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Placenta as a target of trichloroethylene toxicity

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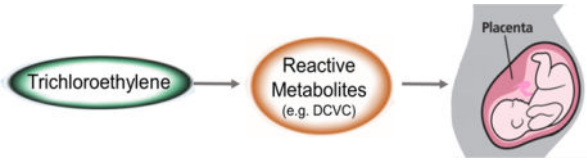
Abstract

Trichloroethylene (TCE) is an industrial solvent and a common environmental contaminant detected in thousands of hazardous waste sites. Risk of exposure is a concern for workers in occupations that use TCE as well as for residents who live near industries that use TCE or who live near TCE-contaminated sites. Although renal, hepatic and carcinogenic effects of TCE have been documented, less is known about TCE impacts on reproductive functions despite epidemiology reports associating maternal TCE exposure with adverse pregnancy outcomes. Toxicological evidence suggests that the placenta mediates at least some of the adverse pregnancy outcomes associated with TCE exposure. Toxicology studies show that the TCE metabolite, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) generates toxic effects such as mitochondrial dysfunction, apoptosis, oxidative stress, and release of prostaglandins and pro-inflammatory cytokines in placental cell lines. Each of these mechanisms of toxicity have significant implications for placental functions and, thus, ultimately the health of mother and developing child. Despite these findings there remain significant gaps in our knowledge about effects of TCE on the placenta, including effects on specific placental cell types and functions as well as sex differences in response to TCE exposure. Due to the critical role that the placenta plays in pregnancy, future research addressing some of these knowledge gaps could lead to significant gains in public health.

Graphical Abstract



Keywords

Trichloroethylene; trichloroethene; placenta; toxicity; adverse pregnancy outcomes

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Conflict of Interest
The authors declare that there are no conflicts of interest.

Environmental significance

Because of its continued industrial use and improper disposal, trichloroethylene (TCE) is a common environmental contaminant detected in thousands of hazardous waste sites. Due to widespread and persistent environmental contamination, TCE poses a threat for human exposure through drinking contaminated water and inhaling volatilized compound. Extensive scientific research has demonstrated potent organ-specific TCE toxicity. Recent epidemiological studies report associations between maternal TCE exposure and adverse pregnancy outcomes. TCE toxicity to the placenta may contribute to adverse pregnancy outcomes due to the placenta's critical role during gestation. Better understanding of TCE impacts on the placenta may provide critical information for assessing and ultimately controlling risks to pregnant women from TCE exposure. This perspective details important research being conducted into the effects of TCE and its reactive metabolites on placenta and provides recommendations for future research.

Introduction

Pregnant women are exposed to a large number of environmental contaminants.[1] For example, perfluorooctanyl sulfonate (PFOS), several polychlorinated biphenyls (PCBs), and several polybrominated diphenyl ethers (OBDEs) were detected in serum of 99–100% of pregnant women included in the U.S. Centers for Disease Control and Prevention (CDC) National Biomonitoring Program.[1] During pregnancy, the placenta provides the essential interface for exchange of oxygen, nutrients, and metabolic wastes between mother and fetus.[2] In addition, the placenta becomes the major source of hormones necessary for pregnancy maintenance, parturition, and maternal and fetal health.[3–5] Because the placenta is critically important for pregnancy success, disruption of placental development and function is an important health concern.[5] Indeed, the summary report from a recent workshop sponsored by the U.S. Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, concludes that, "the majority of adverse pregnancy outcomes can trace their origin to the placenta."[6] Although various environmental contaminants have been detected in placental tissue,[7] our understanding of toxicant effects on placenta is limited. This article explores placental toxicity from exposure to trichloroethylene (TCE), a common and persistent environmental pollutant,[8] in the context of implications for risks to pregnancy. Challenges specifically related to study of the placental as a toxicant target and of trichloroethylene in particular are discussed.

Adverse pregnancy outcomes: a global public health crisis

Adverse pregnancy outcomes are a significant public health concern, especially preterm birth (<37 weeks gestation) and low birth weight (<2500 g).[9, 10] Globally, complications from preterm birth are the number one leading cause of death in children under 5 years of age,[11] with approximately 15 million or 11% of infants born preterm each year.[9] The U.S. has the highest infant mortality rate compared with other developed countries,[11, 12] and disorders related to preterm birth and low birth weight contribute to the majority of infant deaths.[13] Preterm births continued to rise in the U.S., after a brief respite, to 10.02% in

2018.[14] Likewise, the U.S. incidence of low birth weight increased to 8.28% by 2017.[10] Babies born preterm or with low birth weight are more likely to require intensive and prolonged hospitalization, and are at increased risk for chronic health complications.[10, 15, 16] The increased incidence of these adverse pregnancy outcomes and their potential significance for long-term health consequences have resulted in widespread recognition that the high rates of preterm birth and low birth weight constitute a national and global public health crisis.[11, 17–19]

Contribution of environmental contaminants to adverse pregnancy outcomes

Despite considerable research efforts and identification of risk factors associated with adverse pregnancy outcomes, such as genetic factors, infections, contraindicated maternal behaviors and chronic maternal conditions, the specific pathophysiology underlying most adverse pregnancy complications outcomes remains poorly understood.[18] Exposure to environmental contaminants are increasingly thought to contribute to adverse pregnancy outcomes. Indeed, epidemiological studies have detected associations between maternal exposures to environmental contaminants and increased risk for adverse pregnancy outcomes.[20, 21] For example, studies have associated exposure to fine air particulate matter (PM 2.5) with increased risk of pre-eclampsia and preterm birth.[22, 23] Other studies reported that exposure to relatively low concentrations of lead and cadmium were associated with increased preterm birth and small head circumference.[24, 25] Similarly, maternal exposure to polyfluoroalkyl substances (PFAS) was positively associated with preterm birth.[26] Although some studies have established links between environmental contaminants and adverse pregnancy outcomes, many studies have reported conflicting results on the same contaminants.[20] For example, despite dozens of studies on the subject, an association between maternal environmental tobacco smoke exposure and preterm birth remains inconclusive.[27] Despite reasonable evidence that maternal exposure to environmental contaminants contribute to elevated risks of adverse pregnancy outcomes, further investigation into the biological plausibility and specific toxicological mechanism of different environmental contaminants is warranted.

Importance of placenta as a toxicant target organ

Adverse pregnancy outcomes are strongly linked to disorders of the placenta.[6] A leading cause of fetal death in 2014 in the U.S. was identified as “complications of placenta, cord and membranes,”[28] and complications of the placenta are included in the most prevalent identifiable cause of fetal death in the U.S.[12, 28] Furthermore, recent reviews identify the placenta as a key factor in preterm birth[29] and fetal growth restriction.[30] In addition, adaptation of the placenta to changes in its environment is a proposed to play an important role in developmental origins of adult health and disease.[2, 5, 6]

Because the placenta is perfused with maternal blood at a rate up to 700 ml/min by term,[31] any toxicants that are circulating in the mother’s blood are efficiently delivered to the placenta, increasing opportunity for toxicant effects in this organ. In addition, toxicants can move across the placenta between maternal and fetal blood supplies by mechanisms that

include simple passive diffusion and transporter-mediated processes, thereby contributing to increased opportunity for placental toxicant exposure and effects.[32] Moreover, some toxicants can preferentially bioaccumulate in the placenta, as shown for bisphenol A,[33, 34] some PCBs,[35] some co-planar dioxins,[36] and cadmium and other metals.[35]

Because of its significant endocrine functions,[3, 5] the placenta is vulnerable to endocrine disruption.[37, 38] Likewise, because the placenta synthesizes and releases cytokines and prostaglandins, molecules that regulate placental function as well as the onset of labor,[3, 4] disruption of the balance of placental cytokines and prostaglandins could lead to preterm birth or other adverse pregnancy outcomes.[5, 6, 39, 40] Moreover, it has been suggested that early placental formation and development, particularly remodeling of the uterine spiral arteries, may be especially vulnerable to environmental insult.[5, 38, 41, 42] Recent reviews provide evidence that placental insufficiency, which is thought to be a symptom of failed spiral artery remodeling, is known or strongly suspected to contribute to intrauterine growth restriction,[30, 43–45] preterm birth,[29, 46] and pre-eclampsia.[45, 47, 48]

Because proper placental development and function are vital for pregnancy health and success, improved understanding of chemical impacts on the placenta would provide critical insight into environmental risks for pregnancy. Recent reviews highlight the incomplete and uneven knowledge of placental toxicology, with a tendency to focus on endocrine disrupting chemicals.[38, 49]

Trichloroethylene is a pervasive and persistent environmental contaminant

Trichloroethylene (TCE) is an anthropogenic volatile organic chemical with a legacy of environmental contamination.[50] Historically, TCE was used for degreasing metal parts and as a solvent in the manufacture of textiles, lubricants, paints, paint strippers, pesticides and more.[50] Today, over 80% of TCE production is used as a chemical intermediate in closed-system refrigerant manufacturing processes, specifically HFC-134a, a refrigerant used in car air conditioning systems.[50, 51] The other current primary use of TCE is in vapor metal degreasing, although the U.S. Environmental Protection Agency (EPA) proposed banning this application.[52] Within the U.S., the state of Indiana reported the highest release of TCE into the environment from industrial sources, accounting for 35% of total reported releases in the U.S. in 2017 and four times more than the next highest state, Pennsylvania.[50, 53] Due to its low vapor pressure, TCE readily volatilizes into air. In addition, TCE is denser than water, allowing it to form subsurface plumes as a dense non-aqueous phase liquid (DNAPL). As a result of its chemical properties, long-standing industrial use, and past disposal practices, TCE is a common and persistent environmental pollutant found in soil, air and water.[8] It contaminates 1055 of 1750 current and former U.S. EPA-designated National Priorities List (Superfund) sites,[50, 53] and is ranked number 16 in the National Priorities List of the U.S. Agency for Toxic Substances and Disease Registry (ATSDR).[50, 53]

Exposure to trichloroethylene

Exposure in air

Monitoring data from the U.S. EPA indicates that TCE is commonly detected in ambient air, albeit at low concentrations (typically < 1 ppb).[50] Still, high ambient air concentrations have been reported, such as 49 ppb at a site in Oregon in 2011, suggesting that point sources of emissions may elevate air concentrations locally.[50] In addition, indoor air TCE concentrations can become elevated due to TCE release from building materials and household products, volatilization from tap water use (e.g., showering), and migration from surrounding contamination into buildings (vapor intrusion). For example, a recent study detected blood concentrations of TCE that were 50 times higher in people exposed through residential vapor intrusion (>1.6 μg M^{-3} indoor air concentration) compared with residents of households with no detectable TCE vapor intrusion.[54] Numerous studies report higher TCE concentrations in indoor air compared to outdoor air, even for structures located near point source emitters and when no TCE was detected in outdoor air, as summarized.[50] In addition, inhalation exposure is a concern for occupations that involve TCE, particularly those that use TCE in vapor degreasing operations. The U.S. Occupational Safety and Health Administration (OSHA) limits TCE inhalation exposure in the workplace: the permissible exposure limit (PEL) for TCE in air averaged over an 8-hour workday is 100 ppm with an acceptable maximum level of 300 ppm TCE for no more than 5 minutes of any 2-hour period.[55]

Exposure in drinking water

TCE exposure can also occur through ingestion of contaminated drinking water. The U.S. EPA has set a maximum contaminant level of 5 ppb in drinking water,[56] whereas the World Health Organization recommends 20 ppb as a drinking water guideline value.[57] In 2005, the last year of the most recent review of public water systems by the U.S. EPA, TCE was detected in 14.8% of public water supplies that used a surface water source compared to 4.9% that relied on ground water as the source.[50] In the latter review, median levels were 1.6 ppb and 1.1 ppb for public water from surface and ground water sources, respectively, with 159 ppb reported as the highest TCE concentration for a public water supply. A notable contamination of drinking water occurred at the U.S. military base Camp Lejeune in North Carolina in the 1970's and 1980's.[58] TCE contaminated drinking water that was provided to family housing on the base, with a peak concentration of 1,400 ppb detected in 1982.[59] Health effects continue to be studied in the TCE-exposed Camp Lejeune population.[58]

Exposure of pregnant women

Because of the pervasive presence of TCE as an air, water and soil contaminant, and its continued use in industrial processes, pregnant women are likely exposed to TCE. However, estimates of prevalence of exposure of pregnant women are not available.

Trichloroethylene metabolism

The low-molecular weight, lipophilic, and nonpolar properties of TCE allow it to be quickly absorbed into the blood stream from the lungs, gastrointestinal tract, and skin.[60] Once in the

blood stream, the chemical distributes rapidly to highly perfused organs,[60] including liver, kidney and lungs where it is biotransformed. Biotransformation is important for converting TCE to reactive metabolites that can damage cellular macromolecules.[61] There are two major metabolic pathways for TCE in mammals, each pathway involving multiple steps of biotransformation (Figure 1).

Occurring primarily in the liver, an important metabolic pathway of TCE is oxidation by several CYP family of enzymes (notably CYP1A1, CYP1A2, CYP2E1 and CYP3A4) to generate a TCE-epoxide-CYP (TCE-O-CYP) intermediate. The TCE-O-CYP intermediate can generate *N*-hydroxy-acetyl-aminoethanol, chloral (CHL)/chloral hydrate (CH) or TCE-epoxide (TCE-O), which can convert to oxalic acid (OA) or dichloroacetyl chloride (DCAC). CHL/CH is subsequently biotransformed in multiple steps to the several key oxidative metabolites: dichloroacetate (DCA), trichloroacetate (TCA), monochloroacetate (MCA), trichloroethanol (TCOH), and the glucuronide of TCOH, trichloroethanol glucuronide (TCOG).[60, 62] Whereas unstable metabolites from the CYP oxidative pathway include TCE-O-CYP, TCE-O, and DCAC, CH and CHL have been shown to be mutagenic and genotoxic.[63]

In the other important metabolic pathway, TCE is conjugated to glutathione (GSH) as S-(1,2-dichlorovinyl) glutathione (DCVG). The DCVG is further metabolized to *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), which can be metabolized to S-(1,2-dichlorovinyl)thiol (DCVT), N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC), or DCVC sulfoxide (DCVCS).[60, 62] NAcDCVC can be converted to NAcDCVC sulfoxide (NAcDCVCS) by CYP3A enzymes, and DCVT or DCVCS can spontaneously rearrange to chlorothionoacetyl chloride (CTAC) or chlorothioketene (CTK), two unstable compounds that are readily interconverted.[63] In addition to CTAC and CTK, other known unstable metabolites of the glutathione conjugation metabolic pathway of TCE include DCVT.[61] Several downstream metabolites of DCVC – DCVT, CTAC, CTK, and NAcDCVCS – are toxic and mutagenic.[61, 64]

With the exceptions of TCE-O, DCAC, DCVT, DCVCS, CTK, and CTAC, all other TCE metabolites are systemically available.[63] TCE itself has a relatively short plasma half-life of 3–4 days in humans, depending on exposure duration and concentration. Although some TCE is exhaled in its parent form, most TCE is metabolized with elimination from the body in bile and urine.[60, 65] TCE, OA, DCA, TCA, MCA, TCOH, TCOG, NAcDCVC, and NAcDCVCS are known to be recovered in urine, whereas hepatic DCVG can be readily excreted into plasma or bile.[63] Therefore, based on the numerous stable TCE metabolites formed by the CYP oxidative pathway that are recovered in urine, the CYP pathway is known as the quantitatively dominant TCE metabolic pathway. This contrasts with the glutathione conjugation metabolic pathway, which forms fewer metabolites recovered in urine but more unstable and reactive metabolites.[61]

Considerable differences exist among species, tissue, strain within a species, and sex for TCE metabolism, influencing susceptibility to TCE toxicity. Mice are known to have a higher maximal rate of CYP oxidative metabolism than rats, and rats have a higher maximal rate of CYP oxidative metabolism compared to humans.[61] Similarly, mouse tissues exhibit

higher glutathione conjugation rates compared to equivalent rat tissues,[66] and humans have lower activity of γ-glutamyltransferase, which metabolizes DCVG,[60–62] compared to mice and rats.[67] In different organs of mice, liver cells more effectively metabolize CH/CHL via UDP-glucuronosyltransferase compared to lung cells.[68] In rats, liver cells have higher GSH conjugation rates compared to kidney cells.[66] Among 15 different mouse strains, exposure to the same dose of TCE produces variable serum levels of DCVG.[69] Finally, sex-specific difference includes higher abundance of TCE metabolites from the CYP pathway in liver of male compared to female rats, as well as evidence suggesting a faster breakdown of DCVG in males compared to females rats.[70] Because CYPs can be differentially expressed between males and females,[71] inter-individual differences among humans in TCE metabolism can be quite large. TCE metabolism can also be modified by lifestyle factors. For example, ethanol is a known modulator of CYP2E1 expression.[72]

Placental transfer and metabolism of trichloroethylene

Any TCE and TCE metabolites circulating in the mother's blood are efficiently delivered to the placenta. Several studies have demonstrated that TCE and its metabolites readily cross the placenta. In women, early studies that were conducted when TCE was used as an anesthetic gas in obstetric surgery detected TCE in the umbilical vein and umbilical artery, indicating placental transfer.[73, 74] Ghantous et al. demonstrated that unmetabolized radiolabeled TCE as well as radiolabeled TCA derived from TCE metabolism, were detected in the placenta, amniotic fluid and fetal tissues of TCE inhalation-exposed pregnant mice at all stages of gestation.[75] Notably, the glutathione-pathway metabolite DCVC has not been measured directly in placental tissue, which may be attributed to its reactivity and short half-life.[76]

Toxicants that are lipophilic, such as TCE, can transfer across the placenta by simple passive diffusion.[77] On the other hand, various transporters could potentially facilitate placental transfer of some non-lipophilic TCE metabolites.[77, 78] For example, the TCE metabolites DCA and TCA, which circulate in maternal blood,[61] could possibly be transported by placental organic anionic transporters. Additionally, DCVC, which is available systemically[61] and resembles a small peptide in structure, could be transported by placental peptide transferases. Some transporters involved in TCE tissue distribution have been characterized in kidney.[61] However, no research to date has specifically investigated the role of transporters in placental transfer of TCE or its metabolites.

In addition to providing evidence of placental transfer, the findings of Ghantous et al. support occurrence of TCE metabolism in the placenta or fetus independent of maternal metabolism.[75] Consistent with the latter findings, the placenta contains a variety of enzymes capable of metabolizing TCE and its metabolites. In regard to the CYP-dependent oxidative pathway, a range of CYPs are expressed in human placenta.[79] These include CYP1A1 and low levels of CYP2E1,[79] two CYPs that produce the TCE-O-CYP intermediate from TCE,[61] the first step of CYP-dependent TCE metabolism. Low activity of aldehyde dehydrogenase, the enzyme that converts CH/CHL into TCA as part of the CYP-dependent pathway,[61] has also been detected in rat placenta.[80] In regard to the GSH conjugation pathway of TCE metabolism, the human placenta expresses various glutathione S-transferases (GSTs),[81, 82]

the enzymes known to metabolize TCE into DCVG.[61] The human placenta also contains active γ-glutamyltransferase,[83] one of the two enzymes that converts DCVG into DCVC.[61] Cysteinyl-glycine dipeptidase, the other enzyme necessary for DCVG conversion to DCVC,[61] is thought to be relevant to reproduction for its role in GSH metabolism[84] but has not been detected in placenta to date.

Non-reproductive adverse health effects associated with trichloroethylene exposure

TCE has been extensively studied as a renal and liver toxicant because these organs are the primary sites of TCE metabolism via glutathione conjugation and cytochrome P450, respectively, in the body.[8, 60, 70, 85] TCE was recently re-classified by the U.S. EPA,[86] U.S. National Toxicology Program (NTP),[51, 87] and International Agency for Research on Cancer (IARC)[88] as a known human carcinogen. This re-classification was based, in part, on epidemiology studies associating TCE exposure with elevated risk of renal cancer[8, 89] and reports that the glutathione conjugation TCE metabolite, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), causes nephrotoxicity.[60, 90] In addition to liver and kidney toxicity, TCE-induced health effects have been reported in epidemiology and animal studies that indicate neurotoxicity, respiratory distress, and gastrointestinal irritation.[8, 62, 91–94]

Association of maternal trichloroethylene exposure with adverse pregnancy outcomes

Human studies

Although many studies have established TCE as a potent renal and liver toxicant,[8] TCE effects on pregnancy outcomes remain understudied and inconclusive. Epidemiology studies of associations between TCE exposure and adverse pregnancy outcomes report inconsistent findings. Exposure to drinking water contaminated with TCE and other volatile organic chemicals was associated with low birth weight in Woburn, Massachusetts, when exposure in the year of birth as well as the year prior to birth were included in the analysis, but not when the analysis was restricted to the year pregnancy ended.[95] A review of birth records and state databases in northern New Jersey found evidence of an association between TCE in drinking water and low birth weight at term, but not for small for gestational age (weight below the 10th percentile for the gestational age) and very low birth weight (≤1500 g).[96] A study of women exposed to TCE via drinking water in Tucson, Arizona, found a positive association with very low birth weight but no significant association with low birth weight (≤2500 g).[97] In a more recent vapor intrusion study of pregnant women exposed to TCE via indoor air, positive associations were observed for TCE exposure with low birth weight, small for gestational age, and low birth weight at term.[98, 99] Moreover, a study of pregnant women residing at Camp Lejeune, North Carolina, found that TCE exposure via drinking water was associated with measures indicative of impaired fetal growth, including low birth weight at term, reduced mean birth weight, and small for gestational age.[99]

At least some of the inconsistency in findings of published epidemiology reports may be related to chemical co-exposures that are common with TCE. As noted by the U.S. ATSDR,

studies of associations between human health effects and TCE exposure have involved exposure to multiple chemicals but failed to assess possible confounding by chemical co-exposures.[50] Future research of TCE-exposed human populations will likely benefit from advances in methods to study co-exposures, which has become a priory of the National Institute of Environmental Health Sciences.[100, 101]

Animal studies

Exposure of Wistar rats to 100 ppm in air from gestation day 8–21 for 4 hours per day significantly reduced fetal weight at term by approximately 9%.[102] The dose of the prior study is similar to the U.S. OSHA PEL standard of 100 ppm TCE in air averaged over an 8-hour workday. Likewise, in a study of Wistar rats exposed to 480 mg TCE/kg body weight/day during mid-pregnancy (gestation day 6–16) via a food treat, fetal weights were reduced approximately 10% on gestation day 16 without significant reduction of maternal body, liver, or kidney weights.[103] However, a study with Sprague-Dawley rats exposed by oral gavage to 500 mg TCE/kg body weight/day on gestation days 6–15 found no significant reduction in fetal weight on gestation day 21.[104] The doses used in the prior studies with oral exposure are equivalent to an exposure within an order of magnitude of the U.S. OSHA PEL but are several orders of magnitude higher than expected from drinking water exposures. Differences in findings among the rodent studies could be related to differences in strain of rat, exposure regimen, and gestational age at fetal assessment. Regardless, there is sufficient evidence to warrant further evaluation of TCE effects on pregnancy outcomes in toxicology and epidemiology studies.

Placental toxicity of trichloroethylene

Several lines of evidence support the placenta as a target of TCE toxicity, as discussed in this section below and illustrated in Figure 2. This evidence includes toxicokinetics, biotransformation, activation of biochemical and cellular responses, and disruption of placental functions that can lead to adverse pregnancy outcomes, supported by epidemiology studies.

Placental toxicity of the trichloroethylene metabolite DCVC

Experiments in our laboratory indicate that the glutathione pathway metabolite DCVC is particularly toxic to placental cells at low concentrations.[105–107] The DCVC concentrations of 5–20 μM used in the placental cell studies encompass the average maximum concentration of 13.4 μM DCVG measured in the blood of female volunteers exposed to 100 ppm TCE in air for 4 hours.[108] The TCE concentration used in the latter human exposure study is the U.S. PEL concentration limit for occupational TCE exposure averaged over an 8-hour workday.[55] Because DCVG is the precursor of DCVC and precursor conversion to DCVC appears to be in a 1:1 stoichiometric ratio,[60] the range of DCVC concentrations used in placental cell experiments is relevant for human occupational exposures. DCVC disrupts placental cells through multiple molecular and cellular pathways, as described below. In contrast, we observed only nominal cytotoxicity in placental cells exposed to the oxidative metabolites TCA and DCA, even at much higher concentrations than DCVC toxic concentrations (unpublished).

DCVC stimulates pro-inflammatory response in placental cells

DCVC appears to disturb the balance between pro- and anti-inflammatory pathways in placental cells. In a human extravillous trophoblast model, the HTR-8/SVneo cell line, 10 and 20 μM DCVC stimulated generation of reactive oxygen species and the synthesis and release of the pro-inflammatory cytokine IL-6 after 10 and 24 h of exposure, respectively.[107] The DCVC-stimulated IL-6 release from HTR-8/SVneo cells was blocked by treatment with aminooxyacetic acid (AOAA), a renal cysteine conjugate β-lyase (β-lyase) inhibitor,[109, 110] suggesting that the DCVC-stimulated release of IL-6 was due to a reactive metabolite of DCVC generated via β-lyase activity, consistent with reports of DCVC toxicity in other cell types.[61, 109, 110] In addition, DCVC-stimulated release of IL-6 and *IL6* mRNA expression was strongly inhibited by antioxidant treatments,[107] implicating a role for reactive oxygen species in the IL-6 response, also consistent with findings in other cell types.[111, 112] These results suggest that TCE and its metabolites can activate pro-inflammatory pathways in the placenta, potentially via the generation of reactive oxygen species. These findings have significant implications for pregnancy, as an overall imbalance in pro- versus anti-inflammatory signaling in gestational tissues and maternal circulation is thought to be an important contributor to several adverse pregnancy outcomes such as preeclampsia, preterm birth and spontaneous abortion.[113, 114]

DCVC disrupts mitochondrial function and membrane potential

In addition to the findings on reactive oxygen species and the pro-inflammatory response, the prior study with HTR-8/SVneo cells determined that DCVC disrupted the mitochondrial membrane potential with exposures to 5, 10 and 20 μM for 4, 10 and 24 h.[107] Moreover, the mitochondrial membrane transition pore inhibitor bongkrekic acid significantly attenuated but did not completely inhibit IL-6 release.[107] Because mitochondrial dysfunction and depolarization of the mitochondrial membrane are potential initiators as well as responses of increased cellular generation of reactive oxygen species, subsequent studies focused on DCVC effects on mitochondria, cellular energy production and lipid peroxidation.

In follow-up studies, our laboratory recently showed that 20 μM DCVC exposure for 12 h causes adaptive changes to macronutrient and energy metabolism pathway utilization in order to maintain adequate ATP levels.[115] Consistent with energy metabolism disruptions, mitochondrial dysfunction (e.g., proton leak, energy coupling deficiency, and dissipated mitochondrial membrane potential) was also measured in the same cell line, as early as six hours after initiating treatment.[106] In addition, mitochondrial DNA content (a proxy for mitochondrial copy number) was reduced after 12 hours of 20 μM DCVC exposure.[106] Lastly, we reported that DCVC elevated levels of the lipid peroxidation byproduct malondialdehyde.[105] Because optimal mitochondrial functioning is critical for placental extravillous trophoblast cells due to their sizable energy requirements for biological processes such as tissue remodeling,[116–118] mitochondrial disruption could have significant implications for placental health. Indeed, the most widely-reported aspects of mitochondrial dysfunction associated with abnormal placentae are mitochondria-generated reactive oxygen species, lipid peroxidation, and mitochondrial DNA content, as reviewed.[119, 120] Given the critical role that mitochondria play during pregnancy, the effects of DCVC on these processes warrant further study.

DCVC stimulates intrinsic and extrinsic apoptosis pathways

Our laboratory has also shown that DCVC concentrations as low as 20 μM for 12 h induces apoptosis via both the receptor-mediated extrinsic and mitochondrial-mediated intrinsic apoptotic signaling pathways in the HTR-8/SVneo extravillous trophoblast model.[105] Apoptotic cell death can severely impair tissue development and function if it occurs excessively or prematurely. Apoptosis is a programmed cell death that is specifically characterized by signal-induced, energy-dependent breakdown and condensation of nuclei, and fragmented cellular components packaged into apoptotic vesicles in preparation for phagocytosis by immune system cells.[121] During pregnancy, apoptosis plays a vital role in normal implantation, placentation and remodeling of decidual tissue and maternal spiral arteries in early pregnancy.[121, 122] However, excessive apoptosis can disrupt placental development, causing shallow extravillous trophoblast invasion into the uterine wall and insufficient spiral artery remodeling.[48, 123–127] If severe, such changes can lead to pregnancy disorders such as pre-eclampsia and intrauterine growth restriction[122, 128–130] Additionally, increased expression of apoptosis-associated genes has been reported in pregnancies with recurrent miscarriages and failure of blastocyst implantation.[131, 132] Because ample evidence links elevated apoptosis to abnormal placental development, induction of apoptosis in placental extravillous trophoblasts could be a mechanism by which TCE and its metabolites (e.g., DCVC) contribute to poor placentation and early pregnancy disorders if this occurs *in vivo*.

Table 1 summarizes various mechanisms of TCE (and associated metabolite) induced placental toxicity that have been identified thus far. No other published studies were identified that investigated the toxicity of TCE or its metabolites in placenta. It should be noted that which mechanisms predominate likely depend on a number of factors such as level and duration of TCE exposure.

Challenges for advancing understanding of TCE toxicity to placenta

Limitations of experimental models for studying TCE toxicity

One of the most significant challenges for studying placental toxicity is the lack of an adequately relevant animal model.[133] There is considerable variation among placental mammals in uterine and placental structure.[134–136] For example, the uterus of mice and rats has two uterine horns, whereas the human uterus has a single cavity. Moreover, placental differences can include variations in morphology (e.g., discoid in humans and rodents, but cotyledonary in sheep), the degree of invasion into the uterine wall (e.g., deeply invasive in humans and rodents, but superficial in rabbits and sheep), and the histology of cells and cell layers that separate maternal and fetal blood (e.g., humans and rabbits have different histology compared to rodents and sheep).[134–136]

In addition, the endocrine functions of the placenta are critical for pregnancy,[137] yet are widely different across species.[138] As one example, the human placenta acquires the major role of sex steroid hormone production in the first trimester, whereas critical sex hormone production in rats and mice remains in the ovary throughout pregnancy. Moreover, although prostaglandins have a central role in the initiation of labor in mammals,[139] prostaglandin

$F_{2\alpha}$ initiates parturition in mice by stimulating cell death of hormone-synthesizing cells in the ovary, a parturition initiation mechanism that is absent in women.[3, 140] Despite the lack of an animal species that accurately models human pregnancy, our understanding of human pregnancy has been advanced by utilization of animal models, in particular mouse, rat, and sheep, as long as limitations due to inter-species differences are recognized.[135, 141] Moreover, because of the absence of an adequate animal model, placental toxicity investigations must rely on a combination of *in vitro* approaches in addition to animal experimentation, each with its own compromises.[142]

The ideal *in vitro* model for placental toxicity experimentation would involve normal human placental cells and tissue. However, as an internal organ the human placenta is not ethically accessible for study until delivery. Deliveries do not occur on a normal work schedule, making acquisition of fresh placental tissue difficult for *in vitro* models that require live tissue or cells. In addition, because most available placental tissue is from normal term deliveries, experimental findings may be limited and may not reflect responses of the placenta of early and mid-gestation non-labor pregnancy. Placental tissue from first trimester elective pregnancy terminations have contributed valuable insights into early placentation processes like extravillous trophoblast invasion.[143] However, research using tissue from elective pregnancy terminations may be limited by restrictions of research on human fetal tissue. *In vitro* models span the use of intact placental cotyledon diffusion, villous explant cultures, primary cell cultures, and cell lines. In addition, new models are in development to create a “placenta on a chip” that use microfluidic technologies.[144, 145]

Despite their usefulness, *in vitro* models of human placental cells and tissues have shortcomings.[146] Most notably, once removed from the body the cells and tissues are no longer part of integrated physiologic systems, and re-creation of the normal milieu *in vitro* is approximated, at best. Furthermore, tissue disruption to isolate cells for primary monoculture – as well as culture conditions – removes cells from their 3-dimenstional tissue structure and isolates them from other cells with which there is normally contact. Whereas cell lines free the researcher from constraints of tissue availability, derivation from cancerous tissue or induction of immortalization extensively alters genetic and epigenetic profiles.[147–149] Common human placental cell lines such as HTR-8/SVneo, BeWo, JEG-3, and JAR typically retain some but not all unique functional characteristics and molecular markers of trophoblasts and other placental cells *in vivo*.[150] For example, HTR-8/SVneo cells express several markers of extravillous trophoblasts[151] but only express the major histocompatibility protein HLA-G, a marker of extravillous trophoblast cells, when grown on Matrigel.[152] Because *in vitro* models often fail to retain complex cell-to-cell and tissue-tissue interactions present *in vivo*, toxicological assessments with the models must be interpreted with caution.

Combined maternal-fetal origins of placenta

The developmental origin and structural complexity of the placenta create further challenges for understanding toxicant impacts. Foremost, the placenta is an organ that arises from a combination of fetal and maternal cells. As a consequence, toxicants may behave differently in maternal tissues compared to placenta due to genetic differences contributed by the father through the fetus. With respect to TCE metabolism, including bioactivation to harmful

reactive metabolites and detoxication to stable excretable metabolites, genetic differences play a key role in determining individual susceptibility.[61] In particular, genetic differences in genes coding for metabolic enzymes could lead to variations in metabolism within the placenta, depending on maternal or fetal cell origin.

In addition to different genotypes, the combined fetal and maternal origin means that a placenta is not necessarily a female tissue, but rather has a fetal sex which can be male or female. Sex differences are observable not only at the phenotypic level of man versus woman, but resonate at the cellular, molecular, and genetic levels, as well. Likewise, sexual dimorphism is evident in placenta.[153] Thus, toxicants such as TCE, which demonstrates sex-specific toxicity,[61] may exert different effects in placental cells of male fetal origin versus female fetal origin.

Heterogeneity of cell types in placenta

Another challenge for identifying TCE toxicity in the placenta lies in the heterogeneity of cell types that make up this multifunctional organ. The placenta contains a variety of cell types with diverse functions.[5] For example, cytotrophoblasts are proliferative cells that provide structural support to the core of placental villi and can differentiate into specialized trophoblasts that invade and remodel uterine interstitial tissue, spiral arteries, and glands. In addition, cytotrophoblasts fuse to form syncytiotrophoblasts that provide important hormone synthesis and transport functions.[154] In addition, Hofbauer cells are resident placental macrophages of fetal origin that have roles in placental differentiation, placental blood vessel formation, immunoregulation, and more.[155] With respect to TCE, most studies to date have investigated effects of TCE or its metabolites on extravillous trophoblasts,[105–107, 115] placental tissue,[103] or extra placental membranes,[156] despite the potential for significant impacts on pregnancy from disruptions in other cell types. Further research investigating the impacts of TCE on specific placental cell types and on cell-cell interactions using *in vitro* models of human tissue would provide important insight into the mechanism of TCE placental toxicity.

Exposure assessment challenges

Regarding human studies, perhaps the greatest challenge remains in exposure assessment. Identification and quantification of exposure of pregnant women to TCE is challenging, in part, due to lack of validated biomarker(s) specific for TCE exposure. In particular, measurement of the reactive, short-lived, non-excretable intermediate metabolites of TCE glutathione pathway remains problematic, yet this pathway appears to be most relevant for placenta. Identification and measurement of exact protein, nucleic acid, and lipid adducts resulting from formation of the reactive metabolites also remain a challenge. The ability to readily detect such adducts would be crucial to understanding molecular initiating events of TCE metabolites, inform additional mechanisms of action, and help TCE exposure assessment. Consequently, these challenges hamper environmental epidemiology studies with the need to often use imprecise methods for TCE exposure assessment, including estimation, surrogate/proxy measures, and geographic location. More definitive, specific, and easily applicable exposure tools that allow individual exposure estimation would improve human studies of TCE impacts on adverse pregnancy outcomes.

Finally, other structurally similar volatile organic compounds (VOCs) such as perchloroethylene are also associated with adverse pregnancy outcomes[99] and have not been adequately evaluated for placental toxicity. Given their structural similarity and biotransformation to some of the same metabolites, it is plausible that perchloroethylene and possibly other VOCs could act through similar mechanisms as TCE in the placenta.

Summary and Conclusions

Due to its widespread use and presence in contaminated waste sites, TCE exposure is a public health concern with exposures occurring both occupationally and through the broader environment. Although certain toxic effects related to TCE exposure are documented, less is known about reproductive and developmental endpoints. Current evidence suggests that some TCE metabolites have toxic effects on the placenta, with significant health implications for exposed mothers and fetuses. However, there remain significant gaps in our understanding that limit our ability to assess the hazard that TCE presents to placental health. Future research could clarify effects on specific placental cell types and functions, the effect of fetal sex on response to TCE exposure, the true extent of genetic variation in TCE metabolism and kinetics, as well as the potential for synergistic or additive toxicity based on co-exposures with other chemicals. In addition, improved methods of assessing exposures would provide better epidemiological assessments of the risks posed by TCE. Similar gaps in our knowledge exist for structurally similar VOC compounds found in the environment (e.g., perchloroethylene), and future studies should assess these compounds for impacts on placental health. Due to the widespread nature of these chemicals as environmental contaminants and the critical role that the placenta plays in lifelong fetal and maternal health, a more thorough understanding of potential placental toxicity of TCE and other VOCs could lead to significant gains in public health.

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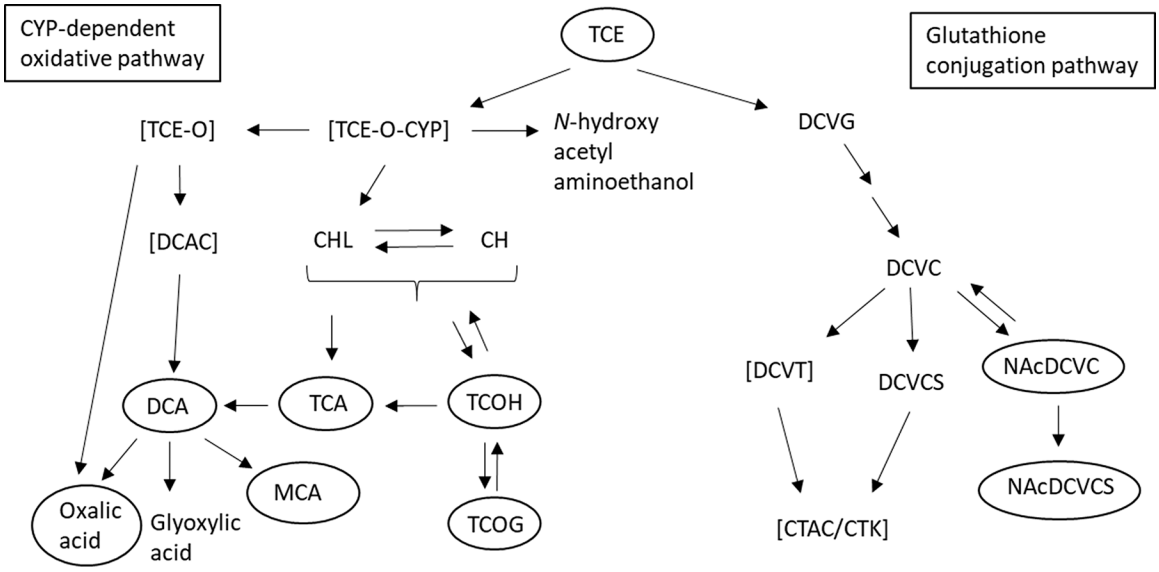

Figure 1. Simplified depiction of two TCE biotransformation pathways.

TCE is metabolized through the CYP oxidation pathway or the glutathione conjugation pathway. Metabolites that are unstable are depicted in brackets whereas metabolites that can be found in urine are circled. With the exception of all unstable metabolites and DCVCS, all other metabolites are systemically available.[61] Abbreviations: CYP, cytochrome P450; 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; 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. This figure was modified from Lash et al.[61]

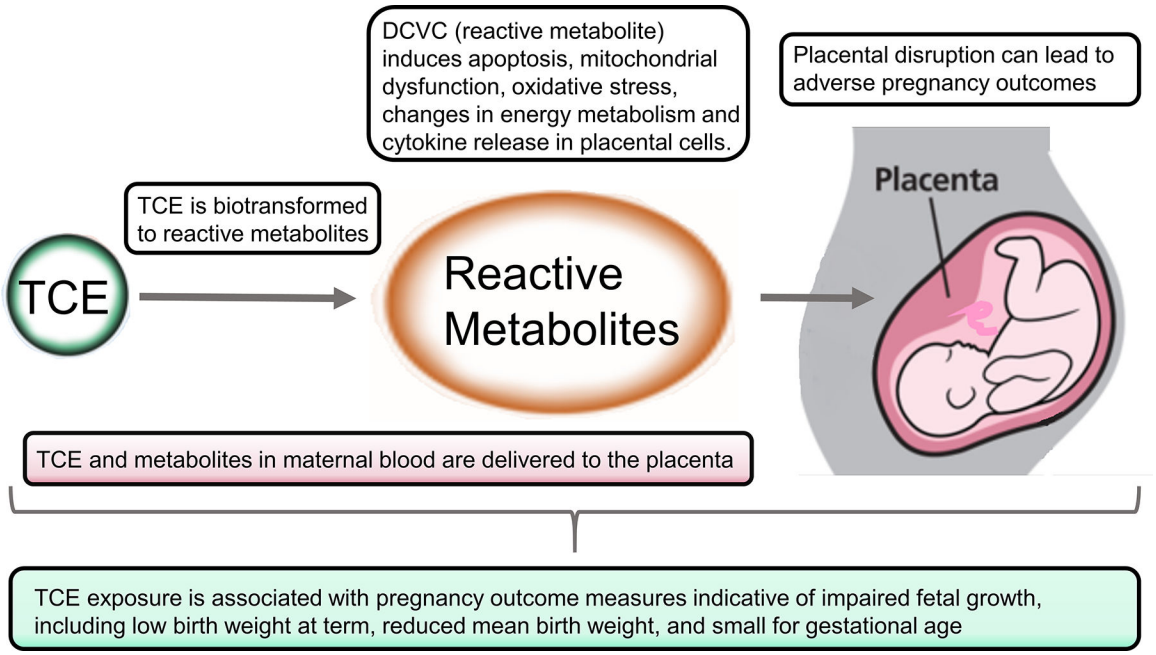

Figure 2.
Key mechanistic evidence suggesting the placenta is a target of TCE toxicity.

Table 1.

Mechanisms of TCE toxicity observed in placental models

Observed mechanism of TCE (or TCE metabolite) toxicity	Model utilized	Potential impact on placenta	Relevant Pregnancy outcome	References
Changes in energy metabolism pathways and macronutrient utilization by cells	HTR-8 cell line	Impaired trophoblast function during tissue remodeling; macronutrients diverted away from fetus to maintain placental sufficiency.	Preeclampsia; intrauterine growth restriction	115
Mitochondrial dysfunction; impaired mitochondrial adaptability	HTR-8 cell line	Insufficient metabolic energy capacity during tissue remodeling; impaired mitochondrial adaptability	Preeclampsia; intrauterine growth restriction	106, 107
Apoptosis; lipid peroxidation	HTR-8 cell line	Insufficient extravillous trophoblast invasion, inadequate remodeling of spiral arteries	Preterm birth; preeclampsia	105
Immunological responses; cytotoxicity	HTR-8 cell line	Increase in pro-inflammatory cytokines (e.g. IL-6), overall shift toward pro-inflammatory state in gestational compartment	Preterm birth; preeclampsia	107
Oxidative stress	HTR-8 cell line; pregnant Wistar rats	Increased ROS in placental cells leading to activation of inflammatory pathways	Preterm birth, preeclampsia	103, 107
Reduced fetal weight	Pregnant Wistar rats	Changes in macronutrient delivery to fetus	Intrauterine growth restriction; small-for-gestational-age; low birth weight	103