue. CYP2H1 expression was significantly increased relative to total RNA (GAPDH) in HH17 hearts after 8 ppb TCE exposure (*p value <0.0001, One-way ANOVA), while PBS and 800 ppb TCE exposure showed no significant change in expression. Real-time PCR results for CYP2H1 showed no significant change in expression at PBS, 8 or 800 ppb TCE exposure

Real-time PCR data were normalized to GAPDH expression within that tissue. We verified that GAPDH expression was not sensitive to TCE exposure by evaluating GAPDH levels in equal aliquots of cDNA from treated and untreated samples. Results shown in Fig. 1a and b illustrate that CYP1A4 was detectable but does not demonstrate significantly altered expression at 8 or 800 ppb TCE exposure in either extra-cardiac or heart tissue.

Lash et al. [22] identified an additional minor cytochrome involved in TCE metabolism in humans and mice, CYP2C9. The avian homolog of this enzyme is CYP2C45 as identified in NCBI's homologene database (Table 2a). CYP2C45 expression was also examined by real-time PCR in HH17 extra-cardiac and heart tissue (Fig. 1c, d). Results show that although detected by PCR, CYP2C45 expression is not significantly altered after TCE exposure in either cardiac or extra-cardiac tissue at either the 8 or 800 ppb TCE doses.

In the course of our study, we identified an additional CYP2C subfamily member, CYP2C18, whose expression after TCE exposure was not addressed in Lash et al. [22].

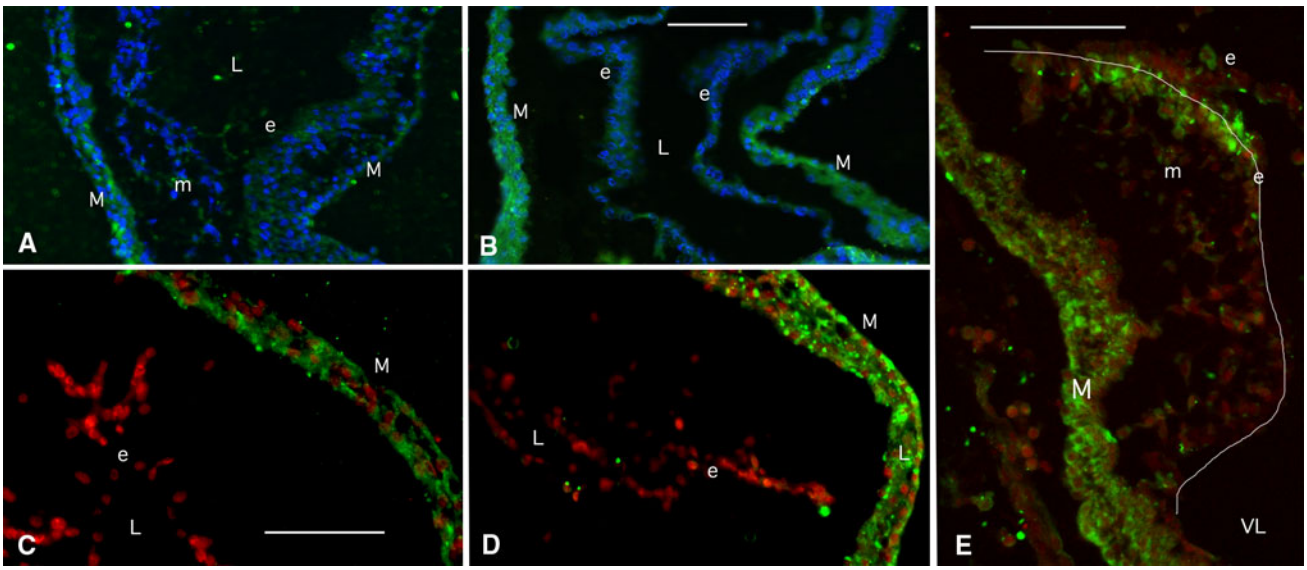

Fig. 2 CYP2C Localization in Embryonic hearts. Representative staining is shown in two control and treatment pairs (**a**, **b** & **c**, **d**) from separate experiments. Each pair was treated together and examined with the same microscope settings. CYP2C antibody expression is observed primarily myocardium of the developing chick heart in control and 8 ppb TCE-treated samples. Some endothelial staining was seen in one control (**a**) while little was seen in the other (**c**). The extracellular matrix lies between the two cell layers. TCE-exposed embryos showed an increase in myocardial staining that corresponds with the PCR data. Increases in endothelial staining were variable and reflect region differences. Small amounts of staining are seen in mesenchymal cells. A sagittal section of a cardiac cushion shows strong staining on the atrial (inflow) side of the cushion where flow becomes constricted (**e**). *Green* = CYP2C, *Blue* is DAPI nuclear stain, *Red* is Topo3 nuclear stain. Outline of the cardiac cushion is indicated by the *white line* in E. *M* myocardium, *e* endothelium, *m* mesenchyme (formed from endothelium), *L* lumen, *VL* ventricular lumen. *Scale marker* indicates 75 μm and the scale is the same in each matched pair (Color figure online)

CYP2C18 expression was previously identified in human heart tissue [38]. The avian homolog of this enzyme is CYP2H1 as identified in NCBI's homologene database (Table 2b). In HH17 heart tissue, CYP2H1 demonstrated significantly increased expression at 8 ppb TCE exposure (One-way ANOVA: $p < 0.0001$), while after 800 ppb TCE exposure, there was no significant change in expression. These data demonstrate that 8 ppb TCE is sufficient to produce a significant increase in the expression of CYP2H1 in the developing chick heart. Although the liver bud can be identified by marker expression as early as stage HH17, cellular organization begins around stage HH25 and albumin expression (as a measure of function) does not begin until stage HH30 [39]. Thus, the liver is insufficiently developed at the stage of collection (HH17) to function as a site of detoxification. This is confirmed by the lack of response in the extra-cardiac tissue sample. Significant upregulation of expression in the heart suggest this organ to be a primary site of phase I enzyme expression in the early embryo.

CYP2C Subfamily Protein Localization

The results above show that a cytochrome P450 enzyme transcript associated with TCE metabolism is expressed in the embryonic heart but not in the rest of the embryo (extra-cardiac tissue). CYP2H1 is significantly induced by 8 ppb TCE exposure in the developing heart. To confirm protein expression in heart tissue and to identify the cells where expression takes place, we undertook an immunostaining procedure on embryonic chick tissue. As we were unable to obtain cytochrome-specific antibodies for the chick, we utilized a cytochrome 2C subfamily specific antibody and verified its ability to work in embryonic chick heart cell lysate by western blot (data not shown). CYP2H1 is an avian member of the CYP2C subfamily (Table 2a) [26]. Embryos were injected with TCE at stage 13 as in the previous experiments and were collected and fixed at stage 17. Sections were stained with anti-CYP2C antibody and photographed. More than 3 exposed and control thoraxes were examined. Cytochrome 2C staining in control sections was seen primarily in the myocardium without regard to regional specificity (Fig. 2a, c). TCE exposure (8 ppb) showed an enhanced signal in the myocardium and endothelium compared to controls (compare 2a & b and 2c & d). Expression in the endothelium was somewhat variable. However, a sagittal section through a TCE-exposed cardiac cushion (valve forming tissue) in the developing AV canal shows a dramatic expression of CYP2C protein in the endothelial cells most proximal to the atrium (Fig. 2e). This is an area of constriction in the heart where blood flows from the atrium, through the AV

canal to the single ventricle. This constriction produces high rates of blood flow and shows endothelial cell proximity to the blood [25]. Endothelial expression appears to vary in the endothelium based upon regional or, perhaps, physiological parameters.

Discussion

TCE exposure, during development, demonstrates cardiac-specific effects for poorly understood reasons. Primary defects associated with human exposures include muscular ventricular septal defects, atrial septal defects, membranous ventricular septal defects, and pulmonary stenosis [40]. Similarly, animal studies show almost identical cardiac defects after TCE exposure during development. In the avian model system, Loeber et al. [23] observed atrial and ventricular septal defects, and Drake et al. [9] observed altered valvuloseptal formation. Rufer et al. [34] were able to observe the formation of cardiac defects in avian embryos post-hatch. In the murine system, Johnson et al. [19] observed both valvuloseptal and myocardial defects after delivery of high doses (1,100 ppm) of TCE in maternal drinking water. Their data show that TCE exposure during the organogenesis stage of development was critical.

A molecular survey in rat embryos exposed to TCE at 100 ppm in maternal drinking water identified Serca2a as transcriptionally downregulated [8]. Further studies in mice and in a rat cardiomyocyte cell line highlighted transcriptional regulation of Serca2a and additional mediators of Ca^{2+} homeostasis by TCE exposure by doses as low as 10 ppb [5, 36]. These results were confirmed in the H2C9 murine cardiomyocyte cell line by Caldwell et al. [6] which show alterations in the expression of the Ca^{2+} pumps, Ryr2 and Serca2a, after low-dose TCE exposure with concomitant altered Ca^{2+} handling. Impaired cardiac output is known to be a mechanism for defective heart development [15, 17]. Reduced calcium fluxes result in reduced cardiac output, and thus, TCE can produce cardiac defects through altered cardiac function. This was confirmed in recent observations that markers of flow within the avian heart (KLF2 and NOS-3) were reduced after TCE exposure in ovo. Analysis of isolated myocytes from these embryos shows TCE to have a persistent effect on myocardial contraction [25].

Thus, we and others have shown that TCE is a specific teratogen in the embryonic heart but there is an unexplained aspect to the dose response. Epithelial mesenchymal transition in vitro is inhibited at 50–250 ppb [3]. Cushion (valve progenitor) proliferation is stimulated at 80 ppb [29], and Caldwell et al. [6] noted a biphasic response curve within a cardiomyocyte cell line. Our published work [25] and that of Drake et al. [10] show that blood flow is more sensitive to 8 than 800 ppb. However, Rufer et al. [34] found that 400 ppb TCE produced more embryo loss than 8 ppb exposure. This suggests that TCE may affect different developmental processes at different exposures or that issues of compartmentalization or metabolism may play a role in the non-monotonic dose–response curve.

Cytochrome P450 expression in the early embryo is not well studied. Previous studies of these enzymes in the embryo focus almost entirely on the role of cytochromes in endogenous retinoic acid metabolism or in response to dioxin exposure. CYP26 family members are expressed in the head region of the chick embryo as early as stage 4 and are postulated to be involved in creating morphogenetic gradients involved in patterning [32]. Mouse embryos null for cytochrome P450 reductase, an electron donor to cytochromes die at E9.5 and show defects in vasculogenesis. The observed abnormalities appear to coincide strongly with known defects from disruption of retinoic acid homeostasis [31]. Dioxin (TCDD) is a teratogen that produces a number of defects including malformation of the heart. TCDD exposure induces CYP1A expression in the endothelium of the zebrafish heart [30]. CYP1A4 is upregulated in isolated embryonic chick cardiomyocytes after dioxin exposure [20]. While these data show that Cytochrome P450s are present in the embryo, they do not provide a context for more widespread xenobiotic metabolism.

The data described here demonstrate that CYP2H1 is significantly upregulated in the heart after TCE exposure and that CYP2C45 is present but uninduced. CYP2H1 upregulation precedes the development of the liver and is consistent with suggestions that the heart is uniquely sensitive to TCE exposure. The early expression of these CYPs as well as CYP1A enzymes is consistent with general observations that environmental xenobiotic exposure can lead to heart defects. Localization of the expression by antibody suggests that there is both myocardial expression and endothelial expression at a stage when these are the two major cell types in the heart. While both cell types appear to upregulate expression, the strong expression seen in the endothelial cells of the atrioventricular canal is interesting as these are precursors of the heart valves. Additionally, these endothelial cells are arranged in the most constricted part of the developing heart where they may serve a surveillance function. Of course, the avian embryo develops in ovo and the expression of phase I enzymes in the heart could be specific to non-placental animals as maternal protection is not available. Though we have not formally explored this expression in mammals, we note that microarray data from the hearts of TCE-exposed murine embryos show upregulation of numerous cytochromes [5].

The regional expression of CYP2H1 in the heart suggests the possibility that the basis for the TCE dose–response curve lies directly in this tissue. It was found that an oxidative metabolite of TCE, trichloracetic acid (TCA) was more toxic than TCE itself in a study of heart defects [18]. Oxidation of TCE by cytochrome enzymes can lead to TCA production [21, 37]. Lumpkin et al. [24] demonstrate that TCA was able to bind to plasma proteins in mice, rats, and humans resulting in an increased half-life of TCA in the bloodstream. This regionally specific metabolism of TCE in the developing heart may imply that the heart is a site of bioaccumulation of the potential oxidative metabolites of TCE: TCA (trichloroacetic acid) and/or DCA (dichloroacetic acid). If these metabolites bind to proteins in the extracellular matrix (ECM) as well as the blood, they may be retained in the heart where they can act on myocardial function. Several investigators have noted that TCE metabolism can be destructive to the very cytochrome P450s catalyzing the metabolic reaction [12, 27, 28]. The unusual dose–response curve may be due to a combination of production of toxic metabolites and the synthesis or destruction of CYPs. The heart may be susceptible at low doses because it contains competent CYPs to metabolize TCE into TCA. However, at higher doses, CYPs are degraded faster than they can be synthesized and less formation of TCA occurs. This suggests that cardio-specific toxicity is most potent prior to the onset of liver development as TCA in the heart would be reduced if TCE is metabolized elsewhere. This suggestion is consistent with the window of toxicity noted by Rufer et al. [34].

In summary, our findings demonstrate that the early heart is a site of phase I enzyme expression in the early embryo. We show that TCE is sufficient to induce upregulation of CYP2H1 and the dose sensitivity of the response recapitulates the non-monotonic response to TCE seen by a number of laboratories. This suggests that CYP2H1 is capable of metabolizing TCE and that local metabolism can mediate the effects of TCE on cardiac teratogenesis. While the basis for the non-monotonic dose–response curve remains unclear, there are a variety of molecular and physiological markers that confirm this response. Future studies will examine transcriptional regulation of perturbed markers in order to resolve a global cell signaling effect of localized cardiac specific metabolism of TCE.

The data uniquely localizing cytochrome expression to the embryonic heart, as well as its induction by TCE exposure, suggest the idea that the developing heart is a unique site of xenobiotic metabolism in the early embryo. Such metabolism could be an important component of the particular sensitivity of the heart to developmental teratogens.

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