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Systematic Review

SYSTEMATIC REVIEW

Phthalate Exposures and Placental Health in Animal Models and Humans: A Systematic Review

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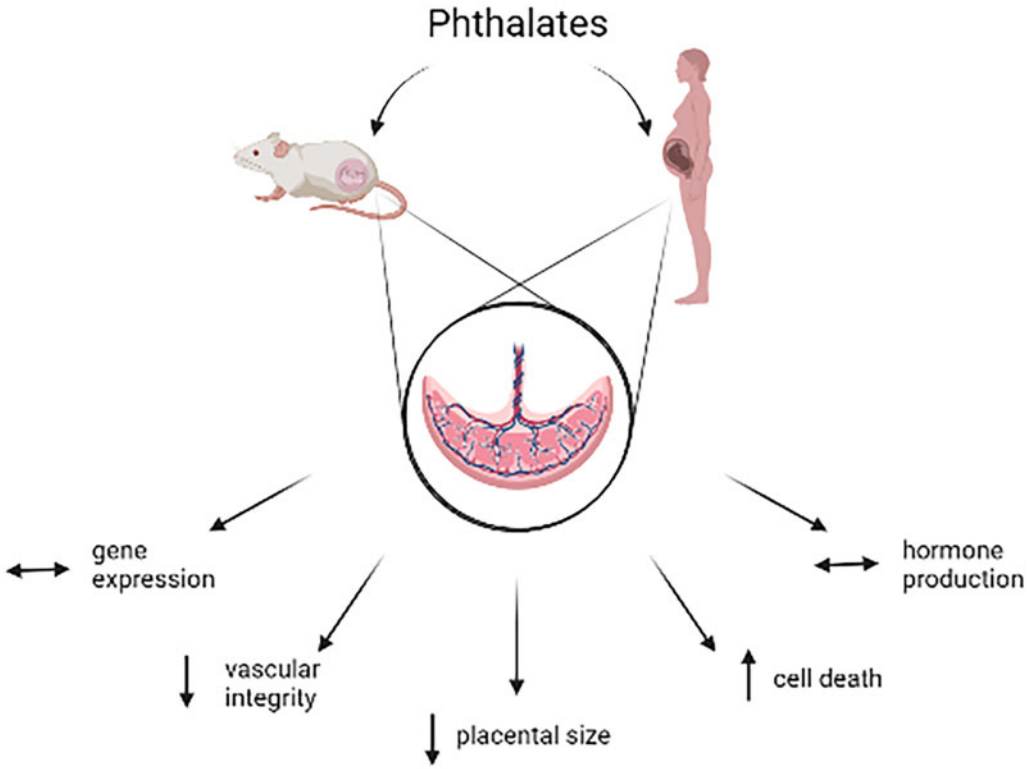
ABSTRACT

Phthalates are ubiquitous compounds known to leach from the plastic products that contain them. Due to their endocrine-disrupting properties, a wide range of studies have elucidated their effects on reproduction, metabolism, neurodevelopment, and growth. Additionally, their impacts during pregnancy and on the developing fetus have been extensively studied. Most recently, there has been interest in the impacts of phthalates on the placenta, a transient major endocrine organ critical to maintenance of the uterine environment and fetal development. Phthalate-induced changes in placental structure and function may have significant impacts on the course of pregnancy and ultimately, child health. Prior reviews have described the literature on phthalates and placental health; however to date, there has been no comprehensive, systematic review on this topic. Here, we review 35 papers (24 human and 11 animal studies) and summarize phthalate exposures in relation to an extensive set of placental measures. Phthalate-related alterations were reported for placental morphology, hormone production, vascularization, histopathology, and gene/protein expression. The most consistent changes were observed in vascular and morphologic endpoints, including cell composition. These changes have implications for pregnancy complications such as preterm birth and intrauterine growth restriction as well as potential ramifications for children's health. This comprehensive review of the literature, including common sources of bias, will inform the future work in this rapidly expanding field.

Key words: phthalates; placenta; pregnancy; endocrine disruptors.

Phthalates are plasticizers present in everyday items such as food containers, cosmetic packaging, toys, and many other plastic-containing products (Schettler, 2006). Because phthalates are not covalently bound to these plastics, they are able to leach from the items that contain them (Paluselli *et al.*, 2019). This characteristic facilitates transfer into the human body, with oral exposure being the most common route (Heudorf *et al.*, 2007). As a result, exposure to these chemicals is



Graphical Abstract

ubiquitous for most human populations, including pregnant women, a population of particular concern due to the developmental vulnerability of the growing fetus (Lyche *et al.*, 2009).

Increasingly, evidence from animal, human, and *in vitro* models demonstrate that phthalates have endocrine-disrupting properties (Qian *et al.*, 2020; van Wezel *et al.*, 2000; Wang *et al.*, 2013). Endocrine disruptors interfere with the action of hormones in biological systems, with ramifications for reproduction, metabolism, neurodevelopment and growth, among other functions (Gore *et al.*, 2015). Among the hallmarks of phthalate exposure are the antiandrogenic effects they elicit in human and animal models (Barakat *et al.*, 2019; Lottrup *et al.*, 2006). They have additionally been associated with altered thyroid, progesterone, and estrogen activity in pregnant women as well as nonpregnant adults and children (Du *et al.*, 2019; Ferguson *et al.*, 2014b; Huang *et al.*, 2016; Long *et al.*, 2021). Phthalates' impacts on pregnancy and the developing fetus have been extensively studied, including associations with preterm birth, which may occur through disruption of endocrine pathways or other mechanisms such as oxidative stress and/or inflammation (Cathey *et al.*, 2021; Ferguson *et al.*, 2014a, 2019; Santos *et al.*, 2021; van *et al.*, 2019; Zhang *et al.*, 2022). Prenatal phthalate exposures have been additionally associated with a range of postnatal child health concerns, including altered reproductive development, neurodevelopment, and asthma (Barakat *et al.*, 2019; Berger *et al.*, 2019; Xu *et al.*, 2020a). Given evidence that phthalates can cross the placenta and are detectable in amniotic fluid (Li *et al.*, 2018), the prevailing view is that their impacts on development occur through direct disruption of fetal physiology (Huang *et al.*, 2009).

However, it is also plausible that in addition to direct impacts on the fetus, phthalates exert indirect effects on the fetus by altering placental development and function (Adibi *et al.*, 2021). The placenta is a major endocrine organ that supports fetal development in numerous ways, including hormone synthesis, metabolism, and nutrient transport to the developing fetus (Evain-Brion and Malassine, 2003). It is formed from embryonic cells that are reprogrammed to form extraembryonic cell types, many of which are subtypes of the trophoblast (Benirschke and Kaufmann, 1990). Some trophoblasts differentiate to become extravillous trophoblasts (EVTs) and can invade the myometrium. EVTs play a role in adhesion, remodeling of spiral arteries and establishing maternal-placental blood flow. Other types of trophoblasts (ie, syncytiotrophoblasts and cytotrophoblasts) form a bilayer along the edge of the placental villi where components of maternal blood are transferred to fetal circulation. The trophoblast layer is also the site of endocrine activity (Benirschke and Kaufmann, 1990; Gingrich *et al.*, 2020). Hormone production by the placenta is imperative for physiological adaptations that occur in the maternal environment during pregnancy to promote fetal growth and development. A number of placental hormones have been well-studied in the context of normal pregnancy maintenance and fetal development, including human chorionic gonadotropin (hCG), progesterone, estrogens, corticotropin-releasing hormone (CRH), and placental lactogen (Napso *et al.*, 2018). The placenta's extensive endocrine activity may also make it a vulnerable target for endocrine disruptors. The resulting changes in hormone production, binding proteins, receptor densities, and metabolism may impact placental phenotype, including size, vascularization, and efficiency, potentially leading to pregnancy complications (eg, preeclampsia) as well as adverse fetal outcomes such as preterm birth and intrauterine growth restriction (IUGR) (Chaddha *et al.*, 2004; Fowden *et al.*, 2015; Ilekis *et al.*, 2016). Even in the absence of these frank pregnancy complications, there may be long-

lasting (or even permanent) impacts of placental impairments on child health including increased risks of reproductive anomalies and adverse neurodevelopment (Park *et al.*, 2018; Sandman *et al.*, 2018; Schneuer *et al.*, 2016). To complement the large and continuously expanding literature suggesting phthalates detrimentally impact the course of reproductive function, pregnancy, and fetal development [summarized in Høyer *et al.* (2018) and Radke *et al.* (2018, 2019)], here we systemically review their specific impacts on the placenta, evaluating both the animal and human literature. Although several thorough reviews on phthalates and the placenta have been previously published (Warner *et al.*, 2021), systematic reviews have the advantage of using explicit, reproducible methods to search and evaluate the literature, with attention to potential sources of bias and confidence in the body of evidence (Rooney *et al.*, 2014). Recognizing that markers of certain placental health may also be measurable even prior to delivery (eg, concentrations of key placental hormones), we expand the scope of placental measures considered beyond that of some prior reviews, which have largely focused on assessments at or after delivery. Placental measures considered in this review include placental morphology, vascularization, hormone production, histopathology, and gene/protein expression.

MATERIALS AND METHODS

Using PRISMA guidelines (Page *et al.*, 2021), a systematic literature review was conducted to identify, evaluate, and summarize articles that report on associations between phthalates and placental measures. As we had originally intended to also include phenols, search terms also included "Bisphenol A," "BPA," and "phenols"; however given the number of potentially eligible records after screening, at the eligibility stage, the choice was made to exclude phenols to ensure we could be sufficiently thorough in our review. The PECO statement provides additional information on the population, exposure parameters, comparator, and outcomes as assessed in both animals and human studies (Table 1). The population for both human and animal studies included pregnant females. Human exposure parameters included phthalates and/or their metabolites measured during gestation, whereas animal studies included phthalate administration during gestation. Reflecting ubiquitous phthalate exposure in human populations, mothers with lower phthalate concentrations served as the comparator for human studies, whereas in animal studies, control groups were the comparator. Given the scope of studies identified as eligible, we organized our review in light of 5 main placental measures of interest: placental hormones, gene/protein expression, morphology, histopathology, and vascularization. The protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on October 5, 2021 (CRD42021235649) and can be accessed at https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=235649.

Eligibility criteria, information sources, and search. Our literature search was designed to identify studies assessing exposure to phthalates in relation to one or more aspects of placental health in animals and/or humans. Our initial search was limited to articles written in English and published between January 1, 2000 and November 22, 2021, a time period chosen due to the more environmentally relevant experimental doses generally used, the lower limits of detection of assays, and the overall higher quality of the more recent literature. We queried 3 databases (PubMed, Scopus, and Web of Science) using terms related to phthalates and placental measures (Table 2). A set of search terms was developed through an iterative process involving input from multiple experts who study phthalates and/or placental health. Phthalate-relevant terms included: plasticizers, phthalates, plastics, di-2-ethylhexyl phthalate (DEHP), and mono-2-ethylhexyl phthalate (MEHP). As we had originally intended to also include phenols in this review, initial search terms also included "Bisphenol A," "BPA," and "phenols"; however given the number of potentially eligible records after screening, the choice was made to exclude phenols to ensure we could be sufficiently thorough in our review. Placenta-relevant search terms included: placenta, hCG, CRH, placentation, trophoblast, junctional zone, giant trophoblast, spongiotrophoblast, syncytiotrophoblast, EVT, labyrinth zone, GCMA [involved in trophoblast fusion to form the syncytiotrophoblast layer (Yu *et al.*, 2002)], and GCM1 [a marker for trophoblasts (Bainbridge *et al.*, 2012)]. Although the placenta may contribute to (and be affected by) pregnancy complications, given our specific interest in the placenta itself, studies of phthalates in relation to pregnancy complications were not included in this review.

Animal studies included experimental phthalate exposures at any dose, over any interval of time, and for any duration during gestation. The only acceptable comparator were animals who were exposed to the vehicle alone as a control (Table 3). Human studies included pregnant mothers of any race/ethnicity, any age, and from any location who had phthalates and/or phthalate metabolites measured during pregnancy or at delivery. Study designs were restricted to cohort studies, case-control studies, cross-sectional, and intervention studies. Case reports and case series were excluded from this review (Table 4).

Study selection and risk of bias. The initial search results from all 3 databases were imported into EndNote X9. The majority of duplicates were automatically removed using the software, followed by a manual removal of any remaining duplicates (Figure 1). Remaining articles were imported into Rayyan QCRTI, a web tool used to screen articles for eligibility. Twenty-nine studies from the initial search were included in the final review plus an additional 6 new articles added during updated searches shortly prior to submission (November 30, 2021) and during the revision process (March 15, 2022) to capture studies that may have been published during manuscript development. A team of 4 reviewers evaluated the search results. Each title and abstract were independently screened by 2 reviewers, and those that were clearly outside of the review topic were excluded. For any titles or abstracts that were of uncertain relevance, the full text was obtained and reviewed to confirm the relevance. Discrepancies were resolved through discussion among the team of reviewers. We additionally cross-checked references from recent related reviews to ensure completeness. The full texts of the final articles selected were downloaded and evaluated for their risk of bias using an adapted version of the U.S. National Toxicology Program's Office of Health Assessment and Translation (OHAT) Risk of Bias Tool for Human and Animal Studies. Following OHAT guidelines, all studies were assessed for their risk of selection, performance, attrition/exclusion, detection, and selective reporting bias. OHAT questions were adapted to be specific to the topic at hand. For example, to characterize phthalate exposure in humans (Table 5), the preferred method is repeat samples of urinary measurements at multiple timepoints (given phthalates' short half-life). Given that, under assessment of detection bias in the OHAT tool, we

Table 1. PECO Statement

	Human Evidence	Animal Evidence
Population	Pregnant women	Pregnant animal models
Exposure	Phthalates and/or phthalate metabolites measured during pregnancy or at parturition	Phthalates administered during varying lengths of duration throughout gestation
Comparator	Humans with lower phthalate and/or phthalate metabolite concentrations	Animals with no exposure to phthalates (controls)
Outcomes	Placental hormones, gene/protein expression, morphology, histopathology, and vascularization	Placental hormones, gene/protein expression, morphology, histopathology, and vascularization

Table 2. Search Strategy to Identify Relevant Papers on Phthalate Exposure and Placental Measures[a]

PubMed 2/5/2021 2000–2021 Journal articles, clinical studies, observational studies, English 2290 results	(placental OR HCG OR CRH OR placenta OR placentation OR trophoblasts OR junctional zone OR giant trophoblasts OR spongiotrophoblasts OR syncytiotrophoblasts OR extravillous trophoblasts OR labyrinth zone OR gcma OR gcm1) AND (plasticizer OR plasticizers OR phthalate OR phthalates OR bisphenol A OR bisphenols OR plastics OR plastic materials OR DEHP OR MEHP OR BPA OR BPS OR phenols)
Scopus 2/5/2021 2000–2021 Journals, articles, English 1430 results	TITLE-ABS-KEY ((plasticizer OR plasticizers OR phthalate OR phthalates OR "bisphenol A" OR bisphenols OR plastics OR "plastic materials" OR dehp OR mehp OR bpa OR bps OR phenols) AND (placental OR hcg OR crh OR placenta OR placentation OR trophoblasts OR "junctional zone" OR "giant trophoblasts" OR spongiotrophoblasts OR syncytiotrophoblasts OR "extravillous trophoblasts" OR "labyrinth zone" OR gcma OR gcm1)) AND (LIMIT-TO (SRCTYPE , "j")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMIT-TO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006) OR LIMIT-TO (PUBYEAR , 2005) OR LIMIT-TO (PUBYEAR , 2004) OR LIMIT-TO (PUBYEAR , 2003) OR LIMIT-TO (PUBYEAR , 2002) OR LIMIT-TO (PUBYEAR , 2001) OR LIMIT-TO (PUBYEAR , 2000)) AND (LIMIT-TO (LANGUAGE , "English"))
Web of Science 2/5/2021 2000–2021 Articles, English 677 results	(TS=((plasticizer OR plasticizers OR phthalate OR phthalates OR "bisphenol A" OR bisphenols OR plastics OR "plastic materials" OR DEHP OR MEHP OR BPA OR BPS OR phenols) AND (placental OR HCG OR CRH OR placenta OR placentation OR trophoblasts OR "junctional zone" OR "giant trophoblasts" OR spongiotrophoblasts OR syncytiotrophoblasts OR "extravillous trophoblasts" OR "labyrinth zone" OR gcma OR gcm1))) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)

[a]After the initial search, the scope of literature was reduced to only studies that included phthalate exposures. Studies that only considered phenols were excluded from consideration.

considered both the biological matrix assayed as well as the number of specimens analyzed. Following OHAT specifications, for each question, risk of bias was rated as: – (definitely high risk), - (probably high risk or not reported), + (probably low risk), ++ (definitely low risk). OHAT criteria can also be used to evaluate animal studies and individual items from the OHAT tool were adapted to the specific topic at hand (Table 6). The adapted OHAT tool was applied to each article by 2 independent reviewers. Any discrepancies in scores were resolved through discussion and consensus among all reviewers. Based on the results of the OHAT review, a score for overall risk of bias was assigned on a scale of 1–3 for each study, with 3 being high risk of bias and 1 being low risk of bias.

RESULTS

Our initial search identified 4397 articles across the 3 databases; after duplicates were removed, 3323 articles remained. Of these, based on the review of the titles and abstracts 3273 articles were excluded due to inconsistency with the PECO statement. The full text of the 50 remaining articles was obtained and reviewed at which point 21 additional articles were excluded and 6 new articles added during the updated searches. Reasons for exclusion were focus on phenols ($n = 17$), review article ($n = 1$), no or mistimed exposure assessment ($n = 2$), and *in vitro* study ($n = 1$). The 35 reviewed papers include 24 human studies and 11 animal studies (Figure 1). All human studies were strictly observational, with no intervention studies identified. In addition, the only animal data available were rodent studies that included phthalate exposure by oral dosing. Here, we summarize the results of those 35 papers by 5 subcategories of placental measures: placental morphology, vascularization, hormones, histopathology, and gene/protein expression.

Morphology

The morphologic characteristics of the placenta (such as weight, size, and shape), while relatively crude measures, may provide insight into adequacy of placental function, including

Table 3. Summary of Animal Studies on Phthalate Exposure and Placental Measures[a]

Placental Measures	Experimental Model & Strain	Dose (Route & Compound)	Exposure Timing	Main Findings	Author (Year)
Morphology	Wistar albino rats	500 mg/kg/day DBP by oral gavage	GD6 to GD18	• DBP treatment significantly reduced placental weight ($p < .05$)	Mahaboob Basha and Radha (2017)
	Wistar albino rats	20, 100, and 500 mg/kg/day DHP or DCHP by oral gavage	GD6 to GD19	• Placental weight in 100 and 500 mg/kg/day DHP and all DCHP treatment groups significantly increased compared with controls ($p < .05$) • Placental diameter decreased in all treatment groups compared with controls ($p < .05$) • Placental thickness decreased in all treatment groups compared with controls	Ahbab *et al.* (2017)
	ICR mice	50 or 200 mg/kg DEHP by oral gavage	GD0 to GD17 GD0 to GD6, GD7 to GD12, or GD13 to GD17	• Placenta weight significantly reduced in GD7–GD12 group exposed to 200 mg/kg DEHP ($p < .01$) • Placenta diameter of male fetuses was significantly reduced in mice exposed to 200 mg/kg DEHP from GD7 to GD12 ($p < .01$) • No significant difference on placental diameter of female fetuses	Shen *et al.* (2017)
	CD-1 mice	125, 250, and 500 mg/kg/day DEHP by oral gavage	GD1 to GD9 or 13	• DEHP significantly reduced placental weight and ratio of placental weight to body weight compared with control group ($p < .01$)	Zong *et al.* (2015)
Vascularization	ICR mice	50 or 200 mg/kg/day DEHP by oral gavage	GD0 to GD15 or 18	• Dose-dependent reduction in placental vascular space in labyrinthine region in DEHP-exposed mice ($p < .01$) • Decrease in labyrinthine microvessel density in DEHP treated mice ($p < .01$) • PlGF and VEGF significantly downregulated in placenta of DEHP-treated mice ($p < .05$)	Yu *et al.* (2018)
	ICR mice	50 or 200 mg/kg DEHP by oral gavage	GD0 to GD6, GD7 to GD12, or GD13 to GD17	• Blood sinusoid area in placental labyrinth layer reduced in mice exposed to 200 mg/kg DEHP in GD7–GD12 group ($p < .01$)	Shen *et al.* (2017)
	Wistar albino rats	20, 100, and 500 mg/kg/day DHP or DCHP by oral gavage	GD6 to GD19	• Decreased and irregular vessel formation in labyrinth in high-dose DHP and all DCHP groups	Ahbab *et al.* (2017)
	CD-1 mice	125, 250, and 500 mg/kg/day DEHP by oral gavage	GD1 to GD9 or 13	• Formation of branched fetal vessels significantly reduced in placenta from 250 and 500 mg/kg DEHP-treated groups in GD13 mice	Zong *et al.* (2015)
Hormone production	ICR mice	50 or 200 mg/kg/day DEHP by oral gavage	GD0 to GD14	• DEHP increased progesterone in maternal serum ($p < .01$) • DEHP increased placental HSD3β1 ($p < .05$)	Zhang *et al.* (2020)
	Albino rats	100 mg/kg/day DEHP by oral gavage	GD0 to GD20	• DEHP decreased progesterone on GD20 ($p < .05$) • No change in maternal estradiol • STAR, HSD, CYP17 expression reduced by DEHP in GD20 rats ($p < .05$) • STAR increased and CYP17 decreased in GD10 rats ($p < .05$)	Saadeldin *et al.* (2018)

Table 3. (continued)

Placental Measures	Experimental Model & Strain	Dose (Route & Compound)	Exposure Timing	Main Findings	Author (Year)
Histopathology	ICR mice	5, 50, or 200 mg/kg DEHP (route?)	GD9 to GD15	• DEHP treatment significantly increased number of micronuclei in sinusoidal cells (50 and 200 mg/kg) and labyrinth trophoblast cells (200 mg/kg) compared with cells in control placentas	Sun *et al.* (2022)
	CD-1 mice	20 μg/kg/day DEHP	GD1 to GD13	• Placental architecture in DEHP exposed mice were normal compared with the control group	Kannan *et al.* (2021)
	ICR mice	50 or 200 mg/kg DEHP by oral gavage	GD0 to GD17, GD0 to GD6, GD7 to GD12, or GD13 to GD17	• Proliferation in labyrinth region significantly reduced in mice exposed to 200 mg/kg DEHP in GD7-GD12 group ($p < .01$)	Shen *et al.* (2017)
	Wistar albino rats	20, 100, and 500 mg/kg/day DHP or DCHP by oral gavage	GD6 to GD19	• Significant degeneration of spongiotrophoblasts in 100 and 500 mg/kg DCHP groups • Increased number and volume of trophoblastic giant cells in 100 and 500 mg/kg DHP and 500 mg/kg DCHP groups • Hemorrhage in labyrinth and basal zones and edema in basal zone in treatment groups (except low dose DHP)	Ahbab *et al.* (2017)
	CD-1 mice	125, 250, and 500 mg/kg/day DEHP by oral gavage	GD1 to GD9 or 13	• DEHP significantly inhibited growth and development of ectoplacental cone • At GD13, significantly smaller spongiotrophoblast area in DEHP-treated mice • Total area of placenta and labyrinth significantly reduced in 500 mg/kg dose group ($p < .01$)	Zong *et al.* (2015)
Gene/protein expression	Wistar rats	500 or 1000 mg/kg/day DEHP by oral gavage	GD7 to GD12	• 951 differentially expressed genes in high-dose group compared with control • 3787 differentially expressed genes in low-dose group compared with control • CYP2R1, SOAT2, and DHCR24 differentially expressed in the placenta of DEHP-treated rats • Somatostatin receptor 4 and 2 significantly enriched in the neuroactive ligand-receptor interaction pathway in DEHP-treated rats	Xu *et al.* (2020b)
	ICR mice	50 or 200 mg/kg/day DEHP by oral gavage	GD0 to GD15 or 18	• Significant downregulation of fatty acid transporter (FATP1) observed in 50 mg/kg/day group ($p < .05$) • No significant changes in FATP4	Yu *et al.* (2018)
	Albino rats	100 mg/kg/day DEHP by oral gavage	GD0 to GD20	• Placental PTEN and AKT1 mRNA were elevated in rats sacrificed on GD10 but reduced in GD20 rats ($p < .05$) after DEHP exposure compared with controls	Saadeldin *et al.* (2018)
	ICR mice	50 or 200 mg/kg/day DEHP by oral gavage	GD0 to GD15 or 18	• Thrα1 and Thrβ1 significantly reduced in DEHP-exposed mice compared with controls ($p < .05$)	Yu *et al.* (2018)

Table 3. (continued)

Placental Measures	Experimental Model & Strain	Dose (Route & Compound)	Exposure Timing	Main Findings	Author (Year)
	CD-1 mice	125, 250, or 500 mg/kg/day DEHP by oral gavage	GD1 to GD9 or 13	• Significant downregulation of placental Glut1 in DEHP-exposed mice compared with controls ($p < .05$) • At GD13, DEHP significantly inhibited Fosl1 mRNA compared with controls ($p < .01$) • DEHP increased Bax casp-3 and -8 mRNA, but decreased Bcl-2 mRNA at GD13 ($p < .05$) • Phosphor-ERK1/2 significantly increased in MAPK signaling pathway in exposed animals • Ascl2 and Esx1 mRNA significantly reduced in all exposed animals ($p < .05$)	Zong *et al.* (2015)
	Sprague Dawley rats	750 or 1500 mg/kg/day DEHP by oral gavage	GD0 to GD19	• Expression of PPARα ($p < .01$ at 1500 mg/kg), PPARγ, CD36, FATP1, HFABP ($p < .05$ at 1500 mg/kg), and CYP4A1 upregulated in placenta after DEHP exposure whereas COX-2 was downregulated • Reduced directional maternal-to-fetal placental transfer of arachidonic acid and docosahexaenoic acid in DEHP-treated animals • Decreased total placental prostaglandins production in DEHP-treated animals ($p < .05$)	Xu *et al.* (2008)

Abbreviations: CD, cluster of differentiation; COX, cyclooxygenase; CYP, cytochrome p450; DBP, dibutyl phthalate; DCHP, dicyclohexyl phthalate; DEHP, di-2-ethylhexyl phthalate; DHCR, dehydrocholesterol reductase; DHP, di-n-hexyl phthalate; ERK, extracellular signal-regulated kinase; FATP, fatty acid transport protein; GLUT, glucose transporter; HFABP, heart fatty acid-binding protein; HSD, hydroxysteroid dehydrogenase; MAPK, mitogen-activated protein kinase; PlGF, placental growth factor; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; SOAT, sterol o-acyltransferase; STAR, steroidogenic acute regulatory protein; THR, thyroid hormone receptor; VEGF, vascular endothelial growth factor.

[a]Some papers are listed more than once as they included placental measures across multiple categories.

Table 4. Summary of Epidemiological Studies on Phthalates in Relation to Placental Measures

Placental Measures	Study Sample and Location	Study Design	Exposure Measures and Timing	Placental Measures and Timing	Main Findings	Author (Year)
Morphology	132 mothers (Massachusetts, USA)	Cohort	Urinary phthalate metabolites (all trimesters) (MEP, MBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MCPP, MCOP, and MCNP)	Placental weight	• Each log-unit increase in prenatal MEP concentrations was overall associated with a ($\beta = 24$ g; 95% CI: −41, −7) decrease in placental weight.	Mustieles *et al.* (2019)
	473 mother-son pairs (Nancy and Poitiers, France)	Cohort	Urinary phthalate metabolites between GA 23 and 29 wks (MCPP, MBP, MiBP, MBzP, MEP, MCNP, MCOP, MEHP, MEHHP, MEOHP, and MECPP)	Placental weight	• MCNP negatively associated with placental weight ($\beta = -10.9$ g; 95% CI: −21.8, 0.09).	Philippat *et al.* (2019)
	2725 mother-newborn pairs (Ma'anshan, China)	Cohort	Urinary phthalate metabolites in first, second, and third trimesters (MMP, MEP, MBP, MBzP, MEHP, MEHHP, and MEOHP)	Placental size and shape	• Placental breadth increased by ($\beta = 0.148$ cm; 95% CI: 0.078, 0.218) with each 1 ln-concentration increase in first trimester MBP. • Placental thickness increased by ($\beta = 0.017$ cm; 95% CI: 0.006, 0.027) with each 1 ln-concentration increase in second trimester MMP. • Placental thickness increased by ($\beta = 0.019$ cm; 95% CI: 0, 0.037) with each 1 ln-concentration increase in third trimester MEHP.	Zhu *et al.* (2018)
Vascularization	1233 women (Rotterdam, Netherlands)	Cohort	Urinary phthalate metabolites in early pregnancy (DNOP, DEHP, LMW, and HMW metabolites)	Placental angiogenic markers in blood during early and mid-pregnancy (sFlt-1 and PlGF)	• Early pregnancy HMW phthalate metabolites associated with sFlt-1 concentrations ($\beta = 0.19$ ng/ml; 95% CI: 0.02, 0.54) and sFlt-1/PlGF ratio ($\beta = 141.72$; 95% CI: 29.13, 373.21) in early pregnancy. • Early pregnancy MEP associated with PlGF in midpregnancy.	Philips *et al.* (2019)
	457 women (Massachusetts, USA)	Nested case-control	Urinary phthalate metabolites at GA 10, 18, 26, and 35 wks (MEHP, MEHHP, MEOHP, MECPP, MBzP, MBP, MiBP, MEP, and MCPP)	Plasma angiogenic biomarkers: PlGF and sFlt-1	• DEHP metabolites associated with lower PlGF and higher sFlt-1/PlGF ratio.	Ferguson *et al.* (2015)
Hsormone production	1018 women (Tennessee, USA)	Cohort	Urinary phthalate metabolites between GA 16 and 39 wks (MiBP, MEP, MMP, MBP, MBzP, MCNP, MCIOP, MCINP, MCPP, MEHP, MEHHP, MEOHP, MECPP, and MCMHP)	Plasma placental CRH	• Phthalate metabolites were associated with higher placental CRH at visit 1 (16–29 wks) and lower placental CRH at visit 2 (22–39 wks)	Barrett *et al.* (2022)
	677 women (Puerto Rico, USA)	Cohort	Urinary phthalate metabolites at GA 16–20 and 24–28 wks (MEHP, MEHHP, MEOHP, MECPP, MEP, MBP, MBzP, MiBP, MHiBP, MCPP, MCNP, MCOP, MHBP, MNP, MONP and phthalate replacements MHiNCH, MCOCH, MECPTP, and MEHHTP)	Serum hormones at GA 16–20 and 24–28 wks (P4, E3, CRH, and SHBG)	• CRH inversely associated with several phthalate metabolites (MCNP, MCPP, MECPP, MEHHP, MEOHP). For example: MCNP; %Δ: −4.08 (95% CI: −7.24, −0.804). Associations stronger in late pregnancy. • No associations with E3. • MCOP associated with lower SHBG (%Δ: 5.66; 95% CI: −11.2, −0.08).	Cathey *et al.* (2019)

Table 4. (continued)

Placental Measures	Study Sample and Location	Study Design	Exposure Measures and Timing	Placental Measures and Timing	Main Findings	Author (Year)
					• MEHHTP associated with lower P4 in late pregnancy: −13.1%Δ (95% CI: −22.3, −2.75). • MCOP was associated with 9.85% (95% CI, −17.0, −2.03) decrease in progesterone.	
	18 women (Czech Republic)	Cohort	Plasma phthalate metabolites in, maternal blood at GA 37 wks and cord blood (MEP, MnBP, MiBP, MBzP, MEHP, 5-OH-MEHP, and 5-oxo-MEHP)	Steroids in maternal plasma at GA 37 wks and cord blood (E1, E2, E3, pregnenolone, and P4)	• No associations between maternal phthalates and maternal hormones. • Higher maternal MnBP associated with higher cord blood E2 ($\beta = 11.3$; 95% CI: 0.18, 22.42) and E3 ($\beta = 111.95$; 95% CI: 28.76, 195.13). • Maternal Σphthalates associated with higher cord E3 ($\beta = 40.54$; 95% CI: 13.80, 67.28).	Kolatorova *et al.* (2018)
	207 women (Chongqing, China)	Cohort	Phthalates in cord blood at delivery (DBP, DiBP, and DEHP)	Cord blood E2, E3, and P4	• DEHP negatively associated with cord blood E2 ($p < .01$). • DBP and DEHP negatively associated with E3 ($p < .05$). • No significant associations with P4.	Huang *et al.* (2018)
	180 women with placental data (New York, USA)	Cohort	Urinary phthalate metabolites at GA 34 wks (MnBP, MBzP, MEHP, MEP, MiBP, MEOHP, MEHHP, MECPP, and MCPP)	Placental mRNA expression of genes related to preeclampsia and gestational diabetes: HSD17β1, CGA, CYP19A1	• CGA reduced in male placentas with high MiBP ($\beta = -2.48$; 95% CI: −3.79, −1.17). • MnBP associated with decreased CGA ($\beta = -2.01$; 95% CI: −3.55, −0.48). • Iinverse association between DEHP-oxo and CYP19A1 in male placentas; opposite positive association in female placentas.	Adibi *et al.* (2017)
	541 women (California, Washington, Minnesota, and New York, USA)	Cohort	Urinary phthalate metabolites in first trimester (MnBP, MBzP, MEHP, MEP, MiBP, MCPP, MCNP, and MCOP)	Serum hCG in first or second trimester	• MnBP, MBzP, and MCOP associated with higher first trimester serum hCG in mothers carrying females and lower hCG in mothers carrying males.	Adibi *et al.* (2015)
	54 women (New York, USA)	Cohort	Urinary phthalate metabolites in third trimester (MEHP, MEOHP, MEHHP, MECPP, MnBP, MiBP, and MBzP)	Placental mRNA expression of hCG and genes involved in steroidogenesis: CYP19, CYP1B1, P450scc, and 17β-HSD	• No significant associations between phthalate metabolites and steroidogenic pathway gene expression. • MEHP, MEOHP, MnBP, MiBP, MBzP, and ΣDEHP metabolites associated with hCG mRNA ($p \leq .05$).	Adibi *et al.* (2010)
Gene/protein expression	202 women (France)	Cohort	Urinary phthalate metabolites between 22 and 29 gestational weeks (MEP, MiBP,	Global DNA methylation in the placenta	• Most DMRs showed increased DNA methylation with phthalate exposure.	Jedynak *et al.* (2022)

Table 4. (continued)

Placental Measures	Study Sample and Location	Study Design	Exposure Measures and Timing	Placental Measures and Timing	Main Findings	Author (Year)
			MnBP, MBzP, MCPP, MCOP, MCNP, MEHP, MEHHP, MEOHP, and MECPP)		• None of the phthalate metabolites were significantly associated with LINE-1 repetitive elements.	
	760 women (Tennessee, USA)	Cohort	Urinary phthalate metabolites in second and third trimesters (MEHP, MEOHP, MEHHP, MECPP, MCMHP, ΣDEHP, MEP, MBP, MCIOP, MIBP, MCINP, MCPP, MMP, MHPP, MINP, and MHXP)	Placental transcriptome at birth including genes and long noncoding RNAs	• Second trimester MCIOP, MEOHP, MECPP associated with expression of multiple genes including *NEAT1* • Third trimester MCIOP and MEP associated with expression of multiple genes • MMP, MCIOP, and MEP associated with gene expression in 27 biological pathways • MCIOP mostly associated with upregulation, MEP associated with downregulation • Some sex differences noted	Paquette *et al.* (2021)
	1660 women (Anhui Province, China)	Cohort	Urinary phthalates metabolites in second and third trimesters (MBzP, MBP, MEHP, MEOHP, MEHHP); ΣPAE calculated as potency-weighted sum of coexposure to DBP, BBzP, and DEHP	Placental mRNA of cytokine IL-6 and activated microphage biomarker CD68	• ΣPAE in each trimester (and average εPAE) negatively associated with IL-6 and CD68 ($p < .05$).	Gao et al. (2022)
	2469 women (Anhui Province, China)	Cohort	Urinary phthalate metabolites in first trimester (MMP, MEP, MBP, MBzP, MEHP, MEHHP, and MEOHP)	mRNAs of placental inflammatory markers: CRP, TNF-α, IL-1β, IL-6, IL-10, MCP-1, IL-8, CD68, and CD206	• MBP positively associated with expression of proinflammatory mediators including IL-6 ($p = .002$) and CRP ($p = .005$). • MBzP associated with increased expression of TNF-α ($p = .015$) and MCP-1 ($p = .039$).	Wang *et al.* (2020)
	10 women (location not reported)	Cohort	Urinary phthalate metabolites shortly before delivery (MiBP), MCPP, MCNP, MCOP, MECCP, MEHHP, MEOHP, MBzP, MBP, MHBP, MHiBP, MEHP, and MEP)	miRNA in placental extracellular vesicles shortly before delivery (37–39 wks gestation)	• MBzP positively correlated with miR_518e ($p = .03$). • No other significant associations.	Zhong *et al.* (2019)
	10 women, 20 placentas (location not reported)	Cross-sectional	Urinary phthalate metabolites on the day before or on the day of delivery (MiBP, MCPP, MCNP, MCOP, MECPP, MEHHP, MEOHP, MBzP, MnBP, MHBP, MHiBP, MiBP, MEHP, MNP, MMP, MEP and 2	Expression of 87 lncRNAs (involved in the regulation of genomic imprinting)	• Strongest correlation between MHiBP and LOC91450 ($R_{spearman} = .88$, $p < .001$). • AIRN, DACT3.AS1, DLX6, DPP10, HOTTIP, LOC143666, and LOC91450 strongly correlated with most metabolites.	Machtinger *et al.* (2018)

Table 4. (continued)

Placental Measures	Study Sample and Location	Study Design	Exposure Measures and Timing	Placental Measures and Timing	Main Findings	Author (Year)
			metabolites of the phthalate alternative MHiNCH and MCOCH)		• H19 negatively associated with most metabolites. • Moderate positive associations for most other phthalates and lncRNAs	
	49 women (Colorado, USA)	Cross-sectional	Urinary phthalate metabolites before termination (MECPP, MCMHP, MEOHP, MEHHP, MCPP, MiBP, MCHP, MiNP, MiDP, MOP, MBP, MHxP, MHpP, MMP, MEP, MBzP, MCHpP, MiPrP, MPeP, MCiOP, MCiNP, and MEHP)	Placental transcription and methylation of genes related to phthalate exposure	• In the high phthalate exposure group, methylation of 39 genes was significantly altered, usually reduced ($p < .005$).	Grindler *et al.* (2018)
	207 women (Chongqing, China)	Cohort	Phthalates in cord blood at delivery (DBP, DiBP, and DEHP)	Placental PPARγ expression	• DiBP, DBP, and DEHP positively associated with PPARγ protein expression ($p < .01$).	Huang *et al.* (2018)
	358 women, 180 placentas (New York, USA)	Cohort	Urinary phthalate metabolites at GA 34 wks (MnBP, MBzP, MEHP, MEP, MiBP, MEOHP, MEHHP, MECPP, and MCPP)	Placental mRNA expression of genes related to preeclampsia, gestational diabetes, fetal growth restriction, and fetal programming: AHR, SLC27A4, PTGS2, and PPARγ.	• MnBP significantly associated with decreased AHR ($\beta = -1.62$; 95% CI: -2.81, -0.43). • Inverse association of MnBP with PPARγ in male placentas (-1.1 $\log_e$ units at highest vs lowest quartile, 95% CI: -2.0, -0.1). • No associations observed with SLC27A4 and PTGS2.	Adibi *et al.* (2017)
	187 mother-infant pairs split between high and low exposure cities (Chenghai and Haojiang, China, respectively)	Cross-sectional	Serum phthalate esters after delivery (BBP, DEHP, DNOP, DEP, and DMP)	Placenta mRNA expression of metallothioneins (MT, MT-1A, MT2A), fatty acid transport and binding proteins (FATP1 and HFABP)	• FATP1 and HFABP mRNA expression in high exposed group were higher than in low exposed group (both $p < .001$), whereas MT-1A was higher ($p = .05$). • DMP and DEHP correlated with higher MT ($\beta = 0.47$, $p = .01$) and MT-2A expression ($\beta = 0.50$, $p = .007$) in males. • DEP positively correlated with MT-1A ($\beta = 0.56$; $p = .03$) and FATP1 expression ($\beta = 0.56$, $p = .03$) in female placentas.	Li *et al.* (2016)
	179 women (Massachusetts, USA)	Cohort	Urinary phthalate metabolites in first trimester (MECPP, MEHHP, MEOHP, MEHP, MCPP, MCOP, MCNP, MBzP, MiBP, MEP, and MnBP)	Expression of 29 placental miRNA regulating gene expression	• miR-142-3p, miR15a-5p, and miR-185 expression associated with $\sum$phthalates ($p < .05$). • 10 individual miRNA were associated with metabolites, most commonly MCOP. • No other significant associations.	LaRocca *et al.* (2016)
	181 mother-newborn pairs (Wenzhou, China)	Nested case-control	Urinary phthalate ester metabolites in third trimester (MBP, MMP, MEHP, and MEOHP)	Placental DNA methylation of growth-related	• Log-unit[a] (10-fold) increase in urinary of MEHHP associated with 2.95% (position 1: $\beta = -2.95$, $p = .005$) and 3.92% (position 2:	Zhao *et al.* (2016)

Table 4. (continued)

Placental Measures	Study Sample and Location	Study Design	Exposure Measures and Timing	Placental Measures and Timing	Main Findings	Author (Year)
				genes: IGF2 and AHRR	$\beta = -3.92$, $p = .039$) decrease in IGF2 methylation. • Log-unit (10-fold) increase in MEOHP concentration associated with 2.88% (position 1: $\beta = -2.879$, $p = .004$) and 4.52% (position 2: $\beta = -4.52$, $p = .013$) decrease in IGF2 methylation.	
	119 mothers (Wenzhou, China)	Case-control	Urinary phthalate metabolites in third trimester (MBP, MMP, MEHP, MEOHP, MEHHP, and ΣDEHP	Placental LINE-1 methylation (marker of global DNA methylation)	• ΣDEHP significantly inversely associated with placental LINE-1 methylation ($\beta = -0.54$, $p = .038$).	Zhao *et al.* (2015)
	196 women (Massachusetts, USA)	Cohort	Urinary phthalate metabolites in first trimester (MnBP, MBzP, MCNP, MCOP, MCPP, MECPP, MEHHP, MEHP, MEOHP, MEP, MiBP, and Σphthalates)	DNA methylation and allele-specific expression of H19 and IGF2 in placenta samples (imprinted genes involved in fetal and placental growth)	• DEHP metabolites significantly associated with decreased IGF2DMR0 methylation in female placenta ($p < .05$). • ΣPhthalates inversely associated with placental H19 methylation ($p < .05$).	LaRocca *et al.* (2014)
	54 women (New York, USA)	Cohort	Urinary phthalate metabolites in third trimester (MEHP, MEOHP, MEHHP, MECPP, MnBP, MiBP, and MBzP)	Placental mRNA expression of PPARγ and AHR genes (involved in trophoblast differentiation)	• MEHP, MEOHP, MnBP, MiBP, MBzP, and ΣDEHP metabolites associated with lower PPARγ and AHR expression ($p \leq .05$).	Adibi *et al.* (2010)

Abbreviations: AHR, aryl hydrocarbon receptor; AHRR, aryl-hydrocarbon receptor repressor; BBP, butyl benzyl phthalate; CD, cluster of differentiation; CGA, chorionic gonadotropin alpha; CRH, corticotrophin-releasing hormone; CRP, C-reactive protein; CYP, cytochrome p450; DBP, dibutyl phthalate; DEHP, di-2-ethylhexyl phthalate; DEP, diethyl phthalate; DiBP, diisobutyl phthalate; DMP, dimethyl phthalate; DNOP, di-n-octyl phthalate; E1, estrone; E2, estradiol; E3, estriol; FATP, fatty acid transport protein; GA, gestational age; hCG, human chorionic gonadotropin; HFABP, heart fatty acid-binding protein; HMW, high molecular weight; HSD, hydroxysteroid dehydrogenase; IGF, insulin like growth factor; IL, interleukin; LINE, long interspersed nuclear element; LMW, low molecular weight; MBP, mono-n-butyl phthalate; MBzP, mono-benzyl phthalate; MCHP, mono-cyclohexyl phthalate; MCHpP, mono(7-carboxy-heptyl) phthalate; MCiNP, mono-carboxy isononyl phthalate; MCiOP, mono-carboxy isooctyl phthalate; MCMHP, mono-[(2-carboxymethyl) hexyl] phthalate; MCNP, mono-carboxyisononyl phthalate; MCOCH, cyclohexane-1,2-dicarboxylicacid monocarboxy isooctyl ester; MCOP, mono-carboxyisooctyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MECPTP, mono-2-ethyl-5-carboxypentyl terephthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHHTP, mono-2-ethyl-5-hydrohexyl terephthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MHBP, mono hydroxybutyl phthalate; MHiBP, monohydroxyisobutyl phthalate; MHpP, mono-2-heptyl phthalate; MHxP, mono-hexyl phthalate; MiBP, mono-isobutyl phthalate; MiDP, mono-(8-methyl-1-nonyl) phthalate; MiNP, mono-isononyl phthalate; MiPrP, mono-isopropyl phthalate; MHiNCH, cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester; MMP, monomethyl phthalate; MnBP, mono-n-butyl phthalate; MNP, mono-isononylphthalate; MONP, mono-oxononyl phthalate; MOP, mono-octyl phthalate; MPeP, mono-pentyl phthalate; MT, metallothioneins; PlGF, placental growth factor; PPAR, peroxisome proliferator-activated receptor; PTGS, prostaglandin-endoperoxide synthase; P4, progesterone; sFlt-1, soluble fms-like tyrosine kinase-1; SHBG, sex hormone-binding globulin; SLC, solute carrier; TNF-α, tumor necrosis factor-α; 5-OH-MEHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; 5-oxo-MEHP, mono-(2-ethyl-5-oxohexyl) phthalate; wks, weeks.

[a]Researchers sampled 4 quadrants of the placenta, positions 1–4.

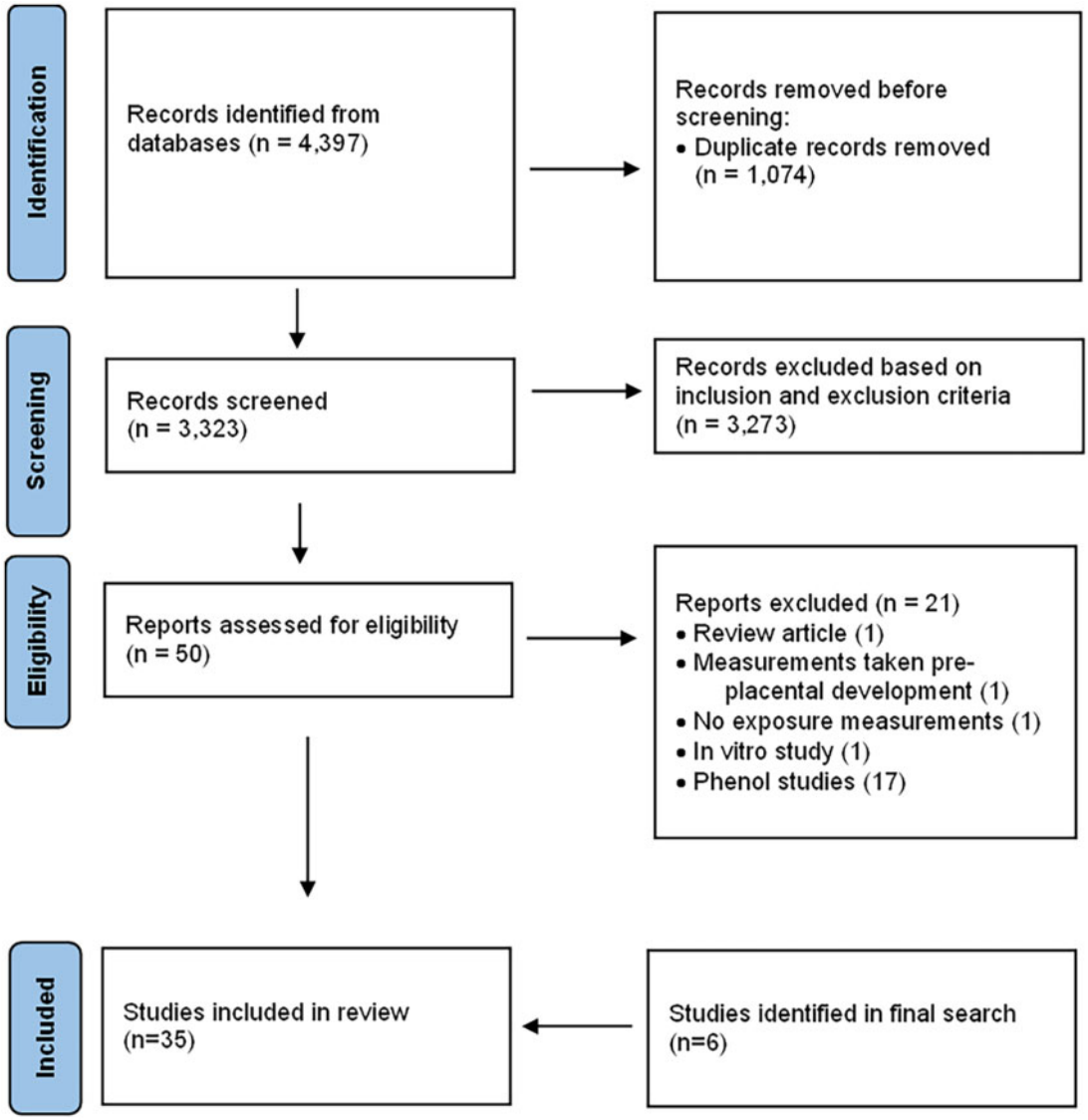


Figure 1. PRISMA flow diagram.

oxygen and nutrient flow, as well as indicate the presence of pathologies (Kishwara *et al.*, 1970). In total, 7 papers (4 animal and 3 human studies) were identified examining phthalate exposures in relation to placental morphology, most of them focused on placental weight as the primary outcome. Of these papers, 5 reported predominantly inverse associations between prenatal phthalate exposure and placental size measures, 1 reported a positive association, and a final paper reported a mix of positive and inverse associations. The particular phthalates and metabolites associated with morphological measures varied across studies. In rodent models, both dibutyl phthalate (DBP) (500 mg/kg/day) and DEHP (125, 200, 250, and 500 mg/kg/day) exposures resulted in lower placental weights compared with controls (Mahaboob Basha and Radha, 2017; Shen *et al.*, 2017; Zong *et al.*, 2015), with DEHP at all doses causing a roughly 25% reduction in placental weight (Zong *et al.*, 2015). Of these, 1 study presented evidence of stronger impacts in males and identified GD7–GD12 as a particularly sensitive window (Shen *et al.*, 2017). The effects of DBP exposure, moreover, appeared to be transgenerational, with reductions in placental weight extending into the F1, F2, and F3 generations (Mahaboob Basha and Radha, 2017).

Two human studies similarly identified inverse associations between phthalate metabolite concentrations during pregnancy and placental weight. A Massachusetts birth cohort ($n = 132$) reported that maternal mono-ethyl phthalate (MEP) was associated with reduced placental weight ($\beta = -24$ g per log unit increase in MEP; 95% CI: -41, -7), but in contrast to the animal studies, observed no associations with DEHP or DBP metabolites (Mustieles *et al.*, 2019). In a French cohort ($n = 473$), midpregnancy mono carboxyisononyl phthalate (MCNP), a metabolite of diisodecyl phthalate, was associated with nonsignificantly lower placental weight ($\beta = -10.9$ g; 95% CI: -21.8, 0.09), whereas no associations were observed for the other 10 metabolites measured (Philippat *et al.*, 2019).

A smaller number of studies have reported positive associations between phthalate exposure and placental weight. For example, a study in Wistar albino rats observed that di-n-hexyl phthalate (DHP) and dicyclohexyl phthalate (DCHP) exposure at GD 7–12 resulted in higher placental weights, but smaller

Table 5. Risk of Bias Assessment for Human Studies on Phthalate Exposures and Placental Measures

	Selection Bias		Confounding Bias	Attrition/Exclusion Bias			Detection Bias				Selective Reporting Bias	Other Bias	Overall RoB Score	Placental Measures
	1. Were the groups comparable in terms of crucial covariates/confounders (Case-control studies)?	2. Were all the participants drawn from the same source population? (cohort)	3. Did the study design or analysis account for important confounding and modifying variables?[a]	4. Was loss to follow-up reported?	5. If so, was there evidence that it was unrelated to the exposure and/or outcome?	6. If there was loss to follow-up reported, was loss of subjects adequately addressed	7. Were phthalate metabolites measured in urine?	8. Were chemicals measured more than once?	9. Was the exposure assessment prospective?	10. Can we be confident in the outcome assessment?	11. Were all measured outcomes reported?	12. Were there any other potential threats to internal validity?		
Jedynak *et al.* (2022)	N/A	++	+	+	+	+	++	-	++	++	++	++	1	GE
Barrett *et al.* (2022)	N/A	++	++	+	-	-	++	++	++	++	++	++	1	Ho
Paquette *et al.* (2021)	N/A	++	++	++	-	+	++	+	++	++	++	++	1	GE
Gao *et al.* (2022)	N/A	++	++	-	—	+	++	++	++	++	++	+	1	GE
Wang *et al.* (2020)	N/A	++	++	+	-	-	++	—	++	++	+	++	2	GE
Cathey *et al.* (2019)	N/A	++	-	-	N/A	N/A	++	+	++	++	++	++	1	Ho
Mustieles *et al.* (2019)	N/A	++	-	+	-	+	++	++	++	++	++	++	1	M
Philippat *et al.* (2019)	N/A	++	-	-	-	++	++	—	++	-	++	++	2	M
Philips *et al.* (2019)	N/A	++	+	++	++	++	++	—	++	+	++	++	1	V
Zhong *et al.* (2019)	N/A	++	—	N/A	N/A	N/A	++	—	-	++	++	-	2	GE
Huang *et al.* (2018)	N/A	++	+	-	N/A	N/A	—	—	-	++	++	-	2	Ho, GE
Grindler *et al.* (2018)	N/A	++	-	N/A	N/A	N/A	++	—	-	++	++	+	2	GE
Machtinger *et al.* (2018)	N/A	++	++	N/A	N/A	N/A	++	—	-	++	++	—	3	GE
Kolatorova *et al.* (2018)	N/A	++	++	-	N/A	N/A	—	++	-	+	—	—	3	Ho
Zhu *et al.* (2018)	N/A	++	+	+	-	-	++	++	++	+	++	++	1	M
Adibi *et al.* (2017)	N/A	++	+	+	-	+	++	—	++	++	++	+	2	GE, Ho
Li *et al.* (2016)	N/A	—	—	N/A	N/A	N/A	—	—	-	-	++	—	3	GE
LaRocca *et al.* (2016)	N/A	++	-	++	—	-	++	—	++	++	++	++	2	GE
Adibi *et al.* (2015)	N/A	+	+	-	++	++	++	—	+	++	++	++	2	Ho
LaRocca *et al.* (2014)	N/A	-	++	-	N/A	N/A	++	—	++	++	++	++	1	GE
Adibi *et al.* (2010)	N/A	++	-	-	++	++	++	—	++	++	++	++	2	Ho, GE
Zhao *et al.* (2016)	++	N/A	-	N/A	N/A	N/A	++	—	—	++	++	++	2	GE
Ferguson *et al.* (2015)	+	N/A	-	N/A	N/A	N/A	++	++	++	++	++	++	1	V
Zhao *et al.* (2015)	++	N/A	-	N/A	N/A	N/A	++	—	—	++	++	++	2	GE

Risk of bias rating levels
Definitely low risk (++)
Probably low risk (+)
Probably high risk or not reported (-)
Definitely high risk (--)
Not applicable

Abbreviations: M, morphology; V, vascularization; Ho, hormones; Hi, histology; GE, gene expression.

[a]Depending on the specific research question, important covariates to consider included gestational age, race/ethnicity, pregnancy complications, mode of delivery (when applicable), and fetal sex.

Table 6. Risk of Bias Assessment for Animal Studies on Phthalate Exposures and Placental Measures

Abbreviations: M, morphology; V, vascularization; Ho, hormones; Hi, histology; GE, gene expression.

placental diameters and reduced thickness (Ahbab *et al.*, 2017). A large Chinese pregnancy cohort study ($n = 2725$) reported associations between phthalate exposure and placental morphological measures; however, associations differed by trimester of exposure, placental measure (eg, breadth, thickness), and metabolite, with no clear pattern emerging (Zhu *et al.*, 2018). In that study, as in 1 animal study (Shen *et al.*, 2017), associations were stronger among male placentas compared with female. The trimester-specific impacts observed in the Zhu *et al.*'s (2018) study echo some of the animal evidence suggesting critical periods during which exposure may impact certain placental parameters. Overall, results of most (but not all) studies suggest that phthalates are associated with decreased placental weight, although data on additional morphological features are limited.

Vascularization

The uterine environment goes through numerous adaptations to sustain a pregnancy, including increased vascularization to support higher flow of oxygen and nutrients between maternal and fetal circulation. When this process is impaired, fetal mortality and pregnancy complications such as preeclampsia and IUGR can arise (Zygmunt *et al.*, 2003). Reflecting its extensive vascularization, the placenta produces angiogenic (eg, placental growth factor [PlGF] and vascular endothelial growth factor [VEGF]) as well as antiangiogenic (eg, soluble fms-like tyrosine kinase 1 [sFlt-1]) markers; imbalances in these markers may signify vascular complications resulting in insufficient blood supply to the placenta and fetus (Herraiz *et al.*, 2015; Reynolds and Redmer, 2001). A total of 6 studies (2 human and 4 animal studies) were identified examining phthalate exposures in relation to placental vascularization, all of which suggested negative associations between phthalate exposure and the vascular integrity of the placenta.

Among pregnant mice that were dosed with DEHP (50 or 200 mg/kg/day), there was a dose-dependent decrease in both the vascular space and microvessel density of the placental labyrinth regions (analogous to the maternal-placental interface in humans) compared with control mice. At both doses, these same mice also showed reductions in placental mRNA concentrations of the angiogenic factors PlGF and VEGF (Yu *et al.*, 2018). A separate study further demonstrated an approximately 50% decrease in blood sinusoid areas of the labyrinth zone in placentas from dams dosed at 200 mg/kg/day DEHP during midgestation (GD7–GD12) (Shen *et al.*, 2017). Furthermore, branching of fetal blood vessels in the placenta was notably reduced at high doses of DEHP exposure (250 and 500 mg/kg/day) (Zong *et al.*, 2015). Some evidence suggests that other less commonly studied phthalates such as DHP and DCHP may similarly compromise vessel integrity at high doses (500 mg/kg/day), with decreased and abnormal vessel formation in the labyrinth region (Ahbab *et al.*, 2017).

Epidemiological evidence further supports impacts on placental vascularization; however in contrast to the animal studies (which primarily examine histopathological measures and gene expression) to date, the human vascular measures have been indirect, primarily consisting of maternal blood biomarkers reflecting angiogenesis. For example, in the Dutch Generation R study ($n = 1233$) investigators observed that sum high molecular weight (ΣHMW) phthalate metabolites measured in midpregnancy were associated with higher maternal blood concentrations of the antiangiogenic marker sFlt-1 ($\beta = 0.19$ units per log unit HMW phthalates; 95% CI: 0.02, 0.54) (Philips *et al.*, 2019), indicating endothelial dysfunction and possibly higher risk of preeclampsia (Palmer *et al.*, 2017). On the

other hand, no associations between phthalate metabolites and maternal sFlt-1 were observed in a nested case-control study ($n = 130$ cases, 352 controls) in Boston (Ferguson *et al.*, 2015). In that study, MEP was associated with higher PlGF concentrations (%Δ 6.79; 95% CI: 0.13, 13.9) whereas ΣDEHP metabolites and the oxidative metabolite MECPP were negatively associated with PlGF (%Δ −6.58; 95% CI:−12.1, −0.69) (Ferguson *et al.*, 2015), indicating impaired placental angiogenesis (Herraiz *et al.*, 2015). Similarly, significantly higher prenatal sFlt-1/PlGF ratio, a characteristic of preeclampsia and placental dysfunction, was observed in subjects who had higher DEHP metabolite concentrations (Ferguson *et al.*, 2015; Herraiz *et al.*, 2015; Philips *et al.*, 2019). In summary, phthalate exposure appears to compromise placental vascularization, and there is a clear need for research to complement molecular results by characterizing human placental vascularization at delivery, as some animal studies have done.

Hormone Production

As endocrine disruptors, phthalates are best known for their ability to alter hormone levels, which is a particular concern during pregnancy as hormone activity changes dramatically to support fetal development (Monneret, 2017; Zoeller *et al.*, 2012). This includes hormone production and secretion by the placenta, a highly active endocrine organ (Napso *et al.*, 2018). We identified 10 studies (3 animal and 7 human studies) that considered the impact of phthalates on placental hormone production. Here, we focus on hormones that are primarily of placental origin during pregnancy, rather than hormones that are produced by multiple organs. These include progesterone, estriol (E3), CRH, hCG, and pregnenolone. We also consider the binding protein, sex hormone-binding globulin (SHBG). In addition, we consider several complementary studies focusing on placental expression of steroidogenic pathway enzymes that regulate hormone biosynthesis such as CYP19, HSD17β3, and CYP17. Although all of the animal studies on this topic have focused on DEHP to date, the human literature has considered a wider array of phthalates.

Several studies have examined phthalates' impacts on progesterone, which plays a key role in early gestation by increasing the production of nitric oxide in uterine endothelial cells, thereby increasing uterine blood flow volume (Dickey and Hower, 1996; Simoncini *et al.*, 2007). After week 10 of human pregnancy, the placenta takes over progesterone production from the ovary to support the uterus during pregnancy (Cable and Grider, 2021). Additionally, it plays a role in the prevention of preterm birth by inhibiting genes responsible for parturition (Chwalisz and Garfield, 1997). Pregnant rats administered DEHP (100 mg/kg/day) showed a significant decrease in maternal serum progesterone levels on GD20 (Saadeldin *et al.*, 2018). A similar trend was observed in a Puerto Rican pregnancy cohort ($n = 677$) whereby an interquartile increase in urinary mono-carboxyisooctyl phthalate (MCOP), a metabolite of diisononyl phthalate (DiNP), was associated with lower progesterone across pregnancy (−9.85%, 95% CI, −17.0, −2.03) (Cathey *et al.*, 2019). By contrast, in a mouse model, after DEHP exposure at 50 and 200 mg/kg/day, progesterone levels on GD15 were significantly higher compared with controls (Zhang *et al.*, 2020). However, in a Chinese cohort ($n = 207$), no significant associations were observed between diisobutyl phthalate (DiBP), DBP, and DEHP with progesterone (Huang *et al.*, 2018). Given the inconsistent results, phthalates' impacts on progesterone production remain unclear.

Progesterone production during early pregnancy is supported by placental hCG, which additionally promotes angiogenesis, differentiation, fetal and uterine growth, and suppresses immune rejection of the fetus as well as myometrial contraction (Cole, 2010). In a multicenter U.S. cohort study ($n = 541$), concentrations of several phthalate metabolites were associated with changes in first trimester hCG, with strong differences observed by fetal sex (Adibi *et al.*, 2015). Specifically, among women carrying female fetuses, hCG was 2- to 4-fold higher among women in the 75th percentile for MCOP, mono-n-butyl phthalate (MnBP), and mono-benzyl phthalate (MBzP) compared with the 25th percentile. However, in women carrying male fetuses, hCG was 0.7- to 1.5-fold lower among women in the 75th percentile for MCOP, MnBP, and MBzP compared with the 25th percentile. No associations with second trimester hCG levels were observed. Associations between phthalate exposure and hCG were further supported by evidence linking multiple urinary phthalate concentrations in late pregnancy to placental expression of hCG and chorionic gonadotropin alpha, one of the genes that encodes hCG, with sex differences again noted ($n = 180$) (Adibi *et al.*, 2017). Overall, hCG may be affected by phthalate exposure, but these changes may be both trimester- and sex-dependent.

Estriol (E3) is the dominant estrogen during pregnancy and is produced almost exclusively by the placenta from the fetal adrenal precursor dehydroepiandrosterone. As a weak estrogen, its function is not fully understood, though it is hypothesized to contribute to placental vascularization and uteroplacental blood flow (Resnik *et al.*, 1974). Clinically, E3 has been widely used to assess fetal well-being, particularly prior to the widespread routine clinical use of ultrasound and other screening biomarkers (Ostergard and Kushinsky, 1971). Importantly, rodents do not produce placental E3, thus the literature is limited to human studies (Hudon Thibeault *et al.*, 2014). In a Puerto Rican cohort, there was no relationship between urinary phthalate metabolites and serum E3 at 16–20 and 24–28 weeks (Cathey *et al.*, 2019); by contrast, 2 other studies observed associations. In a small Czech study ($n = 18$), MnBP ($\beta = 111.95$; 95% CI: 28.76, 195.13) and Σphthalates ($\beta = 40.54$; 95% CI: 13.80, 67.28) were associated with higher cord blood E3 (Kolatorova *et al.*, 2018). The direction of association was reversed, however, in a Chinese cohort ($n = 207$) whereby DBP and DEHP concentrations were associated with lower cord blood E3 (Huang *et al.*, 2018). A notable limitation, however, is that in both the Czech and Chinese studies, phthalates (or their metabolites) were measured in blood, a nonpreferred matrix because of the short half-lives and potential for contamination (Calafat *et al.*, 2013). Thus, there is concern about the validity of these results and the comparability to other studies. SHBG, which is produced by the placenta and can bind sex steroids making them biologically inactive, has also been inversely associated with phthalate exposure, specifically MCOP, in at least 1 study (Cathey *et al.*, 2019). To the extent that phthalates impact estrogen activity in the placenta, either directly or indirectly through effects on binding proteins, receptors, and enzymes, there may be impacts on pregnancy physiology, potentially contributing to complications (Benassayag *et al.*, 1999; Cantonwine *et al.*, 2019).

To examine this further, 1 animal study evaluated the effects of phthalates on the steroidogenic pathway, the biological pathway by which sex hormones and other steroids including estradiol, estrone, progesterone, cortisol, and pregnenolone are produced (Xu *et al.*, 2016). After DEHP administration at 100 mg/kg/day, at GD20 exposed rats showed significant reductions in placental steroidogenic mRNA expression including STAR, HSD17β3, and CYP17 compared with controls. However, in this

same study placental STAR mRNA was significantly higher on GD10 in exposed animals (Saadeldin *et al.*, 2018). Although few human studies have examined these associations, 2 analyses in a New York City-based cohort ($n = 54$ and $n = 358$) reported no significant associations between third trimester urinary metabolites and placental mRNA of the steroidogenic genes CYP19, CYP1B1, P450scc, and 17βHSD (Adibi *et al.*, 2017, 2010). The larger study observed sex differences in the expression of CYP19A1, with a strong negative association with DEHP-oxo (a molar sum of MEOHP, MEHHP, and MECPP as highly correlated metabolites of MEHP) in male placentas, but a positive association in female placentas (Adibi *et al.*, 2017). Additionally, a study in rats suggested that enzymes involved in the steroid biosynthesis pathway upstream of cholesterol may be affected by phthalate exposure (Xu *et al.*, 2020b). Comparisons between animal and human studies are constrained by interspecies differences in placental steroidogenic pathways, such as the lack of placental STAR expression in humans (Sugawara *et al.*, 1995), and lack of estriol production in rodents (Hudon Thibeault *et al.*, 2014)

Only 2 studies have examined associations between phthalate metabolites and CRH, a hormone produced by the placenta (as well as in much smaller quantities by the hypothalamus) that along with progesterone, is important in the onset of labor. High levels of CRH have been associated with spontaneous and preterm labor (Pařízek *et al.*, 2014; Wadhwa *et al.*, 2004). In the Puerto Rican PROTECT pregnancy cohort, CRH was inversely associated with several urinary phthalate metabolites (MCNP, MCPP, MECPP, MEHHP, MEOHP), with stronger association observed during 24–28 weeks gestation compared with 16–20 weeks gestation (Cathey *et al.*, 2019). Similar results were observed in the CANDLE study ($n = 1018$) in late pregnancy, whereby phthalate mixtures were associated with reduced CRH; however in midpregnancy (16–29 weeks gestation), phthalate mixtures were positively associated with CRH, potentially revealing time-dependent (but not sex-specific) effects (Barrett *et al.*, 2022). In summary, despite their well-established role as endocrine disruptors, collectively, results linking phthalates to disruption of placental hormones are inconsistent across both animal models and human studies.

Histopathology

The literature on phthalates in relation to placental histopathology is currently limited to rodent studies. These studies have primarily focused on the fetal side of the placenta including the labyrinth zone, where nutrient and gas exchange take place, and the basal zone, characterized by spongiotrophoblast cells and trophoblastic giant cells (Furukawa *et al.*, 2011). The labyrinth zone, only found in the rodent placenta, is rich in fetal blood vessels; therefore, any defects in this region may impact nutrient transfer and by extension, fetal growth and development (Woods *et al.*, 2018). Spongiotrophoblast cells in the basal zone have a structural function, whereas trophoblastic giant cells are responsible for producing hormones like prolactin to aid in lactation (Furukawa *et al.*, 2011). All these components together play a role in the health of the placenta as well as the health of the fetus.

Collectively, 4 of the 5 histopathology papers identified indicated disorganization of the cellular makeup of the placenta following phthalate exposure in animal models. In the first study, a mouse model was used to study the effect of DEHP (at 125, 250, or 500 mg/kg/day) on the ectoplacental cone, a group of cells formed from the trophectoderm of a blastocyst that eventually develops into the placenta (Müntener and Hsu, 1977; Zong *et al.*, 2015). Phthalate treatment inhibited the growth of the ectoplacental cone at GD9 in a dose-dependent manner, ultimately impacting placental size and development. At GD13, cellular density continued to be affected with a significantly reduced area of spongiotrophoblasts in rodents treated with high doses (100–500 mg/kg/day) of DEHP and DCHP (Ahbab *et al.*, 2017; Zong *et al.*, 2015). Exposure to 200 mg/kg/day DEHP from GD7–GD12 resulted in a 50% reduction in proliferating cells in the labyrinth region. There was also a nonsignificant downward trend in cell proliferation in mice exposed in early and late gestation (Shen *et al.*, 2017). These findings were further corroborated by a mouse study showing a significantly reduced labyrinth area and overall reduced placental area following 500 mg/kg/day of DEHP administration (Zong *et al.*, 2015). By contrast, a single study showed an increase in the size and number of trophoblastic giant cells observed following high doses of DCHP (500 mg/kg/day) and DHP (100 or 500 mg/kg/day) on GD6–GD19 (Ahbab *et al.*, 2017). Mechanistically, this cellular degeneration can be explained by Sun *et al.* (2022), in which the labyrinth trophoblasts and sinusoid cells of placentas from DEHP-treated mice had more micronuclei. Micronuclei are indicative of DNA double-strand breaks that can cause cell cycle arrest and interfere with proliferation (Clarke and Allan, 2009; Fonseca *et al.*, 2019).

In addition to affecting cell number, phthalate exposure may elicit additional damage in placental tissue. In pregnant rats exposed to 20, 100, and 500 mg/kg/day DHP or DCHP, hemorrhage in both the basal and labyrinth zones of the placenta was evident, as was edema in the basal zone (Ahbab *et al.*, 2017). Overall, the histopathological data indicate that phthalates may contribute to reduced surface area for nutrient transport to the fetus and reduced structural support for the placenta as a whole. The one study that showed null results was in mice exposed to a very low, environmentally relevant, daily dose of DEHP at 20 μg/kg/day (Kannan *et al.*, 2021), in contrast to other studies that have mostly relied upon high doses with questionable human relevance. Acknowledging the interspecies differences in placental structures and mechanisms as well the high doses used, the animal literature suggests a need for parallel work in human placentas at delivery, which is currently lacking.

Gene/Protein Expression

There were a large number of papers (5 animal and 15 human) identified that evaluated the effects of phthalates on placental gene and/or protein expression. For clarity, we have synthesized them by pathway and function.

Fatty Acid Homeostasis and Nutrient Transport

The presence of fatty acids in the fetal environment promotes structural integrity of membranes and is an energy source for the fetus. Essential fatty acids (EFA), which are not produced in the body, are only available to the fetus through placental transfer following maternal dietary intake (Haggarty, 2004). Six papers (2 animal and 4 human studies) were identified, and overall, they indicate that fatty acid homeostasis in the placenta may be disrupted by phthalate exposure. One study of pregnant rats exposed to DEHP (750 or 1500 mg/kg/day) examined GD20 expression of placental EFA transporters, EFA metabolic enzymes, and peroxisome proliferator-activated receptor (PPAR) isoforms known to be regulators of EFA homeostasis. Following DEHP exposure, there was a trend toward increased mRNA and protein expression of EFA transporters. Specifically, at 1500 mg/kg/day DEHP, there was increased mRNA expression of fatty acid translocase (FAT) in the labyrinth zone, fatty acid transport protein 1 (FATP1), and heart cytoplasmic fatty acid-binding

protein (HFABP), whereas plasma membrane fatty acid-binding protein (FABPpm) was unaffected (Xu *et al.*, 2008). This rat study further indicated that EFA metabolic enzyme, cytochrome P450 (CYP) 4A1, was also significantly increased after DEHP exposure. However, cyclooxygenase (COX)-2, another metabolic enzyme of interest had reduced protein and mRNA levels in the junctional zone and no change in the labyrinth zone (Xu *et al.*, 2008). Consistent with those results, a human cross-sectional study in China ($n = 187$) reported that placental FATP1 mRNA was 63% higher in women from a geographic region with high phthalate exposure compared with women from a region with low phthalate exposure. Furthermore, HFABP was 178% higher in the more highly exposed group (Li *et al.*, 2016). By contrast, FATP1 mRNA was significantly reduced in a mouse model after lower dose (50 mg/kg/day) DEHP exposure. Inconsistencies across studies may be due to nonmonotonic relationships between exposure and measures or potentially interspecies differences. Additionally, there were no significant associations of urinary phthalates and placental FATP4 mRNA (Adibi *et al.*, 2017; Yu *et al.*, 2018).

Equally important is the ability of the placenta to transfer EFA from the maternal side to the fetal side, to ensure fetal access to EFA. In the same rat study, there was a significant increase in arachidonic acid (AA) and docosahexaenoic acid (DHA) in the placental tissue of DEHP-exposed dams concurrent with significantly lower AA and DHA in fetal plasma compared with controls (Xu *et al.*, 2008). The observed upregulation of EFA transporters may indicate increased transfer from maternal circulation into the placenta, but reduced transfer from the placenta to the fetus. Dose-dependent reductions in prostaglandins following DEHP exposure were also observed in that study (Xu *et al.*, 2008).

PPARs play a role in the regulation of the proteins involved in fatty acid transport and metabolism and have been widely studied in relation to phthalate exposure (Bility *et al.*, 2004; Desvergne *et al.*, 2009; Hardwick *et al.*, 2009). PPARα mRNA and protein expression in the placenta were significantly increased in DEHP-exposed rats, whereas the β isoform showed no changes (Xu *et al.*, 2008). PPARγ was also significantly increased after DEHP exposure (Xu *et al.*, 2008) and this trend was consistent with results of human studies including a cohort of Chinese women ($n = 207$) and a New York City cohort of Dominican and African American women ($n = 54$ and $n = 358$) in which DiBP, DBP, and DEHP were positively associated with placental PPARγ expression (Adibi *et al.*, 2017, 2010; Huang *et al.*, 2018). The γ isoform of the PPAR is of particular interest during pregnancy as it plays an important role in trophoblast differentiation and the development of gestational diabetes (Fournier *et al.*, 2007; Holdsworth-Carson *et al.*, 2010). Collectively, these results point to the ability of phthalates to disrupt the presence and transport of fatty acids in the placenta.

Other nutrient transporters for thyroid hormones and glucose have been examined. The delivery of thyroid hormones across the placenta is important for fetal neurodevelopment and deficient transfer of thyroid hormone has been linked to IUGR (Kilby *et al.*, 1998). Placentas on GD14 that came from mice exposed to 50 and 200 mg/kg/day DEHP showed significant reductions in the protein expression of both THR α1 (thyroid hormone receptor α1) and THRβ1; high dose placentas had <50% expression compared with the controls. Interestingly, the concentrations of total triiodothyronine (TT3) and total thyroxine (TT4) in maternal and fetal blood were unchanged compared with control mice (Yu *et al.*, 2018). Glucose is also important for fetal growth and metabolism and there are several mechanisms that play a role in its homeostasis within the placenta including glucose transporters (Hay, 2006). In the same mouse study, placental Glut1 (glucose transporter 1) mRNA was reduced in a dose-dependent manner following DEHP administration, suggesting that phthalate exposure may affect the transport of essential molecules (ie, fatty acids and glucose) to the fetus (Yu *et al.*, 2018).

Cell Growth/Apoptosis

A small literature (1 human and 3 animal studies) has examined the effects of phthalates on the expression of genes involved in cell growth/progression and apoptosis. Collectively, these studies suggest that phthalates may play a role in deterring placental growth by interfering with pathways involved in the cell cycle and genes that are known to play a role in placental cell growth and differentiation.

In a mouse study, pregnant rats were dosed with 125, 250, or 500 mg/kg/day DEHP during gestation (Zong *et al.*, 2015). Placental expression of genes involved in cell growth and differentiation including Ascl2, Esx1, and Fosl1 was assessed (Baines and Renaud, 2017; Li and Behringer, 1998) on GD9 and 13. Compared with controls, placentas from all dosed animals had significantly lower mRNA expression of Ascl2 and Esx1, whereas Fosl1 only showed significant reductions at GD13 (Zong *et al.*, 2015). Additional work has examined expression of genes involved in apoptosis, including Bax, casp-3, casp-8, and Bcl2 (Ashkenazi, 2008). Expression of proapoptotic genes, Bax, casp-3, and casp-8 mRNA, was significantly increased in the placentas of all treated mice on GD13, whereas no changes were observed in GD9 placentas. On the other hand, expression of the antiapoptotic gene, Bcl2, was significantly reduced (Zong *et al.*, 2015).

Additional pathways involved in cell cycle progression such as the mitogen-activated protein kinase (MAPK) and phosphatase and tensin homolog (PTEN)/protein kinase B (AKT) pathway have also been studied. Erk1/2, a protein involved in the MAPK pathway, has the ability to activate apoptotic factors when phosphorylated (Tan and Chiu, 2013). Phospho-Erk1/2 was significantly increased in the same mouse model mentioned above (Zong *et al.*, 2015), indicating that the MAPK pathway plays a role in inducing apoptosis in the placenta after phthalate exposure. The PTEN/AKT pathway is another regulator of the cell cycle with AKT leading directly to cell cycle progression and PTEN being an inhibitor (Carnero and Paramio, 2014). Following administration of 100 mg/kg/day DEHP to pregnant rats, compared with control placentas, AKT mRNA was significantly increased by almost 50% in placentas of treated rats on GD10, yet reduced by about 80% on GD20 (Saadeldin *et al.*, 2018). The same trend was reported for placental PTEN, which was significantly increased by roughly 50% in treated rats on GD10 but significantly reduced on GD20 (Saadeldin *et al.*, 2018). The smaller reduction in PTEN compared with AKT suggests that phthalate-induced cell cycle disruption may be strongest following exposures in late gestation.

The insulin-like growth factors (IGF) and H19 are also of interest in this context due to their critical role in fetal growth (Fowden, 2003; Gabory *et al.*, 2006). Two human studies in China ($n = 181$) and Boston ($n = 196$) looked at methylation of the IGF2 gene, which leads to silencing of expression by adding methyl moieties to CpG regions of the DNA to prevent protein interactions for transcription (Eden and Cedar, 1994). Phthalates were measured in the third trimester in the Chinese study and during the first trimester in the Boston study and in both studies, urinary DEHP metabolites were significantly inversely associated

with IGF2 methylation (LaRocca *et al.*, 2014; Zhao *et al.*, 2016). In the Boston study, the low molecular weight phthalates and Σphthalates were also inversely associated with IGF2 methylation as well as H19 methylation values (a decrease of 0.46% per log(mol/l); 95% CI: −0.86%, −0.05%), suggesting reduced silencing of these genes and by extension, potentially greater expression (LaRocca *et al.*, 2014). In contrast, in pregnant mice, administration of 200 mg/kg/day DEHP resulted in significant reductions in placental IGF2 mRNA expression (Yu *et al.*, 2018). These placentas also showed significantly lower IGF1 mRNA expression, and fetuses from dosed mothers also exhibited characteristics of fetal growth restriction (FGR) with significantly reduced birth weight (Yu *et al.*, 2018).

Gene Regulation and Epigenetics

In addition to the studies described above assessing methylation of specific candidate genes, 6 studies have examined phthalates' broader impacts on epigenetics and microRNAs (miRNA) expression. For example, a cross-sectional study of pregnancy terminations ($n = 49$) observed altered (generally reduced) methylation of 39 placental genes in women with high versus low phthalate metabolite concentrations (low and high groups determined by quartile of distribution) after a transcriptome-wide analysis evaluating differentially methylated regions (Grindler *et al.*, 2018). On the other hand, a French cohort study revealed mostly increased DNA methylation associated with phthalate exposure ($n = 202$) (Jedynak *et al.*, 2022). Compared with the cross-sectional study that received urine samples and placentas before 12 weeks of pregnancy, the French cohort consisted of urine samples taken between 22 and 29 gestational weeks and placentas taken at birth. This difference may indicate that there is a time-dependent effect associated with phthalate exposure. Another study examined methylation of long interspersed nucleotide elements (LINE), a marker for global DNA methylation. In that Chinese study of 55 FGR cases and 64 controls, ΣDEHP metabolites were significantly inversely associated with LINE-1 methylation in the placenta ($\beta = -0.54$, $p = .04$) (Zhao *et al.*, 2015), whereas the French study reported no significant associations between phthalate metabolites and LINE-1 repetitive elements (Jedynak *et al.*, 2022). Considered together, the studies on phthalate exposure and DNA methylation suggest that phthalates may affect placental gene regulation by differential patterns in DNA methylation, possibly leading to the overexpression or underexpression of genes necessary for placental development.

MiRNA regulate gene expression by inhibiting translation and destabilizing mRNA (Bushati and Cohen, 2007). A cohort study in Boston ($n = 179$) examined the relationship between phthalate metabolites and the expression of 29 placental miRNA, observing that miR-142-3p, miR15a-5p, and miR-185 expression levels were significantly associated with ∑phthalates. Ten of the 29 individual miRNA were also associated with at least 1 metabolite (of 11 measured). MCOP, a metabolite of DiNP, was associated with the greatest number of miRNA (LaRocca *et al.*, 2016). Another cohort study ($n = 10$) measured 20 miRNA in extracellular vesicles from the placenta in maternal circulation and identified miR_518e as significantly associated with MBzP levels, a metabolite of butyl benzyl phthalate. No other correlations were observed (Zhong *et al.*, 2019).

Long noncoding RNAs (lncRNAs) are also known to be regulators of transcription and they have been proposed as markers for toxicological responses (Dempsey and Cui, 2017). A cross-sectional study ($n = 10$) of mothers carrying twins evaluated their 20 placentas to examine associations between 87 lncRNAs and phthalate metabolites measured in urine samples collected at the time of delivery. Most of the lncRNAs were positively correlated with phthalate metabolites with the strongest correlation between mono-hydroxyisobutyl phthalate and lncRNA LOC91450 ($R_{Spearman} = .88$). AIRN, DACT3.AS1, DLX6, DPP10, HOTTIP, LOC143666, and LOC9145H19 also had strongly positive correlations whereas the rest were moderately positive, with the exception of H19, which was negatively associated with most metabolites (Machtinger *et al.*, 2018). Another cohort study in Tennessee ($n = 760$) reported that second trimester MECPP and MEOHP were positively associated with several lncRNAs (Paquette *et al.*, 2021). All 6 studies provide evidence that phthalates may disturb the genomic integrity of the placenta by altering both translational and transcriptional regulation.

Other Genes of Interest

Six additional papers on human studies reported altered expression of genes that did not fall into the above categories. The first paper was a cross-sectional study comparing pregnant mothers in China with low and high phthalate exposure ($n = 187$) as defined by the presence of industrial plastic production in the areas of residence (Li *et al.*, 2016). The authors looked at the association between phthalate esters in umbilical cord blood and metallotheioneins (MTs), proteins involved in the detoxification of toxic metals and antioxidation (Sutherland and Stillman, 2011). DMP was significantly associated with higher MT and MT-2A expression in both male and female placentas, DEHP showed the same associations in female placentas, and DEP was significantly associated with higher MT-1A and FATP1 in female placentas. The upregulation of these genes, furthermore, was associated with reductions in fetal weight and length (Li *et al.*, 2016). Future experiments for this study should aim to measure the metabolites of these phthalates in urine due to the ability of diesters such as DMP and DEHP to hydrolyze once in contact with the blood and epithelium (Calafat *et al.*, 2013).

Additionally, 2 papers examined urinary phthalate metabolites in relation to placental mRNA expression of inflammatory markers. All phthalate metabolites examined in the first trimester were significantly associated with at least 1 inflammatory biomarker, generally in the positive direction. The most consistent associations were observed for MBP which was associated with significantly increased expression of interleukin (IL)-1, IL-6, tumor necrosis factor-α, C-reactive protein, MCP-1, IL-8, IL-10, and cluster of differentiation (CD)68. Overall, associations were stronger in male placentas compared with female placentas (Wang *et al.*, 2020). These results contrast with those of a separate study on neurodevelopment in which second and third trimester phthalate metabolites were correlated with decreased placental IL-6 and CD68 (Gao *et al.*, 2022).

Two papers examined the gene encoding the aryl hydrocarbon receptor (AHR) which plays a role in trophoblast differentiation and disease pathologies such as preeclampsia and FGR (Detmar *et al.*, 2008; Stejskalova *et al.*, 2011). In both studies, phthalate metabolites (predominantly the DEHP metabolites) were associated with significantly lower AHR expression in the placenta (Adibi *et al.*, 2010, 2017).

Lastly, 1 animal study and 1 human study conducted genome-wide analyses of differentially expressed genes in the placenta. Pregnant rats were exposed to 500 or 1000 mg/kg/day DEHP during the middle of gestation for 7 days. Compared with the control group, in the low-dose group, 3787 genes were differentially expressed whereas in the high-dose group, 951 were differentially expressed, with most being downregulated. Of note, somatostatin receptor 2 and 4 were significantly increased

(Xu *et al.*, 2020b). Somatostatin receptors play a role in the development of neurological disorders (Ádori *et al.*, 2015); therefore, their expression in the placenta may also be implicated in adverse neurodevelopmental outcomes following prenatal exposure to phthalates (Xu *et al.*, 2007). Paquette *et al.* (2021), however, reported that DEHP metabolites, in addition to MCIOP, tended to be associated with an overall upregulation of genes, whereas MEP showed an opposite trend.

In total, the studies on gene expression were lacking replication, with many genes studied in only a single analysis, limiting conclusions. Fatty acid homeostasis and methylation were most widely studied; however, results were inconsistent. Several studies provided evidence of heightened expression of genes involved in the cell cycle favoring cell death following phthalate exposures.

DISCUSSION

Overall, the *in vivo* and epidemiological evidence included in this systematic review offers support for the hypothesis that gestational exposure to phthalates impacts some aspects of placental development and function. Phthalate-related alterations were reported for all placental measures considered (eg, morphology, hormone production, vascularization, histopathology, and gene/protein expression). Across both the animal and human studies, most consistent evidence of phthalate-related changes was observed for vascular and morphologic measures, including changes in cell composition and proliferation; however, the need for additional research on both topics is needed, particularly epidemiological studies. Our systematic evaluation of the literature identified not only several strengths of the existing literature but also a number of weaknesses across all placental measures that warrant additional, well-designed research (Table 7). Among the themes that emerged from the literature were an overemphasis on DEHP, issues concerning critical periods of exposure, and inconsistent consideration of variation in associations by fetal/placental sex.

Although numerous phthalates have been studied in relation to placental measures, DEHP has received the most attention and was the ester administered in the majority of the animal studies. Among the large panel of metabolites measured in most human studies, the most consistent adverse impacts were associated with DEHP metabolite concentrations. However, some studies also noted placental alterations associated with the metabolites of DBP, BBzP, DiBP, and DiNP, suggesting a need for future animal studies to examine DEHP as well as other esters of concern. Importantly, like DEHP, these additional esters and their metabolites have been implicated in numerous adverse perinatal and child outcomes including preterm birth, altered neurodevelopment, and asthma (Ferguson *et al.*, 2014a, 2019; Gascon *et al.*, 2015; Navaranjan *et al.*, 2021; Qian *et al.*, 2019; Whyatt *et al.*, 2014; Wu *et al.*, 2020) and it is possible that some of these impacts arise, at least in part, through effects on placental structure and function (Adibi *et al.*, 2021).

Across numerous studies, differences in associations based on the sex of the fetus were reported, with stronger associations typically observed for the placentas of male fetuses. This sex difference is consistent with stronger impacts on male offspring observed in some studies of prenatal phthalate exposure (Bornehag *et al.*, 2018; Swan *et al.*, 2015). The placenta is a fetal-derived organ, and differential patterns of gene expression between male and female human placentas starting in the late first trimester have been reported (Gonzalez *et al.*, 2018); this differential gene expression may modulate susceptibility of the placenta to adverse exposures like phthalates. Sex differences in placental morphology are also evident. In humans, fetoplacental weight ratio (a measure of placental efficiency) tends to be higher in women carrying males compared with females (Matsuda *et al.*, 2015). Sex differences in placental morphology and function may play a role in the increased vulnerability to stressors, including environmental stressors like phthalates, that is often reported in male fetuses (Barrett and Lessing, 2021). Unfortunately, we observed that many studies did not report on sex differences, despite strong evidence that it should be standard practice to consider placental sex in this field.

The rapidly changing physiology of pregnancy may make the placenta more vulnerable to exposures during certain periods; this theme is recapitulated in studies of phthalates and child development, where exposure during certain critical windows in gestation has significant impacts on development, whereas the same exposure during a different gestational window shows no association (eg, Martino-Andrade *et al.*, 2016). However, at present, inconsistencies in timing of exposure across studies have made it difficult not only to compare results across studies but also to determine whether there are critical periods during which phthalates disrupt placental development. Only 1 animal study considered multiple time points during gestation, revealing that the middle (GD7–GD12) may be more sensitive to toxic insults compared with early and late pregnancy (Shen *et al.*, 2017). Similarly, relatively few epidemiological studies have measured phthalates at multiple gestational time points. Therefore, to identify critical gestational windows (if any) we suggest future evaluation of animal models employing single or short-term doses at different gestational time points as well as human studies with repeated exposure measures across pregnancy.

Complicating issues regarding timing of exposure is the considerable potential for exposure misclassification in human studies given the short half-life of phthalates in the body, typically several hours. In our risk of bias assessment, reliance on a single spot urine sample to characterize exposure was a common issue that may reduce internal validity of epidemiological studies and result in biasing estimates toward the null. Other studies used nonpreferred matrices such as blood to characterize phthalate metabolites, despite concerns about contamination and short half-lives (Calafat *et al.*, 2013). Additionally, several studies neglected important factors that can impact placental measures, such as gestational age or pregnancy complications. As usual, loss to follow-up was common in human studies; however, few studies attempted to evaluate whether it could be a source of bias in the results.

Our evaluation of risk of bias in the animal studies demonstrated that a lack of blinding was a common concern. In addition, although several studies noted that allocation to study groups was randomized, the method of randomization and concealment of allocation were missing. Perhaps most importantly, across most animal studies, the use of high phthalate doses that are not environmentally relevant was a major limitation which calls into question the significance to human health. The need for low-dose studies is additionally warranted given evidence that endocrine disruptors often elicit monotonic dose-responses (Andrade *et al.*, 2006; Do *et al.*, 2012; Vandenberg, 2014). All animal experiments employed oral dosing, mostly by gavage. This route of exposure is also not ideal to mimic human exposure because gavage bypasses the oral cavity, where chemicals are often absorbed (Vandenberg *et al.*, 2014). Furthermore, it is a process that adds additional stress to the animals, potentially affecting placental measures.

Finally, although we could not assess it in the current review, for both epidemiological and animal studies, publication

Table 7. Strengths and Weaknesses of Literature on Associations Between Phthalate Exposures and Placental Measures

Placental Measure	Number of Human Studies (Mean RoB Score[a])	Number of Animal Studies (Mean RoB Score[a])	Overall Strengths	Overall Weaknesses
Morphology	3 (1.3)	4 (1.5)	• Outcome measurements collected consistently across studies • Consistent evidence of inverse associations across most studies • Some studies evaluate multiple exposure timepoints • Multiple phthalates considered in animal studies	• Sparse data on endpoints beyond placental weight • Nonenvironmentally relevant doses in animal models • Use of only oral exposure in animal studies
Vascularization	2 (1)	4 (1.8)	• Animal studies report direct vascular assessments and vascular biomarkers • Consistent evidence for impaired vascularization • Multiple phthalates considered in animal studies	• Null results in only study to use low doses • Human studies do not have direct assessments of vasculature • Nonenvironmentally relevant doses in animal models • Use of only oral exposure in animal studies
Hormone production	7 (1.9)	2 (2.5)	• Multiple hormones of interest evaluated • Some consideration of time-dependent effects	• Inconsistent evidence on a wide variety of hormones • Inappropriate matrix (blood) used for phthalate measurement in some studies • Nonenvironmentally relevant doses in animal models • Use of only oral exposure in animal studies • Animals only exposed to DEHP
Histopathology	0 (N/A)	5 (1.8)	• Includes 1 study with an environmentally relevant dose • Some consideration of time-dependent effects • Multiple phthalates considered in animal studies	• No human studies • Although many studies report time frames, there is inconsistency in dosage • Use of only oral exposure in animal studies
Gene/protein expression	15 (1.9)	5 (2.6)	• Consistent with results of histopathology studies • Wide array of gene pathways considered	• Use of only oral exposure in animal studies • Nonenvironmentally relevant doses • Many genes/pathways only considered in 1 study • Animals only exposed to DEHP

[a]Based on Risk of Bias evaluations in Table 5 (human) and Table 6 (animal).

bias is a concern whereby analyses observing no association between phthalate exposures and placental measures may be less likely to be published.

Future Directions

Beyond a need to replicate many of the findings reported in this small but emerging literature, our review identified several important directions for future research. First, future animal studies should be designed with an eye toward translation to human exposure. This would not only include environmentally relevant dosing but also include other exposures models, such as inhalation to account for plastic particles in the ambient air that we breathe and oral routes that do not involve gavage. It would include evaluation of the multiple high-production phthalates to which pregnant women are ubiquitously exposed, extending the current focus on DEHP. In addition, the rodent and human placenta are not always comparable; therefore, there is a need for mammalian models that are more similar to humans such as nonhuman primates. Although there are many similarities regarding their cell makeup, there are also important differences to consider when extrapolating rodent studies to human significance such as the timing of decidualization, timing of when the placenta gets its final structure, and the extent of fetal vessel branching in the zones of blood transfer (Malassiné *et al.*, 2003). In the human placenta, the chorionic villi invade the maternal myometrium whereas in the rodent placenta, the fetal and maternal vessels arrange in a network that allows more efficient nutrient transfer (Dilworth and Sibley, 2013).

Endocrine differences in the placenta are also profound and hinder translational studies. For example, the human placenta

produces significantly more estradiol and progesterone compared with other mammals; in contrast, the ovaries are the primary source of these hormones during rodent gestation (Malassiné *et al.*, 2003). Furthermore, rodents, unlike humans, do not produce estriol at all (Hudon Thibeault *et al.*, 2014). Similarly, only the human placenta produces hCG which is potentially an important target for phthalate toxicity (Evain-Brion and Malassine, 2003). In general, the significant difference in placental hormone production between humans and rodent models limits translational studies of phthalates' endocrine-disrupting properties in the placenta, further suggesting a need for carefully designed human and *in vitro* studies. Despite this, continued interrogation of the rodent placenta in relation to phthalate exposure is important given the human placental development studies are limited to placental measures at delivery (or biomarkers measurable in maternal circulation).

Importantly, to date, most human studies on phthalates and the placenta have been secondary analyses based on extant cohorts that were not specifically designed to study placental measures. As a result, the range of measures available has been limited primarily to what is measurable in banked specimens (eg, placental hormones in maternal circulation) or the clinical record (eg, placental weight at delivery). This has resulted in a wide array of heterogenous placental measures that are often inconsistent with those used in the animal literature. For example, despite animal evidence suggesting visible changes in placental vasculature following phthalate exposure, human studies have relied entirely on angiogenic biomarkers in maternal circulation with no consideration of direct vascular assessments, making cross-study comparisons difficult. Given the growing, suggestive evidence that phthalates may indeed impact placental development, new studies collecting extensive primary data on placental structure and function are warranted, as is more intensive consideration of placental histology and pathology in relation to maternal phthalate exposures. Finally, a main goal of studying the placental impacts of phthalates is to understand the pathways by which they may affect child health and development. Moving forward, it will be important to integrate placental and child outcome data to identify the extent to which the impacts of phthalates on child development are mediated by their effects on the placenta.

In conclusion, given these different mechanisms of placental disturbances, an important next step will be to unify these different observational approaches by looking at a comprehensive set of placental measures within a single study. In isolation, all of these mechanisms appear to be important; therefore, future studies should aim to understand how these diverse types of biomarkers and placental measures may relate to common underlying mechanisms.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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