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Toxicity assessments of selected trichloroethylene and perchloroethylene metabolites in three in vitro human placental models

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ABSTRACT

Residential and occupational exposures to the industrial solvents perchloroethylene (PERC) and trichloroethylene (TCE) present public health concerns. In humans, maternal PERC and TCE exposures can be associated with adverse birth outcomes. Because PERC and TCE are biotransformed to toxic metabolites and placental dysfunction can contribute to adverse birth outcomes, the present study compared the toxicity of key PERC and TCE metabolites in three in vitro human placenta models. We measured cell viability and caspase 3 + 7 activity in the HTR-8/SVneo and BeWo cell lines, and caspase 3 + 7 activity in first trimester villous explant cultures. Cultures were exposed for 24 h to 5–100 μM *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), or 5–200 μM trichloroacetate (TCA) and dichloroacetate (DCA). DCVC significantly reduced cell viability and increased caspase 3 + 7 activity in HTR-8/SVneo cells at a lower concentration (20 μM) compared with concentrations toxic to BeWo cells and villous explants. Similarly, TCVC reduced cell viability and increased caspase 3 + 7 activity in HTR-8/SVneo cells but not in BeWo cells. TCA and DCA had only negligible effects on HTR-8/SVneo or BeWo cells. This study advances understanding of potential risks of PERC and TCE exposure during pregnancy by identifying metabolites toxic in placental cells and tissues.

1. Introduction

The chlorinated hydrocarbons perchloroethylene (PERC) and trichloroethylene (TCE) are chemical solvents routinely used in a variety of industrial applications including chemical manufacturing and metal degreasing [1–3]. Decades of use and improper disposal have resulted in widespread environmental contamination of soil and water with TCE and PERC [2–5]. Consequently, exposure to these chemicals in residential settings through vapor intrusion and contaminated drinking water, and in occupational settings through volatilization, continues to present pressing public health concerns. TCE and PERC have similar chemical structures that differ solely by the fourth chlorine atom in PERC. Although these solvents show overlap in metabolism and toxicity, important distinctions in toxicity have been noted, also [6].

Maternal PERC and TCE exposures have mixed associations with adverse birth outcomes in numerous epidemiology studies, as recently reviewed [7,8]. In pregnant women exposed to both chemicals, TCE exposure was associated with small for gestational age and low birth weight, but PERC exposure was not [9,10]. In contrast, a TCE-focused study reported that TCE exposure was not associated with low birth weight or spontaneous abortion [11], but a PERC-focused study reported that PERC exposure was associated with low birth weight or small for gestational age in older women and in women with previous pregnancy loss [10]. In addition, PERC exposure was associated with placental abnormalities that are important potential mediators of adverse birth outcomes. For example, prenatal PERC exposure was associated with elevated risk of placental abruption, defined as separation of the placenta from the uterine wall before delivery [12]. Moreover, PERC exposure had a dose-dependent increase in odds of stillbirth, due to placental abruption and placental insufficiency [13]. Although no consensus can be reached thus far from epidemiology studies, sufficient evidence exists to warrant investigation into the toxicological

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mechanism of potential associations between PERC and TCE exposure and adverse birth outcomes.

Structural or functional abnormalities of the placenta likely contribute to adverse birth outcomes [14]. The placenta is at risk of injury from maternal exposure to PERC and TCE, in part because it is in direct contact with circulating parent compounds and metabolites in maternal blood [6,15,16]. Furthermore, the placenta is capable of metabolizing xenobiotics [17,18]. It expresses the mRNA of genes that code for enzymes necessary for PERC and TCE glutathione conjugation-derived and cytochrome P450 (CYP) oxidation metabolism [19,20]. These genes include *Kyat1* (cysteine conjugate beta-lyase) [21] from the glutathione conjugation pathway and *CYP1A1/2* [22,23] from the oxidation pathway. Moreover, the placenta possesses cysteine conjugate beta-lyase activity in rat placenta [24] and human placental cells (data not published), suggesting TCE and PERC metabolites may be in contact with placenta. Despite the potential risks of maternal PERC and TCE exposure to the placenta, effects have only minimally been explored. It is also currently unknown if effects could be more attributable to CYP or glutathione conjugate pathway metabolites.

Placental tissues contain an abundance of cell types that together, contribute to the growth, development and function of the placenta. Placental development early during the first-trimester is particularly important for establishing the maternal blood supply to the placenta. During the first-trimester, extravillous trophoblasts of fetal origin invade into the maternal uterine wall from the chorionic villi and facilitate critical remodeling of the spiral arteries necessary to accommodate increased maternal blood flow to the placenta [25,26]. Dysfunctional trophoblasts may jeopardize the fetal blood supply and result in abnormal placental development [27]. Cytotrophoblasts differentiate and fuse into syncytiotrophoblasts, multinucleated cells that make a continuous epithelial border that lines placental villous trees that extend into the maternal myometrium. The syncytium serves as the critical barrier between maternal and fetal blood supply where gas and nutrient exchange occur between mother and fetus. Cytotrophoblast dysfunction may lead to a compromised syncytium, affecting inflammatory factors released into maternal blood, placental hormone signaling, and nutrient/waste exchange [28]. Placental abnormalities arising from impaired extravillous trophoblasts or cytotrophoblast cells may lead to placental insufficiency, a condition that hinders nutrient and gas exchange across the placenta due to inadequate blood flow or compromised syncytium, greatly increasing the risk for adverse birth outcomes such as restricted fetal growth and preterm birth [14,29,30]. In order to test the effects of TCE and PERC metabolites on these specific placental cell types, we use the HTR-8/SVneo cell line to model extravillous trophoblasts [31] and the BeWo cell line to model cytotrophoblasts [32].

Biotransformation has an important role in TCE and PERC-induced toxicity [6,33,34]. TCE and PERC share some metabolic pathways and metabolites. Major and minor pathways are summarized in Fig. 1, with common and distinguishing metabolites of interest highlighted. Both TCE and PERC are metabolized via two primary pathways, the

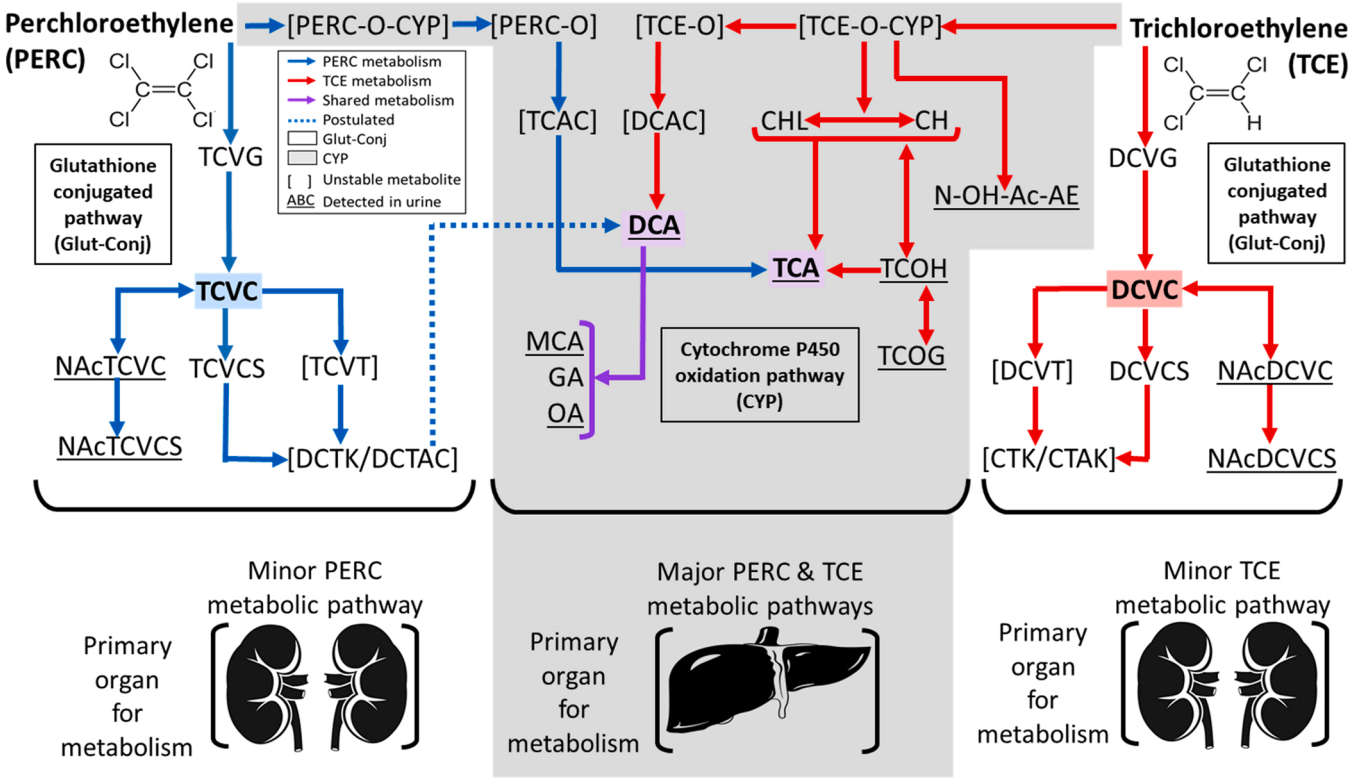


Fig. 1. Perchloroethylene (PERC) and trichloroethylene (TCE) metabolism. Both PERC and TCE are metabolized via two pathways, the cytochrome P450 (CYP) oxidation pathway (white background) and the glutathione conjugation (Glut-Conj) pathway (grey background). The CYP pathway is primarily metabolized in the liver. The Glut-Conj pathway is primarily metabolized in the kidney. Blue denotes PERC metabolism, red arrows denote TCE metabolism, and purple denotes shared metabolism. Metabolites detected in urine are underlined and unstable metabolites are bracketed. Abbreviations: PERC-O-CYP, CYP-bound-PERC-epoxide, PERC-O, PERC-epoxide; TCAC, trichloroacetyl chloride; TCVG, S-(1,2,2-trichlorovinyl) glutathione; TCVC, S-(1,2,2-trechlorovinyl)-L-cysteine; NAcTCVC, N-acetyl-S-(1,2,2-tichlorovinyl)-L-cysteine; NAcTCVCS, NAcTCVC sulfoxide; TCVCS, TCVC sulfoxide; DTAC, dichlorothionoacetyl chloride; DTK, dichlorothioketene. TCE-O, TCE-epoxide; TCE-O-CYP, CYP-bound-TCE-epoxide; DCAC, dichloroacetyl chloride; CHL, chloral; CH, chloral hydrate; DCA, dichloroacetic acid; TCA, trichloroacetic acid; TCOH, trichloroethanol; TCOG, trichloroethanol glucuronide; MCA, monochloroacetic acid; OA, oxalic acid; GA, glyoxylic acid; N-OH-Ac-AE, N-hydroxy-acetyl-aminoethanol, DCVG, S-(1,2-dichlorovinyl)glutathione; DCVC, S-(1,2-dichlorovinyl)-L-cysteine; DCVCS, DCVC sulfoxide; NAcDCVC, N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine; NAcDCVCS, NAcDCVC sulfoxide; CTAC, chlorothionoacetyl chloride; CTK, chlorothioketene.
Modified from [6,8].

cytochrome P450 oxidation pathway and the glutathione conjugation pathway [33,35]. The vast majority of TCE and PERC metabolism occurs through the CYP pathway [6]. In particular, both chemicals are biotransformed to the common metabolite trichloroacetate (TCA) as an excretable product. Excretable dichloroacetate (DCA) is also postulated to be a common metabolite, albeit to a much lesser extent than TCA [6]. Despite having metabolites in common, the intermediary biotransformation steps vary between the two chemicals. In contrast, the glutathione conjugation pathways do not overlap for PERC and TCE. Rather, PERC is metabolized to *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC) and TCE is metabolized to *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC). TCVC and DCVC are subsequently biotransformed by cysteine conjugate beta-lyase to multiple reactive species responsible for forming damaging adducts with cellular macromolecules [34,36]. Formation of oxidative metabolite TCA occurs predominantly in the liver and is implicated in TCE and PERC hepatotoxicity, whereas formation of glutathione conjugation metabolites TCVC and DCVC primarily occurs in the kidney and is linked to renal toxicity [6].

In prior studies, we demonstrated that 24-h DCVC treatment causes cell death with DCVC concentrations as low as 20 μM [37,38] and increases activation of caspase 3 + 7 apoptotic signaling [39] in HTR-8/SVneo cells. In the current study, we confirm our previous DCVC results in HTR-8/SVneo cells and extend our findings by assessing cell viability and caspase 3 + 7 activity in BeWo cells, as well as caspase 3 + 7 activity in first trimester human villous explants, after exposure to TCE and PERC metabolites. We evaluated responses to TCA and DCA as metabolites common to TCE and PERC, in addition to DCVC and TCVC, as metabolites unique to TCE and PERC biotransformation. This study examines the extent to which PERC and/or TCE metabolites contribute to placental cell and tissue injury and the potential contributions of each metabolic pathway to observed toxicity. The results may inform the development of therapeutic and/or dietary interventions potentially capable of targeting the metabolites responsible for TCE and PERC toxicity. We hypothesize that the glutathione conjugation-metabolites DCVC and TCVC will be more toxic to placental cells than oxidation metabolites TCA and DCA.

1.1. Materials and methods

The overall experimental design is summarized in Table 1, showing placental models and exposure regimens used to compare responses to common and distinguishing PERC and TCE metabolites.

1.1.1. Tissue and Cell Culture Reagents

Cell and tissue culture reagents including RPMI 1640 culture medium, advanced DMEM/F-12 culture medium, F12-K Nutrient Mixture Kaighn's Modification culture medium, 10,000 U/mL penicillin and 10,000 μg/mL streptomycin (P/S) mixed solution, Antibiotic-Antimycotic (Anti-Anti) mixed solution, and fetal bovine serum (FBS) were purchased from Gibco, a division of Thermo Fisher Scientific (Waltham, MA, USA). Phosphate buffered saline (PBS) and 0.25% trypsin were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). TCA and DCA were purchased from MilliporeSigma (St. Louis, MO, USA). TCVC was purchased from Toronto Research Chemicals (North York, ON, Canada). DCVC was synthesized as a powder by the University of Michigan Medicinal Chemistry Core as previously described[40]. High-performance liquid chromatography analysis was used to determine purity (98.7%). A stock solution of 1 mM DCVC was prepared by dissolving DCVC in phosphate buffered saline and stored in small aliquots at − 20 °C to minimize freeze/thaw cycles. The chemical purity of the DCVC stock solution was confirmed nuclear magnetic resonance (NMR).

Table 1
Summary of *ins vitro* placental models and exposures to PERC and TCE metabolites.

Metabolites	Live Cell Viability	Caspase 3 + 7 Activity	Proliferation (DNA quantification)
DCVC (5–100 μM)	HTR-8, BeWo	HTR-8, BeWo, Explants	NA
TCVC (5–100 μM)	HTR-8, BeWo	HTR-8, BeWo	NA
TCA (5–200 μM)	HTR-8, BeWo	HTR-8, BeWo	BeWo
DCA (5–200 μM)	HTR-8, BeWo	HTR-8, BeWo	BeWo

1.1.2. Cell culture (HTR-8/SVneo and BeWo)

HTR-8/SVneo cells, an immortalized human placental cell line that models extravillous trophoblasts, was a gift from Dr. Charles H. Graham (Queen's University, Kingston, Ontario, Canada). The cells were cultured as previously described [31,38,41]. Briefly, cells were cultured between passages 78–84 in RPMI 1640 medium without phenol red supplemented with 10% FBS and 1% P/S. Cells were maintained at 37 °C in a 5% CO_2 humidified incubator prior to and during experiments to ensure optimal cell growth. Cells were grown to 70–90% confluence 24 h after plating prior to use in experiments.

BeWo cells, a choriocarcinoma-derived human placental cell line that models cytotrophoblasts [32], was obtained from American Type Culture Collection (ATCC). Cells were sub-cultured in F12-K Nutrient Mixture Kaighn's Modification medium for population maintenance. For experiments, cells were cultured in DMEM/F12 without phenol red. All media were supplemented with 10% FBS and 1% (P/S). Cells were maintained at 37 °C in a 5% CO_2 humidified incubator prior to and during experiments to ensure optimal cell growth. Cells were grown to 70–90% confluence 24 h after plating prior to use in any experiments.

Because proliferation rates between HTR-8/SVneo and BeWo cells may vary and to facilitate better interpretation between experimental models and metabolites, results were reported as a percentage of negative control rather than raw numbers.

1.1.3. Cell line validation

Microsatellite genotyping via fragment analysis was performed using AmpFLSTR Identifiler Plus PCR Amplification Kit run on an 3730XL Genetic Analyzer (Applied Biosystems; Waltham, MA, USA). The short tandem repeat profiles generated for our HTR-8/SVneo or BeWo cells, respectively, were compared to the short tandem repeat profile for cells identified as HTR-8/SVneo (ATCC® CRL-3271™) or BeWo (ATCC® CCL-98™) by the American Type Culture Collection [42,43]. The short tandem repeat profiles were an exact match for HTR-8/SVneo cells (CSF1PO: 12, D13S317: 9,12, D16S539: 13D5S818: 12, D7S820: 12, TH01: 6,9.3, vWA: 13,18, TPOX: 8, Amelogenin: X) [42] and BeWo cells (CSF1PO: 11,12, D13S317: 9,11, D16S539: 13,14 D5S818: 10,11 D7S820: 10,12, TH01: 9,9,3, vWA:16, TPOX: 8, Amelogenin: X,Y) [43].

1.1.4. Explant tissue procurement and culture

First trimester placental tissues were acquired from three donors as discarded tissues after first trimester pregnancy terminations performed by collaborating Wayne State University physicians at a Michigan family planning clinic with Institutional Review Board approval (Wayne State University IRB# 013515MP4E). Donors provided written informed consent prior to obtaining tissues and no donor or fetal characteristics were shared with the research team. Tissues were transported to the Wayne State University laboratory in ice-cold PBS. Tissues were processed as previously described [44]. Chorionic villi were dissected under a microscope into approximately 20 mg (wet) pieces. Villous explants were placed one per well, free-floating in clear 24-well cell culture plates, and allowed to acclimate in a 5% CO_2 humidified incubator for 24 h prior to all experiments, as previously described [45]. The explant culture medium was DMEM/F12 supplemented with 10% FBS and 1% Anti-Anti.

1.1.5. Measurement of cell viability and caspase 3 + 7 activity in HTR-8/SVneo and BeWo cells

ApoLive-Glo™ Multiplex Assay Kit (Promega; Madison, WI, USA) was used to measure cytotoxicity of test chemicals in the HTR-8/SVneo and BeWo cell lines. This assay sequentially measures the relative abundance of live cells and caspase 3 + 7 activity in a single multiplexed assay. Live cell proxy measurements were conducted with the cell-permanent compound glycyl-phenylalanyl-aminoflourocoumarin (GF-AFC), which generates a fluorescence signal proportional to the number of living cells. Caspase 3 + 7 activity was measured using an engineered substrate designed with a targeted amino acid sequence specific to the caspase of interest. Following cleavage of the substrates by caspases 3 + 7, the resulting molecule, aminoluciferin, reacts with luciferase to generate a luminescence signal proportional to the magnitude of caspase activity.

The assay was performed according to the manufacturer's recommended protocol. Cells (HTR-8/SVneo and BeWo) were seeded at a density of 10,000 cells per well in a white clear-bottomed 96-well plate and allowed to adhere for 24 h. Cells were then treated for 24 h with medium alone (control) or test chemicals diluted in RPMI 1640 supplemented with 10% FBS and 1% P/S (HTR-8/SVneo) or DMEM/F12 supplemented with 10% FBS and 1% P/S (BeWo). Test chemical concentrations were as follows: DCVC (5, 10, 20, 50 and 100 μM), TCVC (5, 10, 20, 50 and 100 μM), TCA (5, 10, 20, 50, 100 or 200 μM) and DCA (5, 10, 20, 50, 100 or 200 μM). Following exposure, GF-AFC was added directly to the media and incubated for 1 h. Fluorescence signal for viable cells was measured with a SpectraMax M2e Multi-Mode Microplate Reader (Molecular Devices; San Jose, CA, USA) (emission: 400 nm; excitation: 505 nm). After viability measurements, Caspase-Glo substrate was added to the plates and incubated at room temperature for 1 h. Luminescence signal was quantified using the Glomax Multi Plus detection system (Promega). Camptothecin (4 μM), a toxic compound which targets the enzyme topoisomerase I and causes double-strand DNA breaks during replication, [46,47] was included as a positive control for each experiment. Three independent experiments were conducted with each test chemical, with 3 replicates per treatment in each experiment.

1.1.6. Measurement of caspase 3 + 7 activity in villous explants

The Caspase-Glo® 3/7 Assay System (Promega) was used to measure caspase 3 + 7 activity in villous explants because the tissue model is incompatible with the viability portion of ApoLive-Glo™ Multiplex Assay. Villous explants were added one per well as free-floating tissue cultures in clear 24-well cell culture plates and allowed to acclimate in a 5% CO_2 humidified incubator for 24 h. Explants were treated with DMEM/F12 medium supplemented with 10% FBS and 1% Anti-Anti (control) or medium plus DCVC (5, 10, 20, 50 and 100 μM) for 24 h. Villous explants for treatment groups within each experiment were from the same placenta. The assay was performed according to the manufacturer's recommended protocol, as described above. Three independent experiments were performed with placentas from 3 separate donors.

1.1.7. DNA quantification in BeWo cells

DNA was quantified in BeWo cells using the CyQUANT® Cell Proliferation Assay (Invitrogen). BeWo cells were plated in tissue culture-treated 96-well white, clear-bottom plates at a density of 10,000 cells in 100 μL medium per well and allowed to adhere for 24 h before treatment. Cells were treated with medium alone (negative control), camptothecin (4 μM; positive control), TCA (5, 10, 20, 50, 100 or 200 μM) or DCA (5, 10, 20, 50, 100 or 200 μM) for 24 h. The assay is based on a proprietary dye that binds cellular DNA and emits a fluorescence signal proportional to the number of cells. The assay was performed according to the manufacturer's protocol. Briefly, after treatment, medium was removed and 100 μL of dye-binding solution (ratio of 1 μL of CyQUANT® dye to 0.5 mL of 1x HBSS buffer) was added per well. Cells were incubated in the dark at 37 °C for 1 h. After incubation, fluorescence was measured (excitation: 485 nm; emission 530 nm) using a SpectraMax M2e Multi-Mode Microplate Reader (Molecular Devices). Three independent experiments were conducted per chemical, with three replicates per treatment in each experiment.

1.1.8. Statistical analysis

The technical replicates were averaged within each experiment. Data are summarized as means ± standard deviation (SD). To test for an overall effect of treatment, we tested for differences in means by any treatment group using one-way ANOVA tests. To test for an effect for each treatment group, we followed up significant ANOVA tests with Tukey's post-hoc tests for comparison of means for each treatment group relative to the control group. To further evaluate sensitivity between cell models, percent changes for viability and caspase 3 + 7 measurements (compared to controls) for respective metabolite concentrations were statically tested for significant differences between cell models using t-tests or one-way ANOVA. Two-way ANOVAs were also conducted to test for significant differences in chemical sensitivity between cell models. Fold-change magnitudes were log-transformed prior to statistical testing. GraphPad Prism software version 8 (GraphPad Software Inc; San Diego, CA, USA) was used for analyses and plotting. N = number of independent experiments. $P < 0.05$ was considered statistically significant.

2. Results

Results showing measures of toxicity for selected TCE and PERC metabolites in three in vitro placental models are summarized in Table 2.

2.1. Effects of TCE metabolite DCVC on cell viability and caspase 3 + 7 activity

The HTR-8/SVneo cells were more sensitive to DCVC-induced cytotoxicity than BeWo cells, exhibiting significantly decreased cell viability (a proxy for live cell abundance) and increased caspase 3 + 7 activity at lower DCVC concentrations (Fig. 2). Specifically, 24-h exposure to DCVC decreased HTR-8/SVneo cell viability 17% at 20 μM ($P = 0.03$), 34% at 50 μM ($P < 0.03$) and 38% at 100 μM ($P < 0.02$), relative to the non-exposed, negative control (Fig. 2A, one-way ANOVA $P < 0.0001$). BeWo cells, on the other hand, required 100 μM DCVC to reduce cell viability, which was significantly reduced 33% ($P = 0.03$) compared to 5 μM DCVC treatment but was not significantly different from negative controls ($P = 0.06$, not significant) (Fig. 2C, one-way ANOVA $P = 0.03$). Similarly, caspase 3 + 7 activity increased in HTR-8/SVneo cells after 24-h exposure to DCVC at 20 μM (38% decrease; $P < 0.0003$), 50 μM (46% decrease; $P < 0.0001$) and 100 μM (78% decrease; $P < 0.0001$) relative to negative controls (Fig. 2B, one-way ANOVA $P < 0.0001$). In comparison, BeWo cells exhibited increased caspase 3 + 7 activity at 50 μM DCVC (36% increase, $P < 0.05$) and 100 μM DCVC (200% increase, $P < 0.0001$) compared to negative controls, but not at lower DCVC concentrations (Fig. 2D, one-way ANOVA $P < 0.0001$). Interestingly, the HTR-8/SVneo cell responses to 100 μM DCVC approached levels observed with the positive control, camptothecin, but this was not the case with BeWo cells. Similar to BeWo cells, caspase 3 + 7 activity increased in the placental villous explants with exposure to 50 μM DCVC (94% increase, $P < 0.03$) and 100 μM DCVC (92% increase, $P < 0.04$), relative to negative controls (Fig. 2E, one-way ANOVA $P < 0.0001$). For technical reasons, cell viability could not be evaluated in the explant model with the assay used with the cell lines. Overall, these results demonstrated that DCVC caused substantial toxic effects in all three placental models tested.

Table 2
Overall summary of results.

			HTR-8/SVneo		BeWo	Villous	Explants
Metabolite	**Endpoint**	Direction of Change	Concentrations with significant ($P < 0.05$) change, compared to control	Direction of Change	Concentrations with significant ($P < 0.05$) change, compared to control	Direction of Change	Concentrations with significant ($P < 0.05$) change, compared to control
DCVC	Viability	↓	20, 50,100 μM	↓	100 μM	NA	NA
	Caspase	↑	20, 50,100 μM	↑	50, 100 μM	↓	50, 100 μM
TCVC	Viability	↓	50, 100 μM	=	ø	NA	NA
	Caspase	↑	100 μM	=	ø	NA	NA
TCA	Viability	=	ø	↑	50, 100 μM	NA	NA
	Caspase	=	ø	=	ø	NA	NA
	Proliferation	NA	NA	=	ø	NA	NA
DCA	Viability	↑	200 μM	=	ø	NA	NA
	Caspase	↓	10, 20, 50, 100, 200 μM	=	ø	NA	NA
	Proliferation	NA	NA	=	ø	NA	NA

2.2. Effects of PERC metabolite TCVC on cell viability and caspase 3 + 7 activity

The PERC-specific glutathione conjugation-derived metabolite TCVC demonstrated cytotoxicity at elevated concentrations in HTR-8/SVneo cells but not BeWo cells (Fig. 3). TCVC exposure for 24-h decreased HTR-8/SVneo cell viability 12% at 50 μM ($P < 0.01$) and 27% at 100 μM ($P < 0.01$), relative to non-exposed, negative controls (Fig. 3A, one-way ANOVA $P < 0.0001$). Caspase 3 + 7 activity increased in HTR-8/SVneo cells only at the highest concentration of TCVC tested, 100 μM (50% increase; $P < 0.01$), relative to negative controls (Fig. 3B, one-way ANOVA $P < 0.0001$). In contrast, BeWo cells treated with TCVC for 24 h did not exhibit significant modification of cell viability (Fig. 3C) or caspase 3 + 7 activity (Fig. 3D) at concentrations up to100 μM, despite substantial responses to the positive control, camptothecin. Due to a limited availability, TCVC toxicity was not tested in first trimester placental explant cultures. Similar to DCVC treatment, these results demonstrated HTR-8/SVneo vulnerability to a glutathione conjugation-derived metabolite, albeit TCVC, that was less cytotoxic than DCVC at equivalent concentrations.

2.3. Effects of the common TCE and PERC metabolites TCA and DCA on cell viability and caspase 3 + 7 activity

Because of similarities in their chemical structures, TCE and PERC share common metabolic pathways and metabolites that include the cytochrome P450 pathway metabolites TCA and DCA. Unlike DCVC and TCVC, TCA treatment for 24 h had no significant effect on either HTR-8/SVneo cell viability (Fig. 4A) or caspase 3 + 7 activation (Fig. 4B). Moreover, BeWo cell viability estimates increased after 24-h exposure to 50 μM TCA (33% increase, $P = 0.005$) and 100 μM TCA (41% increase, $P = 0.0007$), but significant effects were not observed at 200 μM TCA nor at lower TCA concentrations (Fig. 4C, one-way ANOVA $P = 0.002$). Exposure to 5–200 μM TCA for 24 h elicited no significant effects on BeWo cell caspase 3 + 7 activity (Fig. 4D). To further explore the apparent TCA-stimulated increase of live BeWo cells, cellular proliferation was also measured but no significant changes were observed (Fig. 4E).

Similarly, 24-h exposure to DCA concentrations up to 200 μM failed to produce evidence of cytotoxicity in either HTR-8/SVneo or BeWo cells (Fig. 5). Instead, HTR-8/SVneo cells treated with 200 μM DCA exhibited slightly increased estimated cell viability by 6% ($P < 0.02$) relative to negative controls (Fig. 5A, one-way ANOVA $P = 0.0016$), and exposure to 10–200 μM DCA decreased caspase 3 + 7 activity 15–21% ($P < 0.05$), relative to negative controls (Fig. 5B, one-way ANOVA $P = 0.0013$). In BeWo cells, no significant changes in cell viability and caspase 3 + 7 activity were detected following treatment with 5–200 μM DCA (Figs. 5C and 5D, respectively). No significant changes in cellular proliferation were observed in BeWo cells exposed to 5–200 μM DCA for 24 h (Fig. 5E). As in prior experiments, the positive control camptothecin elicited substantial responses. Overall, these results demonstrated that treatment with TCA and DCA caused minimal effects in placental cell lines compared to glutathione conjugation-derived metabolites DCVC and TCVC.

2.4. Comparisons of toxicity results across metabolite concentrations and placental models using statistical analyses

Line graphs depicting percent changes for viability and caspase 3 + 7 measurements (compared to controls) for respective metabolite concentrations, and statistical analyses for results between cell models are summarized in Fig. 6. Two-way ANOVAs for DCVC viability and caspase activity, respectively, revealed significant differences in percent changes for concentrations (viability: $P < 0.0001$, caspase: $P < 0.0001$) and cell models (viability: $P = 0.02$, caspase: $P = 0.1$). DCVC concentrations 20 and 50 μM had the largest percent change differences between HTR-8/SVneo and BeWo cells for DCVC viability (Fig. 6 A), however, only the difference between the 50 μM (t-test, $P = 0.02$) measurements was statistically significant. The largest differences in percent change for DCVC caspase 3 + 7 activity (Fig. 6B) between the two cells lines plus explants were observed in 20 (one-way ANOVA, $P = 0.004$), 50 (one-way ANOVA, $P = 0.01$) and 100 μM (one-way ANOVA, $P = 0.0006$), and all differences between respective measurements were significant. Two-way ANOVAs for TCVC viability and caspase activity, respectively, were not significantly different for concentrations and cell models. TCVC concentrations 50 and 100 μM had the largest percent change differences between HTR-8/SVneo and BeWo cells for TCVC viability (Fig. 6 C), however, only the difference between the 100 μM (t-test, $P = 0.01$) measurements was statistically significant. For TCVC caspase 3 + 7 (Fig. 6D), none of the differences in percent change were significant. These results further confirm that HTR-8/SVneo cells were more sensitive than BeWo cells to DCVC and TCVC toxicity, and that DCVC was more toxic to the cells than TCVC.

Two-way ANOVAs for DCVC viability and caspase activity, respectively, revealed significant differences in percent changes for concentrations (viability: $P = 0.01$, caspase: $P = 0.8$) and cell models (viability: $P < 0.0001$, caspase: $P = 0.006$). The largest percent change differences for dual metabolite TCA viability (Fig. 6E) were observed in 20 (t-test, $P = 0.001$), 50 (t-test, $P = 0.002$) 100 (t-test, $P = 0.004$) and 200 μM (t-test, $P = 0.01$), and all differences between respective measurements were significant. Only the difference in the 100 μM (t-test, $P = 0.02$) measurements was significantly different for TCA caspase 3 + 7 activity (Fig. 6 F). Two-way ANOVAs for DCVC viability and caspase activity, respectively, revealed significant differences in percent changes for cell

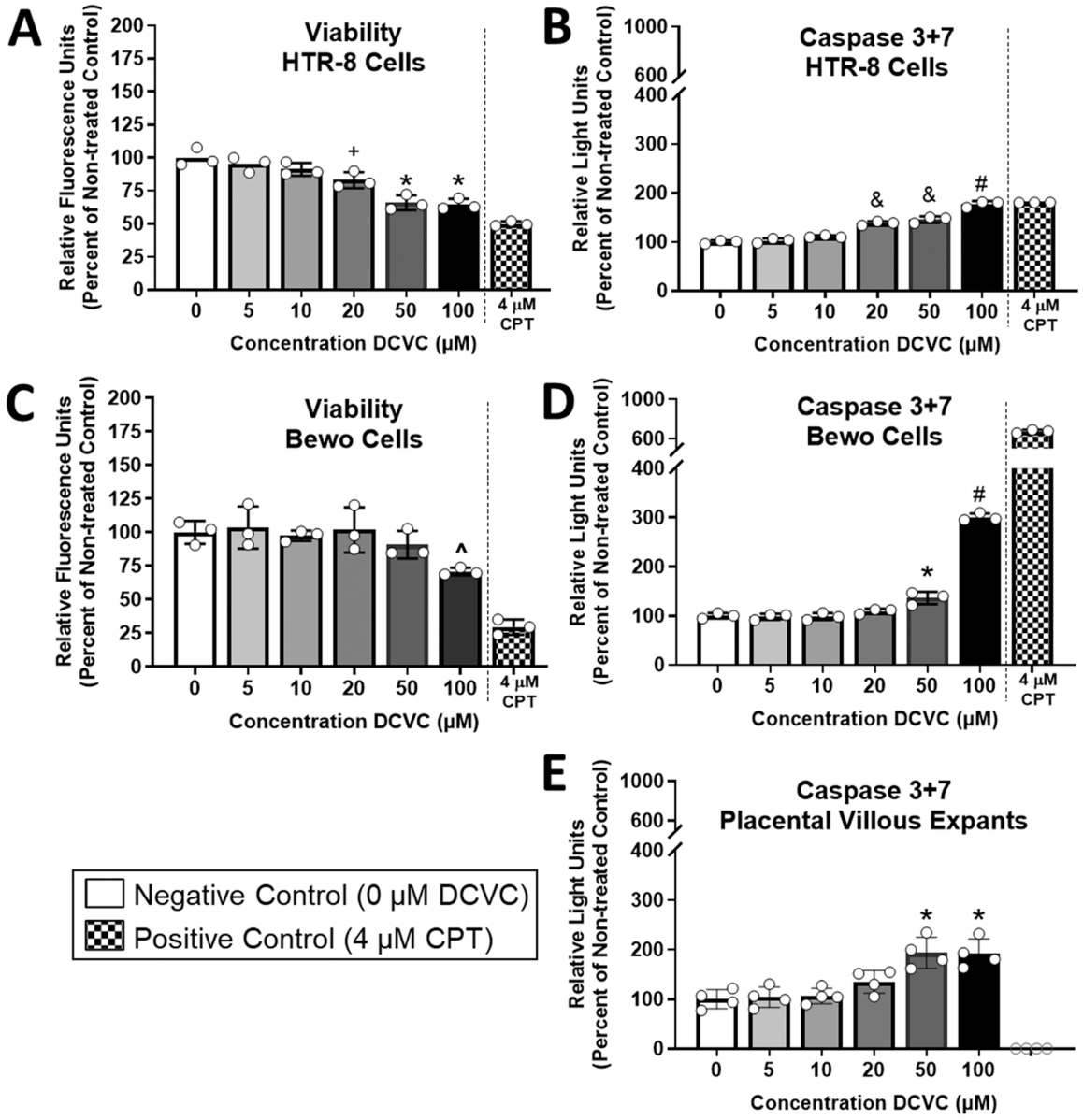


Fig. 2. Effects of TCE metabolite DCVC on cell viability and caspase 3 + 7 activity.HTR-8/SVneo cells, BeWo cells, and first trimester placental villous explants were treated with 0 (negative control), 5, 10, 20, 50, or 100 μM DCVC for 24 h. Cell viability and caspase 3 + 7 activity were measured with the ApoLive-Glo™ Multiplex Assay Kit. A) HTR-8/SVneo cell viability (one-way ANOVA, $P < 0.0001$). B) HTR-8/SVneo cell caspase 3 + 7 activity (one-way ANOVA, $P < 0.0001$). C) Bewo cell viability (one-way ANOVA, $P = 0.03$). D) BeWo cell caspase 3 + 7 activity (one-way ANOVA, $P < 0.0001$). E) Caspase 3 + 7 activity in placental villous explants. Camptothecin (CPT) (4 μM) was included as a positive control. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison of means. The positive control data were not included in the statistical analysis. Bars represent means ± SD. Open circles represent data points from individual experiments (some points overlap). $^{+}$Significantly different compared to 0 μM DCVC (negative control) ($P < 0.05$). $^{\#}$Significantly different compared to 0 (negative control), 5, 10, 20 and 50 μM DCVC ($P < 0.05$). *Significantly different compared to 0 (negative control), 5, 10 and 20 μM DCVC. $^{\&}$Significantly different compared to 0 (negative control), 5 and 10 μM DCVC ($P < 0.05$). $^{\wedge}$Significantly different compared to 5 and 20 μM DCVC ($P < 0.05$). N = 3 independent experiments, with three replicates per treatment in each experiment.

models (viability: $P < 0.0001$, caspase: $P < 0.0001$), but not concentrations. DCA metabolite concentrations 20 (t-test, $P = 0.03$) and 50 μM (t-test, $P = 0.03$) had the largest differences in percent changes between HTR-8/SVneo and BeWo cells for DCA viability (Fig. 6 G) and both were statistically significant. For DCA caspase 3 + 7 activity (Fig. 6H), percent change differences for 20 (t-test, $P = 0.01$) and 50 μM (t-test, $P = 0.02$) were significant, however, very small. Although percent change differences for TCA and DCA viability measurements were large, as well as statistically significant, due to metabolite effects in BeWo cells, they do not demonstrate a trend towards toxicity because viability was increased in BeWo cells, rather than decreased. Taken together, our data failed to reveal toxic effects of oxidative metabolites TCA and DCA in the placental cell lines tested.

3. Discussion

Multiple epidemiology studies report associations between exposure of pregnant women to PERC and TCE with elevated risk of adverse birth outcomes [9,10,12,13]. The present study assessed differences in toxicity of key PERC and TCE metabolites in three human in vitro placental models: HTR-8/SVneo cell line derived from first trimester placenta as an extravillous trophoblast model; BeWo cell line derived

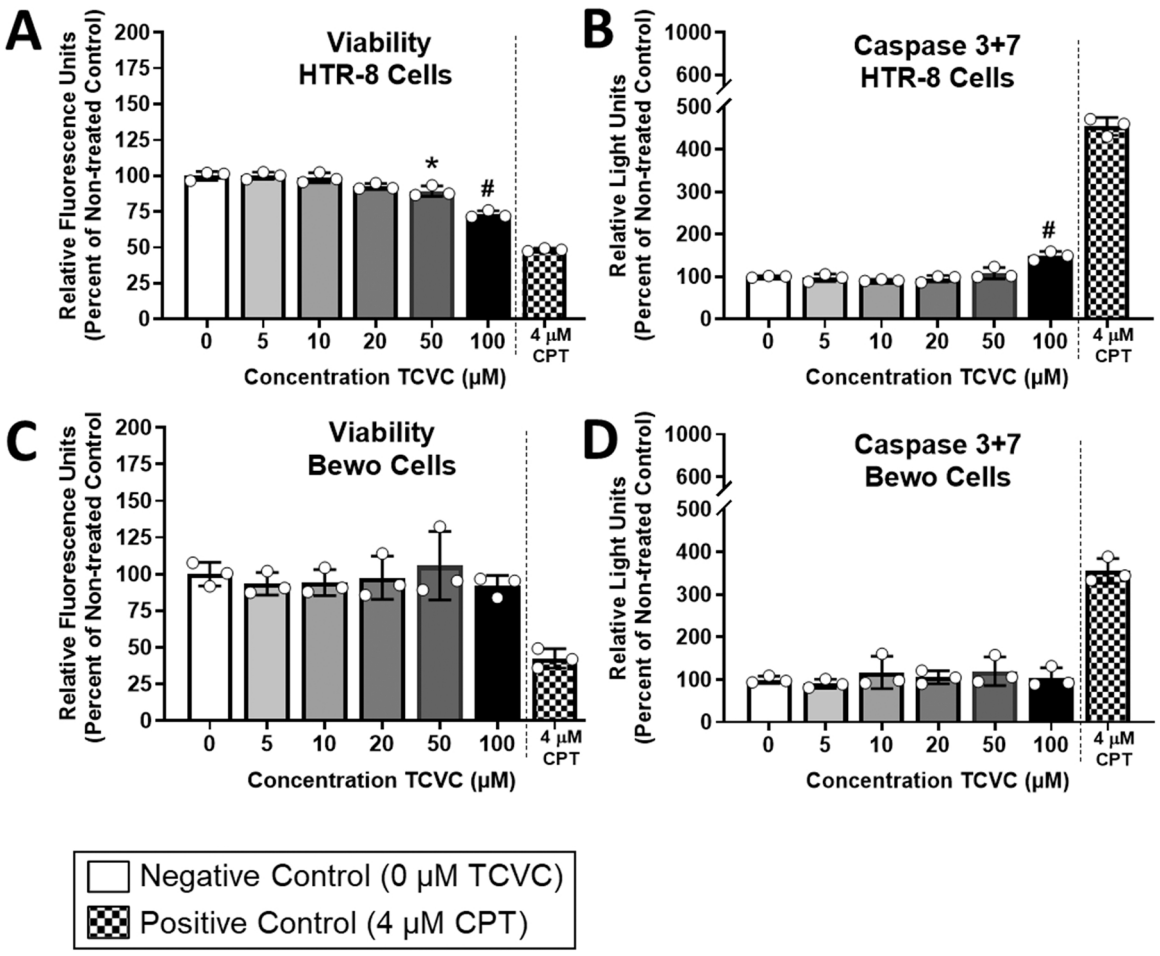


Fig. 3. Effects of PERC metabolite TCVC on cell viability and caspase 3 + 7 activity. HTR-8/SVneo cells and BeWo cells were treated with 0 (negative control) or 5–100 μM TCVC for 24 h. Cell viability and caspase 3 + 7 activity were measured as in Fig. 2. A) HTR-8/SVneo cell viability (one-way ANOVA, $P < 0.0001$). B) HTR-8/SVneo cell caspase 3 + 7 activity (one-way ANOVA, $P < 0.0001$). C) BeWo cell viability. D) BeWo cell caspase 3 + 7 activity. Camptothecin (CPT) (4 μM) was included as a positive control. Data were analyzed statistically as in Fig. 2. * $P < 0.05$ compared to 0 μM (negative control). N = 3 independent experiments, with three replicates per treatment in each experiment.

from a choriocarcinoma as a cytotrophoblast model; and first trimester placental villous explant culture to model integrated tissue. The metabolites selected for comparison included TCA and DCA, which are formed in oxidative biotransformation of both PERC and TCE, and the glutathione conjugation pathway metabolites TCVC and DCVC, which are distinct metabolites of PERC and TCE biotransformation, respectively. Toxicity was determined by measuring cell viability and caspase 3 + 7 activity. Cell viability is a proxy for the number of living cells, while caspase activity is an indicator of apoptotic activation because caspases are specialized proteins that dismantle apoptotic cells [48]. These two endpoints were used as benchmarks for placental toxicity because aberrant cell death and apoptosis in extravillous trophoblasts and the syncytium are key pathological features of placental dysfunction associated with intrauterine growth restriction [49–51] and pre-eclampsia [50–52].

Although the CYP oxidation pathway metabolites TCA and DCA are implicated as prominent proximate toxic metabolites in the liver [53–55], in this study, TCA and DCA exhibited no evidence of overt toxicity in any placental model at concentrations up to 200 μM. In contrast, the glutathione conjugation pathway metabolites DCVC and TCVC, which are important for kidney toxicity [6], decreased cell viability and activated apoptotic caspases 3 + 7 in at least one placental model at concentrations ranging from 20 to 100 μM. This is consistent with our previous observations that DCVC concentrations as low as 20 μM caused significantly decreased live cell viability, increased cell death [38] and elevated caspase 3 + 7 activity [39] in HTR-8/SVneo cells.

Differences in sensitivity to DCVC and TCVC were also observed between in vitro placental models. Specifically, HTR-8/SVneo cells were more sensitive to DCVC than the other placental models, and HTR-8/SVneo cells, but not BeWo cells, exhibited toxicity in response to TCVC exposure. Sensitivity differences between cell types may be due to distinctive gene expression, differences in mitochondrial activity, detoxification ability and/or membrane permeability. For example, because BeWo cells are capable of fusing to make the syncytium, the barrier that protects the placenta, BeWo cells may be less permeable to toxicants than HTR-8/SVneo cells. Another potential explanation for differences in sensitivity may be due to specific characteristics of each cell line. For example, BeWo cells, originally derived from cancer cells, may be more resistant to toxic injury than HTR-8/SVneo cells through advanced adaptation mechanisms such as enhanced repair/increased tolerance to DNA damage, antiapoptotic potential, and enzymatic deactivation; the same way some cancers are more resistant to chemotherapy treatment than others [56].

Despite inherent differences in cell lines, the results in this study highlight potential specific vulnerabilities in normal cells to DCVC and/or TCVC insult in the placenta, which is consistent with DCVC and TCVC toxicity in other tissue types such as kidney [6]. It has been well-documented that DCVC [57–60] and TCVC [33,61,62] cause cell-specific toxicity to renal proximal tubular cells because of proximity

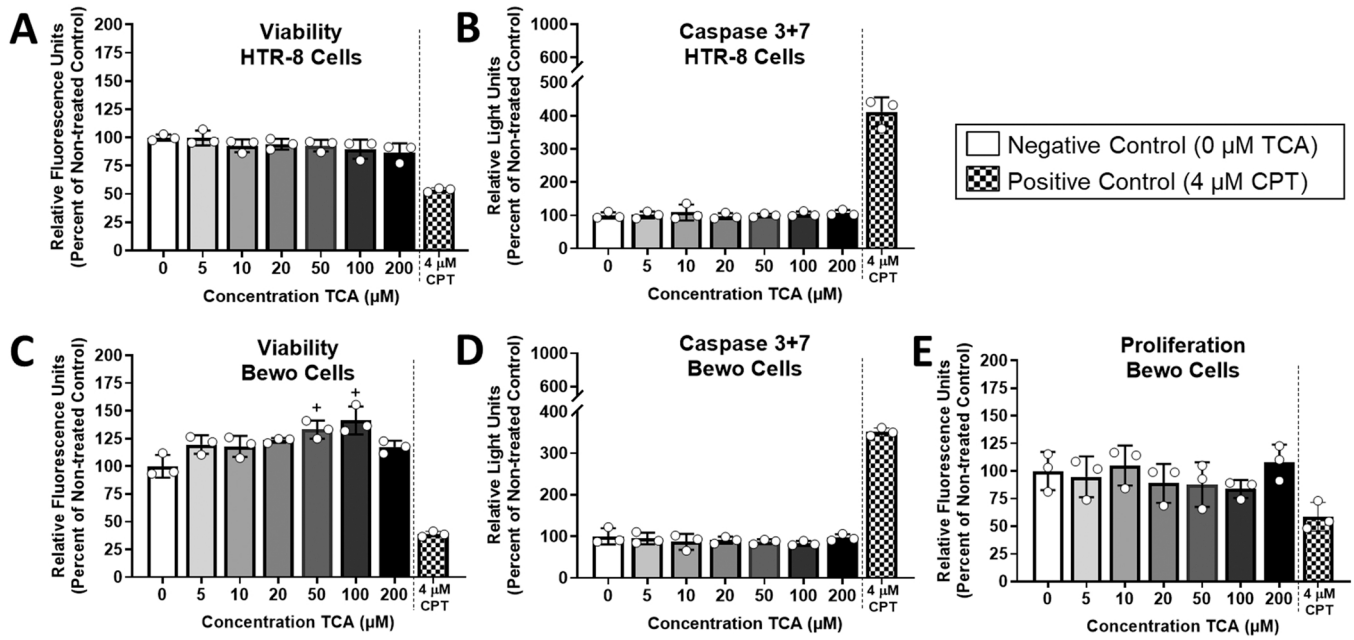


Fig. 4. Effects of PERC and TCE metabolite TCA on cell viability, caspase 3 + 7 activity and proliferation. HTR-8/SVneo cells and BeWo cells were treated with 0 (negative control), 5, 10, 20, 50, 100, or 200 μM TCA for 24 h. Cell viability and caspase 3 + 7 activity were measured with the ApoLive-Glo™ Multiplex Assay Kit. Cellular proliferation was measured with the CyQUANT® Cell Proliferation Assay. A) HTR-8/SVneo cell viability. B) HTR-8/SVneo cell caspase 3 + 7 activity. C) BeWo cell viability (one-way ANOVA, P = 0.002). D) BeWo cell caspase 3 + 7 activity. E) BeWo cell proliferation. Camptothecin (CPT) (4 μM) was included as a positive control. Data were analyzed statistically as in Fig. 2. $^{+}$Significantly different compared to 0 μM DCVC (negative control) (P < 0.01). N = 3 independent experiments, with three replicates per treatment in each experiment.

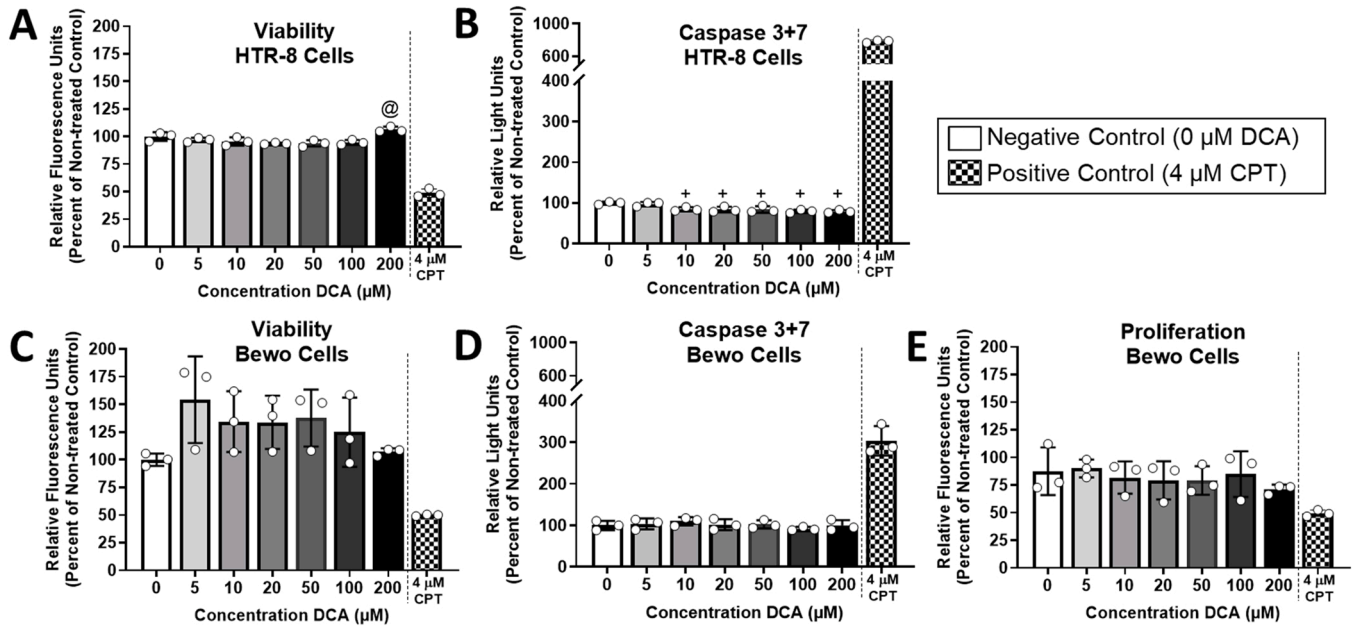
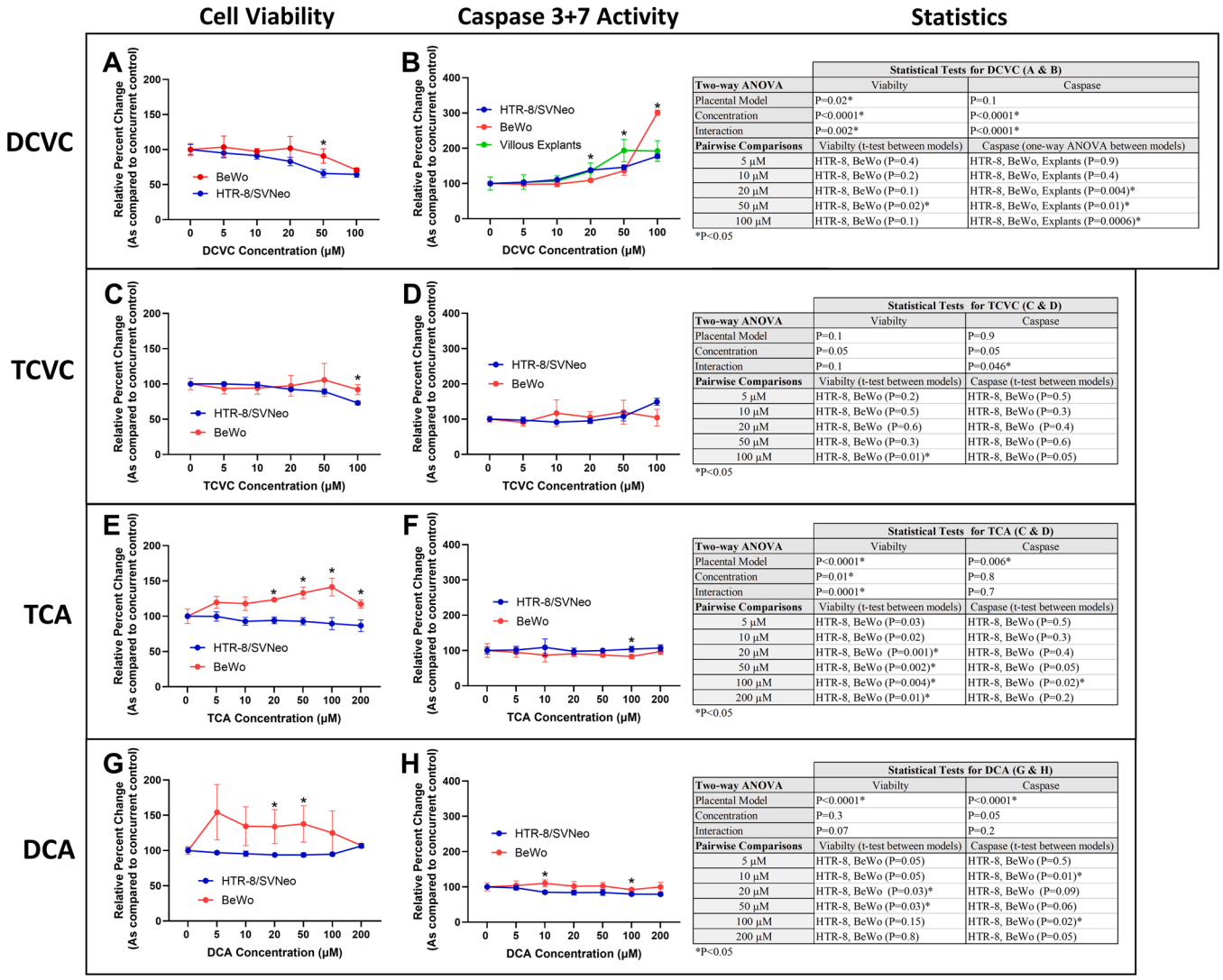


Fig. 5. Effects of PERC and TCE metabolite DCA on cell viability and caspase 3 + 7 activity. HTR-8/SVneo cells and BeWo cells were treated with 0 (negative control), 5, 10, 20, 50, 100, or 200 μM DCA for 24 h. Cell viability and caspase 3 + 7 activity were measured with the ApoLive-Glo™ Multiplex Assay Kit. Cellular proliferation was measured with the CyQUANT® Cell Proliferation Assay. A) HTR-8/SVneo cell viability (one-way ANOVA, P = 0.002). B) HTR-8/SVneo cell caspase 3 + 7 activity (one-way ANOVA, P = 0.001). C) BeWo cell viability. D) BeWo cell caspase 3 + 7 activity. E) BeWo cell proliferation. Camptothecin (CPT) (4 μM) was included as a positive control. Data were analyzed statistically as in Fig. 2. $^{+}$Significantly different compared to 0 μM DCVC (negative control) (P < 0.01). $^{@}$Significantly different compared to 0 (negative control), 5, 10 20, 50 and 100 μM DCA (P < 0.02). N = 3 independent experiments, with three replicates per treatment in each experiment.

to circulation, proximal tubular reabsorption and transport, and expression of cysteine conjugate beta-lyase, the enzyme compulsory for DCVC and TCVC bioactivation to reactive metabolites [63,64]. Regarding the placenta, cell-specific vulnerability to DCVC and TCVC is entirely plausible because of high perfusion, mRNA expression [19] and enzymatic activity of cysteine conjugate beta-lyase [24], and metabolic activity [20,65]. Because our in vitro results show potential cell-specific vulnerability in cell lines and the glutathione-conjugation metabolites



Statistical Tests for DCVC (A & B)		
Two-way ANOVA	Viabilty	Caspase
Placental Model	P=0.02*	P=0.1
Concentration	P<0.0001*	P<0.0001*
Interaction	P=0.002*	P<0.0001*
Pairwise Comparisons	Viabilty (t-test between models)	Caspase (one-way ANOVA between models)
5 μM	HTR-8, BeWo (P=0.4)	HTR-8, BeWo, Explants (P=0.9)
10 μM	HTR-8, BeWo (P=0.2)	HTR-8, BeWo, Explants (P=0.4)
20 μM	HTR-8, BeWo (P=0.1)	HTR-8, BeWo, Explants (P=0.004)*
50 μM	HTR-8, BeWo (P=0.02)*	HTR-8, BeWo, Explants (P=0.01)*
100 μM	HTR-8, BeWo (P=0.1)	HTR-8, BeWo, Explants (P=0.0006)*

*P<0.05

Statistical Tests for TCVC (C & D)		
Two-way ANOVA	Viabilty	Caspase
Placental Model	P=0.1	P=0.9
Concentration	P=0.05	P=0.05
Interaction	P=0.1	P=0.046*
Pairwise Comparisons	Viabilty (t-test between models)	Caspase (t-test between models)
5 μM	HTR-8, BeWo (P=0.2)	HTR-8, BeWo (P=0.5)
10 μM	HTR-8, BeWo (P=0.5)	HTR-8, BeWo (P=0.3)
20 μM	HTR-8, BeWo (P=0.6)	HTR-8, BeWo (P=0.4)
50 μM	HTR-8, BeWo (P=0.3)	HTR-8, BeWo (P=0.6)
100 μM	HTR-8, BeWo (P=0.01)*	HTR-8, BeWo (P=0.05)

*P<0.05

Statistical Tests for TCA (C & D)		
Two-way ANOVA	Viabilty	Caspase
Placental Model	P<0.0001*	P=0.006*
Concentration	P=0.01*	P=0.8
Interaction	P=0.0001*	P=0.7
Pairwise Comparisons	Viabilty (t-test between models)	Caspase (t-test between models)
5 μM	HTR-8, BeWo (P=0.03)	HTR-8, BeWo (P=0.5)
10 μM	HTR-8, BeWo (P=0.02)	HTR-8, BeWo (P=0.3)
20 μM	HTR-8, BeWo (P=0.001)*	HTR-8, BeWo (P=0.4)
50 μM	HTR-8, BeWo (P=0.002)*	HTR-8, BeWo (P=0.05)
100 μM	HTR-8, BeWo (P=0.004)*	HTR-8, BeWo (P=0.02)*
200 μM	HTR-8, BeWo (P=0.01)*	HTR-8, BeWo (P=0.2)

*P<0.05

Statistical Tests for DCA (G & H)		
Two-way ANOVA	Viabilty	Caspase
Placental Model	P<0.0001*	P<0.0001*
Concentration	P=0.3	P=0.05
Interaction	P=0.07	P=0.2
Pairwise Comparisons	Viabilty (t-test between models)	Caspase (t-test between models)
5 μM	HTR-8, BeWo (P=0.05)	HTR-8, BeWo (P=0.5)
10 μM	HTR-8, BeWo (P=0.05)	HTR-8, BeWo (P=0.01)*
20 μM	HTR-8, BeWo (P=0.03)*	HTR-8, BeWo (P=0.09)
50 μM	HTR-8, BeWo (P=0.03)*	HTR-8, BeWo (P=0.06)
100 μM	HTR-8, BeWo (P=0.15)	HTR-8, BeWo (P=0.02)*
200 μM	HTR-8, BeWo (P=0.8)	HTR-8, BeWo (P=0.05)

*P<0.05

Fig. 6. Comparisons of toxicity results across metabolite concentrations and placental models. Line graphs depicting percent changes in viability and caspase 3 + 7 measurements (compared to controls) for respective metabolite concentrations are displayed. Percent changes for viability and caspase 3 + 7 measurements (compared to controls) for respective metabolite concentrations were statically tested for significant differences between cell models using t-tests or one-way ANOVA. Two-way ANOVAs were also conducted to test for significant percent change differences between metabolite concentrations and cell models. Percent changes were log-transformed prior to statistical testing. *Asterisks indicate significantly different percent changes (compared to controls) between cell models for respective metabolite concentrations ($P < 0.05$). Results for HTR-8/SVneo appear as blue lines, BeWo cells appear as red lines and villous explants appear as a green line (DCVC caspase 3 +7 only).

are known to exhibit cell-specific vulnerability in normal kidney cells, a critical need exists for future studies to investigate the effects of DCVC and TCVC on relative cell abundance and specific cell type in placental tissue.

Because evidence shows that PERC and TCE metabolism occurs primarily through the CYP oxidation pathway in the liver, much attention has been paid to harmful effects of TCA and DCA as bioactive metabolites of PERC and TCE [6,66]. Between the two metabolites, TCA is considered the major metabolite in PERC- and TCE-exposed rodents based on concentrations measured in serum and urine. In humans, TCA is readily measured in serum and urine but DCA has not been detected directly, further confirming TCA as the major metabolite [35,67]. The lack of overt toxic effects of TCA and DCA on placental cell lines in the present study suggests some resilience against placental injury by these toxicants. These results are generally consistent with other studies evaluating TCA effects on placenta-related outcomes. Direct TCA exposure in mice did not affect placental weight [68]. Moreover, TCA did not affect pathogen-stimulated immune response in term gestational membranes, whereas DCVC inhibited pathogen-stimulated TNF-α release [69]. Nonetheless, prior studies showed that TCA exerts adverse effects in pregnant laboratory animals. TCA can cross the placenta and accumulate in fetuses of pregnant mice exposed to TCE or PERC [15]. Other studies showed that TCA exposure disrupted fetal development, increasing central nervous system abnormalities [70], decreasing fetal weight [71] and increasing cardiac defects [72–74], although the latter has been disputed [71,75,76]. Although our experiments do not support 3overt TCA and DCA toxicity in placental cell lines, which contrasts from the effects seen with the glutathione conjugation metabolites, further investigation into potential sublethal effects of TCA and DCA may be warranted.

The present study found evidence of increased abundance of live BeWo cells following TCA treatment, consistent with increased hepatocyte proliferation found in previous studies investigating TCA- and DCA-induced liver carcinogenicity in rodents [54,55]. Another study reported inhibited apoptosis in rat hepatocytes exposed to TCA or DCA [77], similar to the modest but significantly decreased caspase 3 + 7

activity observed with DCA exposure of HTR-8/SVneo cells. However, no changes in BeWo cell DNA quantity were observed, suggesting the increase in live BeWo cells occurred by non-nuclear event(s), which remain unclear.

3.1. Metabolite concentrations

The metabolite concentrations used in this study were selected based on relevance to plausible human exposure levels and to establish preliminary concentration thresholds of overt toxicity for metabolites in the different placental models. The glutathione conjugation-derived metabolite concentrations used in our study, 5–100 μM, are relevant to the Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) for PERC and TCE of 100 parts-per-million (ppm) averaged over an 8-hour work day [78,79]. In an occupational exposure study, TCE concentrations up to 229 ppm were reported in 80 exposed workers (29% women) wearing aerosolized monitoring devices [80]. In addition, female volunteers exposed by inhalation to the PEL of TCE for 4 h had an average peak blood concentration of 13.4 μM for the metabolic precursor to DCVC, *S*-(1,2-dichlorovinyl)glutathione (DCVG) [81], a concentration comparable to the lower end concentrations used in this study. Although TCVC has not been directly measured in human serum, NAcTCVC, the downstream metabolite of TCVC following biotransformation via N-acetyltransferase, was measured at the mean value of 422 μM in the urine of human volunteers exposed to 40 ppm PERC for 6 h via inhalation, which is considerably higher than the concentrations used in this study [67]. Under the same conditions, the latter study also measured TCA, the major CYP-derived PERC and TCE metabolite. They reported a mean value of 3.04 μM TCA in plasma, which is comparable to the lowest concentrations used in this study, and 40.8 mM TCA in urine, which is several orders of magnitude higher than the highest concentrations used in this study [67]. Since DCA has not been measured in human serum or urine [35,67], we used the same large range of concentrations for DCA as TCA. Although more recent studies have measured TCE and PERC metabolites in mouse samples using advanced technologies [66], [82,83], differences between rodent and human metabolism of TCE and PERC [6,34] limit the direct relevance of metabolite concentrations derived from mice to our study.

3.2. In vitro placental models

The current study is limited by the use of in vitro cultured cell lines and tissues to model the placenta, which may not fully represent biological interactions that occur in vivo. When using tissue explants, villi structure remains intact, although critical interactions are lost between fetal and maternal cells that play a pivotal role in placental development. Cell lines, on the other hand, consist of using a single cell type with no tissue structure intact. HTR-8/SVneo is an immortalized cell line that exhibits a combination of molecular markers and phenotypical characteristics unique to extravillous trophoblasts [84]. Molecular markers include: cytokeratin 7, histocompatibility antigen class I G (HLA-G) (when grown on Matrigel) and α5β1 integrin dimers [85–89]. Phenotypical characteristics include mesenchymal proteomic profile [90] and stress-induced altered invasion [87,91,92]. BeWo is a choriocarcinoma hormone-producing cell line that models cytotrophoblasts when unsyncytialized and syncytiotrophoblasts when syncytialized [32, 93,94]. Although the BeWo cells used in the current study were unsyncytialized, BeWo cells syncytialized with forskolin display characteristics consistent with syncytiotrophoblasts in vivo including cell fusion and multinucleation [93,95], as well as secretion of human chorionic gonadotrophin [93].

Although the HTR-8/SVneo and BeWo cell lines both originate from human placenta, the processes of immortalization and tumorigenesis inevitably change some cellular characteristics relative to primary cells. For example, HTR-8/SVneo cells have differential genetic and epigenetic profiles compared to primary extravillous trophoblasts [96,97], including aneuploidization [98]. BeWo b30 cells, a BeWo clone, have altered expression of placental lactogen, placental alkaline phosphatase and Schwangerschafts protein 1, and exhibit a brush border [93], similar to syncytiotrophoblasts in vivo. However, BeWo choriocarcinoma cells may possess detoxification mechanisms acquired during oncogenic transformation that are not available in non-cancerous trophoblasts [99, 100]. Despite these limitations, HTR-8/SVneo and BeWo cells have been used extensively by placental biologists to model interactions of placental cells with toxic substances in vitro [65,84]. Moreover, cell lines are a useful tool in toxicology studies because of their availability, minimal time investment and low cost [65]. Overall, the in vitro models used here contribute new information to the potential effects of chlorinated hydrocarbon metabolites on placental toxicity.

Focused assessments of DCVC-induced caspase 3 + 7 activity in human primary placental villous explants.

Due to extremely limited availability, another limitation of this study is that the first trimester placental villous explant model was only used to evaluate the toxicity of one metabolite, DCVC. DCVC was selected for the villous explant model because it proved to be the most potent toxic metabolite in the HTR-8/SVneo cell line, which specifically models and was originally derived from first trimester placental tissue.

4. Conclusion

In summary, toxicological evaluation showed that 24-h treatment with the glutathione conjugation-derived metabolites DCVC and TCVC differentially affected HTR-8/SVneo cells, BeWo cells and villous explants (DCVC only), causing toxicity in HTR-8/SVneo cells at a lower concentration than BeWo cells and villous explants. Moreover, HTR-8/SVneo cells were more sensitive to TCE-derived DCVC than PERC-derived TCVC. Lastly, the CYP oxidation-derived metabolites TCA and DCA did not have overt toxic effects on placental cell lines. These findings suggest that the glutathione conjugation biotransformation pathway may be more directly involved in the mechanism of placental toxicity than the oxidative pathway because its metabolites are more toxic to placental cells. Moreover, despite their chemical similarity, TCE may pose a greater risk for placental toxicity due to metabolic formation of DCVC, which does not occur for PERC. This study advances our understanding of how maternal exposure to PERC and/or TCE may contribute to placental injury by identifying relative toxicity of key metabolites in placental cells and tissues. This important knowledge will future studies using primary cell or tissue culture models, as well as in vivo studies, will clarify the results reported here and further probe the specific biological and molecular signaling pathways underpinning chlorinated hydrocarbons toxicological mechanisms.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval and consent to participate

Placental tissues were acquired as discarded tissues at the time of termination at a Michigan family planning clinic with Institutional Review Board approval (Wayne State University IRB# 013515MP4E) and written informed consent by patients, obtained by collaborating Wayne State University physicians.

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