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CONTEMPORARY REVIEW

Using Experimental Models to Assess Effects of Bisphenol A (BPA) and Phthalates on the Placenta: Challenges and Perspectives

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ABSTRACT

The placenta is critical for all aspects of fetal development. Bisphenol A (BPA) and phthalates are endocrine disruptors with ubiquitous exposure in pregnant women—their effects on the placenta is an area of growing research interest. Therefore, our objectives were to (1) summarize research related to the effects BPA or phthalates on placental outcomes in animal and cell models, and (2) evaluate the challenges for using such models to study the impacts of these chemicals on placental endpoints. Overall, studies in cells and animal models suggest that BPA and phthalates impact placental hormones, some epigenetic endpoints, increase inflammation and oxidative stress, and decrease cell viability and nutrient transfer. However, few animal or cell studies have assessed these outcomes at concentrations relevant to humans. Furthermore, it is unclear whether effects of BPA/phthalates on the placenta in animal models mediate fetal outcomes, as most studies have dosed after the earliest stages of placental and fetal development. It is also unclear whether effects of these chemicals are sex-specific, as few studies have considered placental sex. Finally, while there is substantial evidence for effects of mono-(2-ethylhexyl) phthalate (the major metabolite of di-(2-ethylhexyl) phthalate), on placental endpoints in cells, little is currently known about effects of other phthalates to which pregnant women are exposed. Moving forward, these limitations will need to be addressed to help us understand the precise mechanisms of action of these chemicals within the placenta, and how these reported perturbations impact fetal health.

Key words: BPA; endocrine disrupting chemicals; phthalates; placenta.

Bisphenol A (BPA) and phthalates are endocrine disrupting chemicals found in food packaging and personal care/household products (Mervish *et al.*, 2014; Schettler, 2006). Although life-course exposures to these chemicals can adversely impact health, pregnancy is an especially sensitive window, and epidemiological studies demonstrate that pregnant women have widespread exposure to these chemicals (Fisher *et al.*, 2015; Yan *et al.*, 2009). In placental mammals, the establishment and maintenance of a successful pregnancy, and the offspring's lifelong health, rely on finely tuned developmental trajectories of placental and fetal tissues (reviewed in Godfrey, 2002 and Jansson and Powell, 2007). As we have reviewed elsewhere (Strakovsky and Schantz, 2018), recent human studies suggest associations between BPA or phthalate exposures and numerous placental molecular endpoints. These include hormone-related mRNA expression (Adibi *et al.*, 2010, 2017), micro-RNA expression (LaRocca *et al.*, 2016; Zhong *et al.*, 2018), long noncoding RNA expression (Machtinger *et al.*, 2018), and DNA methylation (Grindler *et al.*, 2018; LaRocca *et al.*, 2014; Nahar *et al.*, 2015; Zhao *et al.*, 2015, 2016). Furthermore, there is

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evidence that phthalate exposure in humans is also associated with changes to placental anthropometry (length, breadth, surface area, and thickness) (Zhu *et al.*, 2018). However, the current literature in humans is still limited in its ability to pinpoint specific mechanisms by which these chemicals target the human placenta to cause these effects and to ultimately impact placental function.

These knowledge gaps are due, in part, to the challenges in assessing phenotypic and molecular endpoints within the large and heterogeneous human placenta, with studies showing that even the most careful sampling schemes to assess gene expression can result in great within-placental variability (Adibi *et al.*, 2009). Other challenges for making mechanistic evaluations within the human placenta thus far have included the following: (1) the fact that chemical exposures have been assessed at various points in pregnancy, whereas placental tissues are typically only available at birth, and (2) lack of reliable information about the role of fetal sex in modifying associations of exposures with placental molecular endpoints. As will be discussed throughout this review, there are undoubtedly challenges for modeling placental outcomes in experimental animal and cell models. Nevertheless, given the difficulties that exist in human placental sampling, such models are invaluable for unraveling the mechanisms of action of environmental chemicals within the placenta. The primary goal of this review is to address these challenges and opportunities for modeling placental structural, functional, and molecular outcomes in response to BPA and phthalates.

ANIMAL MODELS

Given the challenges in both human exposure assessment and placental sampling, animal models are important for assessing the underlying mechanisms behind the associations observed in humans. First, unlike in humans, the small size of the rodent placenta allows for histological evaluation of whole placentas as well as for site- and structure-specific molecular analysis in response to chemical exposures. Second, rodent models provide excellent opportunities for sex-specific evaluations of the placental-fetal unit, given that each rodent pup develops with its own placenta. As will be described below, important factors to consider when designing such models include the structural, functional, and epigenetic differences between species, exposure window, chemical dose, and placental sex, among others (Tables 1 and 2).

Animal Model Selection

Although the rodent is the most-widely utilized animal model in studies assessing the effects of BPA and phthalates on the placenta, there are important developmental and structural differences between human and rodent placentas (reviewed in Carter, 2007) (cited and reviewed in **Figure 1**). These differences include placental hormone synthesis and function (**Figure 1A**), trophoblast invasion, remodeling of spiral arteries that carry blood and nutrients to the fetus, the timing and process of decidualization and placental development, the formation and branching of villi (**Figure 1B**), and the fetal: placental weight ratio, which is driven by fundamental species differences in maternal-fetal blood flow (**Figure 1C**).

Despite these differences, there are numerous reasons for using rodent models to study the effects of environmental chemicals on the placenta. First, like humans, rodents develop a single discoid placenta. Humans and rodents also both have a hemochorial placenta, in which fetal chorionic villi are directly bathed by maternal blood (**Figure 1D**). In regard to epigenetic regulation, aspects of placental imprinting differ between rodents and humans (Monk *et al.*, 2006). However, given that genomic imprinting is important for both human and rodent placental development (Frost and Moore, 2010), rodent studies have been useful for assessing the capacity of environmental chemicals to disrupt placental imprinting. Other useful models of human placental development also exist, and include nonhuman primates and sheep. However, few studies using these models have assessed the effects of endocrine disruptors on placental endpoints (except 1 study in sheep discussed later), therefore here, we have focused primarily on currently available studies in rodents.

Exposure Timing

Developmental toxicity studies targeting specific windows of fetal organ development are valuable for gaining insight into fetal organogenesis. However, the role of the placenta in mediating these outcomes is difficult to assess if dosing begins after the earliest stages of implantation and placental development/trophoblast invasion (as early as gestational day [GD] 3.5 in mice) (Yamanaka *et al.*, 2006). Furthermore, these studies do not accurately model exposures in humans, as women are exposed to BPA and phthalates on a daily basis, prior to and throughout pregnancy.

Of the studies described here, only one began dosing prior to mating, whereas others started on GD 0 or 1, or much later (Tables 1 and 2). However, there is evidence for window-specific effects of phthalates on placental pathology. A recent study in ICR mice dosed with high-dose (500 mg/kg) di-(2-ethylhexyl) phthalate (DEHP) found decreased placental weight and diameter (males only) and decreased blood sinusoid area and cell proliferation (males and females analyzed together) only in dams dosed at GD7-12, but not GD0-6 or GD13-17 (Shen *et al.*, 2017). Timing of exposure is especially significant when assessing placental epigenetic endpoints, as the earliest rapid stages of placental cell differentiation occur under strict epigenetic control (Maltepe *et al.*, 2010). In JF1 mice, neither BPA (0.2 mg/kg/day) nor DEHP (750 mg/kg/day) gavaged from GD8.5 to GD12.5 had major effects on loss-of-imprinting or the expression of imprinted genes (Kang *et al.*, 2011). BPA also had no effect on either placental or embryonic loss-of-imprinting in pregnant C57BL/6 dosed from GD5.5 to GD12.5 (Susiarjo *et al.*, 2013). However, when the C57BL/6 mice were dosed with BPA beginning two weeks prior to mating until GD9.5 or GD12.5, BPA disturbed loss-of-imprinting, altered the mRNA expression of several imprinted genes, reduced placental CpG and average global DNA methylation, and decreased DNA methylation of 1 imprinted gene (Susiarjo *et al.*, 2013). Although germline imprints (those passed to future generations) resist the first wave of active demethylation immediately following fertilization (Sanz *et al.*, 2010), the earliest regulation of placental imprinting occurs, in part, independently of DNA methyltransferase 1 (DNMT1) mechanisms that regulate extra-placental imprinting (Court *et al.*, 2014). These early dynamic processes may be especially sensitive to disruption by environmental chemicals. Therefore, given the role of epigenetics in regulating transcription, downstream translation, and ultimately placental development, it is critical that studies assessing the effects of chemicals on the placenta begin dosing prior to mating or immediately following fertilization.

Bisphenol dose and compound selection. Animal studies assessing nonplacental endpoints suggest a nonmonotonic dose-response for BPA, likely reflecting effects on both endocrine and

Table 1. Effects of Bisphenol A (BPA) Exposure on Placental Endpoints in Rodent Models

Model or Strain	BPA Dose	Dosing Mode	Dosing Window (GD)	Sex	Summary of Outcomes	Ref.
ICR mice	2.5 μg/kg	Oral	6.5–17.5	Male	Effects on nuclear receptor mRNA expression, including hormone receptors: YES	Imanishi *et al.* (2003)
				Female	Effects on nuclear receptor mRNA expression, including hormone receptors: YES (some outcomes different compared with males.)	
ICR mice	10 mg/kg	SC	0–7	NR	Effects on placental weight, anatomy, and structure: YES	Tachibana *et al.* (2007)
ER-luc mice	50 mg/kg	Oral	8–15	Combined	Effect on estrogen receptor gene activity: YES	Ter Veld *et al.* (2009)
	6.66 μl/g bw	IP	14			
JF1 mice	0.2 mg/kg	Oral	8.5–12.5	NR	Effects on placental imprinting: NO	Kang *et al.* (2011)
Mating between C57BL/6(B6) and B6 or B7 mice	10 μg/kg	In diet (estimated exposure from 50 μg/kg or 50 mg/kg in diet)	2 weeks premating—GD9.5	NR	Effects on loss-of-imprinting and expression of imprinted genes: YES Effects on global methylation: NO	Susiarjo *et al.* (2013)
	10 mg/kg				Effects on loss-of-imprinting and expression of imprinted genes: YES Effects on global and CpG-specific methylation: YES Effects on placental anatomy and structure: SOME	
			2 weeks premating—GD12.5			
	10 mg/kg		5.5–12.5		Effects on loss-of-imprinting: NO	
ICR mice	2 mg/kg	Oral	13–16	NR	Effects on hormonal mRNA expression: NO Effects on protein expression of downstream pathways: SOME	Tan *et al.* (2013)
	20 mg/kg				Effects on hormonal mRNA expression: NO Effects on protein expression of downstream pathways: SOME	
	200 mg/kg				Effects on hormonal mRNA expression: YES Effects on protein expression of downstream pathways: YES	
CD-1 mice	0.5 mg/kg	Oral	1–11	Combined	Effects on placental anatomy and structure: SOME Nuclear protein content of growth-related protein: YES	Tait *et al.* (2015)
	50 mg/kg				Effects on placental anatomy and structure: YES Nuclear protein content of growth-related protein: NO (unique gene signature compared with low dose)	
ICR mice	50 mg/kg	SC	11.5–16.5	Combined	Effects on mRNA expression of some metal cation transporters: SOME	Lee *et al.* (2016)
Kunming mice	5	SC	0.5–5.5	NR	Effects on proteins related to trophoblast migration: YES	Lan *et al.* (2017)[a]
	40				Effects on proteins related to trophoblast migration: YES	
Polypay× Dorset sheep	0.5 mg/kg	SC	30–100	Combined	Effects on placental weight: (NO), number of placentomes: NO, tissue composition: NO Effects on syncytialization-related protein and mRNA: NO, binucleate cells: NO	Gingrich *et al.* (2018)
	BPS: 0.5 mg/kg				Effects on placental weight: (NO), number of placentomes: NO, tissue composition: NO Effects on syncytialization-related protein and mRNA: YES, binucleate cells: YES	

BPA, bisphenol A; BPS, bisphenol S; GD, gestational day; IP, intraperitoneal; LOI, loss of imprinting; ND, not detected; NR, not reported; prot, protein; SC, subcutaneous.

[a]Authors also showed histological data and reported (at 5, 10, and 40 mg/kg BPA) increased ITGB1 and ITGA5, a shift in the sizes of the layers, and increased presence of glycogenosomes and vacuoles by BPA, but without quantification or statistical comparisons.

Table 2. Effects of Phthalate Exposure on Placental Endpoints in Rodent Models

Model or Strain	Phthalate Dose (mg/kg)	Phthalate	Dosing Mode	Dosing Window (GD)	Sex	Summary of Outcomes	Ref.
Sprague Dawley rats	750	DEHP	Oral	0–19	Combined	Effects on lipid-related mRNA or protein expression in junctional zone: SOME	Xu *et al.* (2008)
						Effects on prostaglandin-related mRNA or protein expression in junctional zone: SOME	
						Effects on lipid-related mRNA or protein expression in the labyrinth: SOME	
						Effects on overall prostaglandin-related mRNA expression in junctional zone: NO	
						Effects on overall prostaglandin production: YES	
	1500					Effects on lipid-related mRNA or protein expression in junctional zone: SOME	
						Effects on prostaglandin-related mRNA or protein expression in junctional zone: SOME	
						Effects on lipid-related mRNA or protein expression in the labyrinth: YES	
						Effects on prostaglandin-related mRNA expression in junctional zone: NO	
						Effects on overall prostaglandin production: YES	
						Effects on overall fatty acid distribution: SOME	
ER-luc mice	100	DEHP	Oral	8–15	Combined	Effect on estrogen receptor gene activity: NO	Ter Veld *et al.* (2009)
		DIHP				Effect on estrogen receptor gene activity: YES	
	6.66 μl/g bw	DEHP	IP	14		Effect on estrogen receptor gene activity: NO	
		DIHP				Effect on estrogen receptor gene activity: YES	
JF1 mice	750	DEHP	Oral	8.5–12.5	NR	Loss-of-imprint: SOME	Kang *et al.* (2011)
CD-1 mice	125	DEHP	Oral	1–9	NR	Effects on placental anatomy and structure: YES	Zong *et al.* (2015)
						Effects on development-, apoptosis-, and vascularization-related genes: SOME	
				1–13		Effects on placental weight, anatomy, and structure: SOME	
						Effects on vascularization: NO	
						Effects on development-, apoptosis-, and vascularization-related genes: YES	
	250			1–9		Effects on placental anatomy and structure: YES	
						Effects on development-, apoptosis-, and vascularization-related genes: SOME	
				1–13		Effects on placental weight, anatomy, and structure: YES	
						Effects on vascularization: YES	
						Effects on development-, apoptosis-, and vascularization-related genes: YES	
	500			1–9		Effects on placental anatomy and structure: YES	
						Effects on development-, apoptosis-, and vascularization-related genes: SOME	
				1–13		Effects on placental weight, anatomy, and structure: YES	
						Effects on vascularization: YES	
						Effects on development-, apoptosis-, and vascularization-related genes: YES	

Table 2. (continued)

Model or Strain	Phthalate Dose (mg/kg)	Phthalate	Dosing Mode	Dosing Window (GD)	Sex	Summary of Outcomes	Ref.
ICR mice	200	DEHP	Oral	0–6 or 7–12 or13–17	Combined and by sex	Effects on placental weight and anatomy: YES—in males and if exposed on GD7-12 Effects on placental structure and proliferation: YES—on GD7-12	Shen *et al.* (2017)
Wistar rats	500	DBP	Oral	6–18 to F0	Combined	Effects on placental weight: YES—in undosed F1, F2, F3 generations.	Mahaboob Basha and Radha (2017)
Wistar rats	20	DHP	Oral	6–19	NR	Effects on placental shape: SOME Effects on markers of placental damage: NO Effects on protein content of estrogen receptors: YES Effects on marker of proliferation: YES	Ahbab *et al.* (2017)
	100					Effects on placental shape: SOME Effects on markers of placental damage: SOME Effects on protein content of estrogen receptors: YES Effects on marker of proliferation: YES	
	500					Effects on placental shape: YES Effects on markers of placental damage: SOME Effects on protein content of estrogen receptors: YES Effects on marker of proliferation: YES	
	20	DCHP				Effects on placental shape: YES Effects on markers of placental damage: SOME Effects on protein content of estrogen receptors: YES Effects on marker of proliferation: YES	
	100					Effects on placental shape: SOME Effects on markers of placental damage: SOME Effects on protein content of estrogen receptors: YES Effects on marker of proliferation: YES	
	500					Effects on placental shape: YES Effects on markers of placental damage: YES Effects on protein content of estrogen receptors: YES Effects on marker of proliferation: YES	

–, no effect observed; DEHP, di(2-ethylhexyl)phthalate; DHP, di-n-hexyl phthalate; DCHP, dicyclohexyl phthalate; GD, gestational day; IHC, immunohistochemistry; IP, intraperitoneal; NR, not reported; prot, protein.

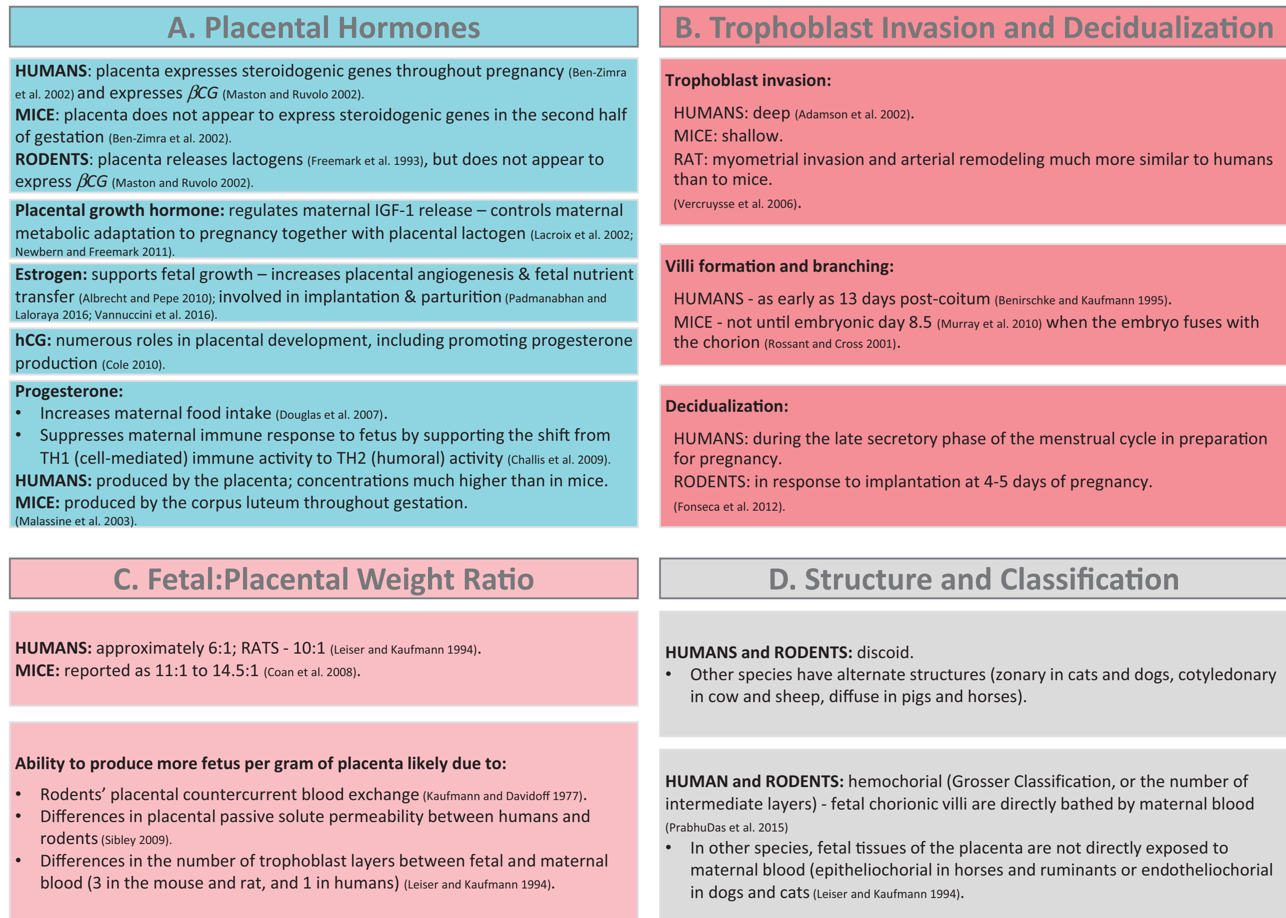

Figure 1. Species differences (specifically focusing on humans and rodents) in A, placental hormones, B, trophoblast invasion and decidualization, C, the fetal: placental weight ratio, and D, placental structure and classification (Adamson *et al.*, 2002; Albrecht and Pepe, 2010; Benirschke and Kaufmann, 1995; Ben-Zimra *et al.*, 2002; Challis *et al.*, 2009; Coan *et al.*, 2008; Cole, 2010; Douglas *et al.*, 2007; Fonseca *et al.*, 2012; Freemark *et al.*, 1993; Kaufmann and Davidoff, 1977; Lacroix *et al.*, 2002; Leiser and Kaufmann, 1994; Malassine *et al.*, 2003; Maston and Ruvolo, 2002; Murray *et al.*, 2010; Newbern and Freemark, 2011; Padmanabhan and Laloraya, 2016; PrabhuDas *et al.*, 2015; Rossant and Cross, 2001; Sibley, 2009; Vannuccini *et al.*, 2016; Vercruysse *et al.*, 2006).

non-endocrine pathways. Placental studies, however, have thus far utilized relatively high doses and limited dose-response curves. Despite this, several studies do suggest a dose-response relationship for BPA. For example, in ICR mice, most effects on placental mRNA and protein expression were observed only at the higher BPA doses (200 and 20 mg/kg vs 2 mg/kg) (Tan *et al.*, 2013). In a study using CD-1 mice, 0.5 versus 50 mg/kg/day BPA also had dose-dependent effects on placental size, size of the spongiotrophoblast layer, total areas of maternal blood spaces, and embryonic labyrinthine capillaries. In this study, of all genes affected by 0.5 or 50 mg/kg BPA (compared with control), only 77 were shared between the 2 treatment groups, with 0.5 mg/kg BPA generally having a larger effect than 50 mg/kg. The 2 BPA doses also led to different significantly enriched KEGG pathways and different affected protein hubs (Tait *et al.*, 2015). BPA has also been shown to dose-dependently affect epigenetic endpoints in other tissues (Ho *et al.*, 2015), and while the Susiarjo *et al.* study demonstrated dose-dependent effects on placental imprinting and DNA methylation, and included a lower dose of BPA (estimated 10 μg/kg bw/day), additional studies are warranted using wider ranges of doses, including those more in line with human exposures (estimated to be well <1–2 μg/kg bw/day in the general population; Teeguarden and Hanson-Drury, 2013).

Only one animal study has assessed the effects of bisphenols other than BPA. Interestingly, a study in sheep found that bisphenol S (BPS), which is currently being used as a replacement for BPA in many products, altered the expression of syncytialization-related proteins/mRNA and decreased the number of trophoblast-derived binucleate cells, whereas the same dose of BPA had no effect on these outcomes (Gingrich *et al.*, 2018). Pregnant women are exposed to various bisphenols (Kolatorova *et al.*, 2018; Wan *et al.*, 2018), so additional studies in animals may be warranted to investigate the effects of these compounds on the placenta.

Phthalate dose and compound selection. Animal studies assessing effects of phthalates on placental endpoints are also limited, have utilized relatively high doses, and have primarily focused on 1 phthalate, DEHP. In pregnant CD-1 mice, DEHP (125, 250, or 500 mg/kg/day) had dose-dependent effects on total placental and labyrinthine area, whereas all DEHP doses altered placental weight, spongiotrophoblast area, small-branched fetal vessels, proliferation in several placental zones, and the mRNA expression of numerous genes (Zong *et al.*, 2015). In Sprague Dawley rats, DEHP (750 or 1500 mg/kg/day) dose- and zone-specifically induced placental mRNA and protein expression of *Ppara* and *Pparg*, the expression of several fatty acid transport-related

genes/proteins, and placental content of several long-chain polyunsaturated fatty acids. However, both doses induced cytochrome p4504A1 (*CYP4A1*) mRNA and protein, and reduced labyrinthine cyclooxygenase (COX)-2 protein and prostaglandin formation from placental homogenates (Xu *et al.*, 2008). It is likely that the lack of a dose-response in these studies was due to the high phthalate doses used in these experiments, which were chosen because previous studies had used similar doses, and not because they were relevant to human exposures. Authors discussed that approximate plasma concentrations of mono-(2-ethylhexyl) phthalate (MEHP) (the major metabolite of DEHP) in healthy women at term are 2.05 ± 1.47 μg/ml (referenced from Latini *et al.*, 2003). However, authors of this animal study discussed unpublished results showing that 750 and 1500 mg/kg DEHP treatment led to maternal MEHP plasma concentrations of 65 or 136 μg/ml (respectively). Given that these internal doses are greatly out of the range of human exposures, additional studies using lower concentrations of DEHP are needed to corroborate these findings.

Two more-recent studies reported placental outcomes in response to phthalates other than DEHP, but also at relatively high doses. In Wistar rats, 500 mg/kg DBP dosed to the F0 generation decreased placental weight in F1, F2, and F3 generations, accompanied by numerous reproductive and developmental disturbances in offspring (Mahaboob Basha and Radha, 2017). Another study in Wistar rats found that all doses (20, 100, 500 mg/kg) of di-n-hexyl phthalate (DHP) or dicyclohexyl phthalate (DCHP) decreased placental protein expression of PCNA, PPARγ, ERα, ERβ, and AR. However, effects on placental weight, diameter, and length differed by chemical and dose, as did most pathological findings within the trophoblasts, spongiotrophoblast, and basal zone (Ahbab *et al.*, 2017). Although this study included a dose-response curve, even the lowest concentration (20 mg/kg) may not be relevant to human exposures, as urinary MCHP (the major metabolite of DCHP) was below the level of detection for most people in the recent NHANES (CDC, 2017), and little is known about human DHP exposure.

Based on these findings, studies with more extensive dose-response curves that are more in line with human exposures will be essential to begin unraveling the mechanisms responsible for associations of phthalates and BPA exposures with placental outcomes in humans. This task is complicated by species differences in toxicokinetics, exposure, metabolism, and elimination rates of both BPA and phthalates (Doerge *et al.*, 2011; Rusyn *et al.*, 2006; Silva *et al.*, 2007; Thayer *et al.*, 2015). In humans, exposure is estimated based on internal doses of parent compounds or excretion of their metabolites. In animals, however, doses of parent compounds are known, but not the circulating or excreted concentrations of chemicals and their metabolites. Establishing exposure "dose" in humans is difficult given the multiple exposure pathways for BPA and phthalates and the paucity of data on human pharmacokinetics of these compounds. Therefore, in future animal studies, assessment of circulating and excreted concentrations of chemicals and their metabolites will be critical to allow exposure/dose comparisons between humans and model animals.

Sex-specific placental outcomes. The placenta develops from both maternal and fetal tissues, and consequently has sexually dimorphic responses to environmental and dietary cues (Rosenfeld, 2015). Studies in humans do suggest sex-specific associations between exposure to BPA or phthalates and placental outcomes (Adibi *et al.*, 2017; LaRocca *et al.*, 2014). However, of the animal studies described here, only 2 specifically discussed "placental sex." In ICR mice, 200 mg/kg DEHP decreased placental weight and diameter in male but not female placentas (Shen *et al.*, 2017), whereas 2.5 μg/kg/day BPA differentially affected placental mRNA expression depending on placental sex, either in magnitude or direction (Imanishi *et al.*, 2003). As previously discussed, rodent models are especially useful for assessing sex-specific associations between placental and fetal outcomes, as each pup develops with its own placenta. Given the reported sexually dimorphic associations of BPA/phthalates with placental outcomes in humans and some previous animal studies, future animal models should leverage the rodent fetal-placental unit to assess sex-specific placental effects of BPA and phthalates.

IN VITRO SYSTEMS

Cell culture experiments are indispensable for establishing mechanisms of action of BPA and phthalates on placental function. In placental *in vitro* models, these functional/phenotypic endpoints include standard measures of cell health (viability, proliferation, necrosis), and placenta-specific measures of function and development (apoptosis, migration, invasion, and differentiation). Similar to cancer, appropriate placental development is characterized by increased migration, invasion, differentiation (Knofler and Pollheimer, 2013), and apoptosis (which is exaggerated in instances of placental disease; Sharp *et al.*, 2010), thereby providing phenotypic readouts for the effects of BPA and phthalates. As will be discussed in following sections, similar to animal models, chemical concentration and length of exposure are critical for assessing effects of these chemicals, as is model selection. Models utilized in currently available studies include classic immortalized cell lines, as well as more physiologically relevant placental primary cultures to more-accurately model placental exposures (Tables 3 and 4).

Cell Models

Models assessing the impacts of environmental chemicals on placental molecular signaling have included immortalized cell lines, primary trophoblasts, cytotrophoblasts, microsomes, chorionic villi explants, and placental macrophages. For both immortalized and primary cells, the differences between their signaling and physiological responses to treatments represent fundamental differences in physiological functions of cells from which they are derived (Bilban *et al.*, 2010). For example, in human HTR-8/SVneo placental cells, BPA had no effect on cell viability or proliferation, whereas a range of BPA concentrations decreased cell migration and invasion, reflecting the invasive nature of these first-trimester-derived trophoblasts (Spagnoletti *et al.*, 2015). The invasive nature of HTR-8/SVneo cells was confirmed in another study, showing that BPA increased MMP-9 (but not MMP-2) protein, involved in cell invasion and migration (Lan *et al.*, 2017), and both proteins were increased by BPA in BeWo cells, a first-trimester-derived trophoblast choriocarcinoma cell line (Wang *et al.*, 2015).

Differences in gene transcription underlie the functional differences between placental cell lines and cell types, and may likely regulate their response to environmental chemicals. For example, in both JEG-3 choriocarcinoma immortalized cells and PL30 cells derived from third-trimester placentas, BPA concentration-dependently inhibited *CYP19* gene expression, but the baseline expression of *CYP19* appeared to be much greater in JEG-3 than in PL30 cells, reflecting the innate differences of an important hormonal regulator between these cells (Huang and Leung, 2009). A microarray study in several

Table 3. Effects of Bisphenol A (BPA) in Placental *In Vitro* Models

Cell Model	μM BPA[a]	Treatment Length (h)	Summary of Outcomes	Ref.
JEG-3	25	10 min, 30 min, 1, 2	Effects on aromatase activity: YES	Nativelle-Serpentini *et al.* (2003)
		18	Effects on aromatase activity: YES	
	50	10 min	Effects on aromatase activity/mRNA: NO	
		30 min, 1	Effects on aromatase activity: NO	
		2	Effects on aromatase activity: YES	
		18	Effects on aromatase activity: YES	
	100	10 min, 2	Effects on aromatase activity: YES	
		30 min, 1	Effects on aromatase activity: NO	
		18	Effects on aromatase activity: YES	
BeWo	0.1, 10, 50	approximately 1	Effects on measure of drug efflux: YES	Jin and Audus (2005)
Microsomes @term	500	15 min	Effects on aromatase activity: YES	Benachour *et al.* (2007)
1° Cytotrophoblasts	8.8×10^{-4}, 8.8×10^{-3}	24	Effects on measure of apoptosis: NO, necrosis: YES, *TNF-α* mRNA: YES, TNF-α prot: NO	Benachour and Aris (2009)
	0.0876, 0.438		Effects on measure of apoptosis: YES, necrosis: YES, *TNF-α* mRNA: YES, TNF-α prot: YES	
	0.876		Effects on measure of apoptosis: YES, necrosis: NO, *TNF-α* mRNA: NO, TNF-α prot: NO	
	8.76, 87.6		Effects on measure of apoptosis: YES, necrosis NO, *TNF-α* mRNA: NO, TNF-α prot: YES	
	438, 876		Effects on measure of apoptosis: YES, necrosis YES, *TNF-α* mRNA: NO, TNF-α prot: YES	
JEG-3	1	24	Effects on aromatase mRNA/protein expression or promoter activity, aromatase activity: NO	Huang and Leung (2009)
PL30			Effect on aromatase mRNA: NO	
JEG-3	5		Effects on aromatase mRNA/protein expression or promoter activity, aromatase activity: YES	
PL30			Effect on aromatase mRNA: NO	
JEG-3	25		Effects on aromatase mRNA/protein expression or promoter activity, aromatase activity: YES	
PL30			Effect on aromatase mRNA: YES	
JEG-3	50		Effects on aromatase mRNA/protein expression or promoter activity, aromatase activity: YES	
PL30			Effect on aromatase mRNA: YES	
JEG-3, PL30	100		Effects on aromatase mRNA/protein expression or promoter activity, aromatase activity: YES	
3A, HTR-8/Svneo	1.095	6 days	Effects on miR-146a expression: NO	Avissar-Whiting *et al.* (2010)
3A	10.95		Effects on miR-146a expression/survival after miR-146a overexpression: NO	
HTR-8/Svneo			Effects on miR-146a expression: NO	
3A	109.51		Effects on miR-146a and other miR expression: YES; altered survival after miR-146a overexpression: NO	
TCL-1			Effects on miR expression: NO	
HTR-8/Svneo			Effects on miR-146a and other miR expression: YES	

Table 3. (continued)

Cell Model	μM BPA[a]	Treatment Length (h)	Summary of Outcomes	Ref.
JEG-3	1×10^{-3}	24	Effects on proliferation: NO (also in BeWo cells)	Morice *et al.* (2011)
		48	Effects on viability or proliferation: NO (also in BeWo cells)	
	0.01	24	Effects on proliferation: NO (also in BeWo cells)	
		48	Effects on viability or proliferation: NO (also in BeWo cells)	
	0.1	1 or 6?	Effects on mRNA of cell cycle-related genes: SOME	
		8	Effects on mRNA of an apoptosis-related gene: YES	
		24	Effects on proliferation: YES (NO in BeWo)	
		48	Effect on viability: NO, apoptosis: YES, proliferation: YES (NO in BeWo)	
	1	1 or 6?	Effects on mRNA of cell cycle-related genes: SOME	
		8	Effects on mRNA of an apoptosis-related gene: YES	
		24	Effects on proliferation: YES (NO in BeWo)—potentially through Errγ	
		48	Effects on proliferation: YES (NO in BeWo), viability: NO, apoptosis: YES	
	10	24	Effects on proliferation: YES (NO in BeWo);	
		48	Effects on proliferation: YES (NO in BeWo), viability: NO	
	100	48	Effects on viability: YES	
JEG-3	1, 5	24	Effects on CRH mRNA/protein expression and promoter activity: NO, upstream proteins: SOME	Huang and Leung (2012)[b]
	25		Effects on CRH mRNA/protein expression and promoter activity: YES, upstream proteins: SOME	
	50		Effects on CRH mRNA/protein expression and promoter activity: YES, upstream proteins: YES	
1° Villi	5×10^{-4}	24	Effect on β-hCG in media with/without BPA-conditioned decidualized stromal cells (DSCs): NO;Effect on proinflammatory cytokine MIF: YES—not if co-cultured with BPA-conditioned DSCs	Mannelli *et al.* (2014)
		48	Effect on β-hCG in media with/without BPA-conditioned decidualized stromal cells (DSCs): NO	
BeWo	0.01	48	Effect on proliferation: NO, invasion into endometrial cells YES; invasion-related mRNA/prot: YES, DNA methylation-related mRNA/prot: SOME	Wang *et al.* (2015)
	0.1		Effect on proliferation: NO	
	1		Effect on proliferation: YES, invasion into endometrial cells: YES; invasion-related mRNA/prot: YES, DNA methylation-related mRNA/prot: SOME	
	10		Effect on proliferation: YES, invasion-related prot: YES, DNA methylation-related mRNA/prot: SOME	
	100		Effect on proliferation: NO, invasion into endometrial cells: YES, invasion-related mRNA/prot: YES, DNA methylation-related mRNA/prot: YES	
	1000		Effect on proliferation: YES	
1° Trophoblasts	0.438	24	Effects on 11B-HSD2 activity, mRNA, prot: NO	Rajakumar *et al.* (2015)
	1.095, 2.19, 4.38	24	Effects on 11B-HSD2 activity, mRNA, prot: YES	
	8.76	3	Effects on 11B-HSD2 activity, mRNA, prot: NO	
		6, 12	Effects on 11B-HSD2 activity, mRNA, prot: YES	
		24	Effects on 11B-HSD2 activity, mRNA, prot: YES Effects on nutrient transport- and hormone-related mRNA: YES	

Table 3. (continued)

Cell Model	μM BPA[a]	Treatment Length (h)	Summary of Outcomes	Ref.
HTR-8/Svneo	1×10^{-9}	24, 48	Effect on viability: NO, proliferation: NO, migration: NO, invasion: NO, DNA content: NO	Spagnoletti *et al.* (2015)
		72	Effect on viability: NO, proliferation: NO, migration: NO, invasion: NO, DNA content: YES	
	1×10^{-7}	24, 48	Effect on viability: NO, proliferation: NO, migration: NO, invasion: NO, DNA content: YES	
		72	Effect on viability: NO, proliferation: NO, migration: NO, invasion: YES, DNA content: YES	
	1×10^{-5}	24	Effect on viability: NO, proliferation: NO, migration: NO, invasion: NO, DNA content: NO, interaction w/ HUVECs: NO	
		48	Effect on viability: NO, proliferation: NO, migration: YES, invasion: YES, DNA content: YES (not reversed by ICI), interaction w/ HUVECs: NO	
		72	Effect on viability: NO, proliferation: NO, migration: YES, invasion: YES, DNA content: YES; differentiation: NO, cell cycle-related protein: NO	
	1×10^{-3}	24	Effect on viability: NO, proliferation: NO, migration: NO, invasion: NO, DNA content: NO	
		48	Effect on viability: NO, proliferation: NO, migration: YES, invasion: YES, DNA content: YES	
		72	Effect on viability: NO, proliferation: NO, migration: YES, invasion: YES, DNA content: YES; differentiation: YES, cell cycle-related protein: YES	
	0.1	24	Effect on viability: NO, proliferation: NO, migration: NO, invasion: NO, DNA content: NO	
		48	Effect on viability: NO, proliferation: NO, migration: YES, invasion: YES, DNA content: YES	
		72	Effect on viability: NO, proliferation: NO, migration: YES, invasion: YES, DNA content: YES; differentiation: YES, cell cycle-related protein: NO	
1° in first trimester	1×10^{-3}	24, 48	Effects on chemical efflux-related protein: NO	Sieppi *et al.* (2016)
1° at term		24	Effects on chemical efflux-related protein/mRNA: NO	
		48	Effects on chemical efflux-related protein: YES—partly reversed by ICI mRNA: NO	
1° in first trimester	0.1	24, 48	Effects on chemical efflux-related protein: NO	
1° at term		24	Effects on chemical efflux-related protein: NO; mRNA: YES	
		48	Effects on chemical efflux-related protein/mRNA: NO	
JEG-3	0.01–500	24	Effects on cytotoxicity at 138–218 μM: YES; ROS generation: YES; aromatase activity at IC50=71 μM: YES	Perez-Albaladejo *et al.* (2017)
HTR-8/Svneo	0.1	24	Effects on migration: NO	Lan *et al.* (2017)
	1, 10, 50		Effects on migration: YES	
	0.1	48	Effects on proliferation: NO, migration: YES; invasion-related proteins: NO; adhesion-related protein: YES (NO mRNA), mesenchymal proteins/mRNA: NO	
	1		Effects on proliferation: NO, migration: YES; invasion-related proteins: SOME; adhesion-related protein: YES (NO mRNA), mesenchymal proteins/mRNA: NO. Effects potentially through ERK signaling	
	10, 50		Effects on proliferation: NO, migration: YES; invasion-related proteins: YES; adhesion-related protein and mRNA: YES, mesenchymal proteins/mRNA: NO	
1° villi in first trimester	50	48, 72	Effects on migration: YES; adhesion-related protein: NO	

[a]Doses from studies were converted to μM to facilitate comparison across studies.
[b]Authors also reported increased binding of CRE at the *CRH* promoter and CREB phosphorylation by BPA, but quantified data were not presented to include in this dose-response table.

Table 4. Effects of Phthalates in Placental *In Vitro* Models

Cell Model	μM Phthalates	Phthalate	Treatment Length (h)	Outcomes	Ref.
HRP-1	25, 50, 100, 200	DEHP, MEHP, EHA	mRNA:4 Prot: 12	Effects on lipid-related mRNA/prot expression: SOME (also some differences by phthalate metabolite).	Xu *et al.* (2005)
	50	DEHP, MEHP, EHA	2, 4, 8, 12, 24	Effects on lipid-related mRNA/prot: SOME (also some differences by phthalate metabolite; effects on mRNA tended to precede protein response, without consistent time-specific patterns)	
			24	Effects on fatty acid distribution: SOMEEffects on transport of arachidonic acid: YES (MEHP, EHA), and docosahexaenoic acid: YES (DEHP)	
HRP-1	50	DEHP	24	Effects on free fatty acid: YES, cholesterol esters: NO, diacylglycerol: NO, triacylglycerol: YES, phosphatidylcholine: NO, phosphatidylethanolamine: YES, phosphatidylserine: YES, lyso-phosphatidylcholine: YES, sphingomyelin: NO, saturated fatty acids: YES, monounsaturated fatty acids: NO, polyunsaturated fatty acids: YES, ω3 fatty acids: SOME, ω6 fatty acids: SOME, ω7 fatty acids: NO, ω9 fatty acids: NO	Xu *et al.* (2006)
		MEHP		Effects on free fatty acids: YES, cholesterol esters: YES, diacylglycerol: YES, triacylglycerol: YES, phosphatidylcholine: NO, phosphatidylethanolamine: YES, phosphatidylserine: NO, lyso-phosphatidylcholine: YES, sphingomyelin: YES saturated fatty acids: YES, monounsaturated fatty acids: YES, polyunsaturated fatty acids: YES, ω3 fatty acids: SOME, ω6 fatty acids: SOME, ω7 fatty acids: YES, ω9 fatty acids: YES	
		EHA		Effects on free fatty acids: YES, cholesterol esters: YES, diacylglycerol: YES, triacylglycerol: YES, phosphatidylcholine: YES, phosphatidylethanolamine: YES, phosphatidylserine: NO, lyso-phosphatidylcholine: NO, sphingomyelin: NO, saturated fatty acids: YES, monounsaturated fatty acids: YES, polyunsaturated fatty acids: YES, ω3 fatty acids: YES, ω6 fatty acids: YES, ω7 fatty acids: YES, ω9 fatty acids: YES↑, ↑, –	
HTR-8/ Svneo	11.25	MEHP	1	Effect on ROS production: NO	Tetz *et al.* (2013)
	22.5		1	Effect on ROS production: NO	
			24	Effects on marker of apoptosis: NO, viability: NO	
			48	Effect on viability: NO	
	45		1	Effect on ROS production: YES	
			24	Effects on marker of apoptosis: NO, viability: NO	
			48	Effect on viability: NO	
	90		1	Effect on ROS production: YES	
			4, 8	Effect on *PTGS2* (*COX2*) mRNA: YES	
			24	Effects on marker of apoptosis: NO, viability: NO; *PTGS2* (*COX2*) mRNA: NO, other oxidative stress-related mRNA: NO	
			48	Effect on viability: NO	
	180		1	Effect on ROS production: YES	
			4, 8	Effect on *PTGS2* (*COX2*) mRNA: YES	
			24	Effects on marker of apoptosis: YES, viability: NO, *PTGS2* (*COX2*) mRNA: YES, other oxidative stress-related mRNA: SOME	
			48	Effect on viability: YES	

Table 4. (continued)

Cell Model	μM Phthalates	Phthalate	Treatment Length (h)	Outcomes	Ref.
HTR-8/ Svneo	10	MEHP	72	Effect on viability: NO	Meruvu *et al.* (2015)
	25		2, 4, 8	Effect on ROS production: YES	
			72	Effects on ROS production: NO, cytotoxicity: NO	
	50		2	Effect on ROS production: YES	
			4, 8	Effects on ROS production: YES, miR-16: NO	
			24	Effect on miR-16: NO	
			48	Effects on apoptosis: NO, miR-16: NO	
			72	Effects on viability: NO, cytotoxicity: NO, miR-16: NO, ROS production: NO	
	100		2	Effect on ROS production: YES	
			4	Effects on ROS production: YES, miR-16: YES; *BCL-2* mRNA: YES	
			8	Effects on ROS production: YES, miR-16: NO	
			24	Effects on miR-16: NO, *BCL-2* mRNA: NO	
			48	Effects on apoptosis: YES, miR-16: YES, *BCL-2* mRNA: NO	
			72	Effects on ROS production: NO, viability: NO, cytotoxicity: NO; miR-16 inhibition prevents MEHP-induced ↓BCL-2/BAX	
	180		2	Effect on ROS production: YES	
			4	Effects on ROS production: YES, miR-16: YES; *BCL-2* mRNA: YES	
			8	Effects on ROS production: YES, miR-16: YES	
			24	Effects on miR-16: YES; *BCL-2* mRNA: YES	
			48	Effectss on apoptosis: NO, miR-16: YES; *BCL-2* mRNA: YES	
			72	Effects on ROS production: YES, cytotoxicity: YES (180 and 360 uM); Effect on viability: YES; miR-16 inhibition prevents MEHP-induced ↓BCL-2/BAX	
	360		72	Effects on ROS production: YES, cytotoxicity: YES	
THP-1, 1° placental macrophages (PM)	10, 45	MEHP	24	Effects on PGE2 release (PM): NO, PGF2 release (PM): NO	Tetz *et al.* (2015)
	90		24	Effects on PGE2 release (PM): NO, PGF2 release (PM): YES	
	180		2, 4	Effect on PGE2 release (PM): NO	
			8	Effects on PGE2 release (PM): NO, COX1 prot (THP-1 and PM): NO, COX2 prot (THP-1 and PM): YES	
			24	Effects on PGE2 and PGF2 release (PM): YES; COX and COX-2 inhibition decreased MEHP-stimulated PGE2 release (THP-1 and PM)	
HTR-8/ Svneo	25	MEHP	24	Effects on viability: NO, ROS production: YES	Meruvu *et al.* (2016)
			48	Effect on viability: NO	
	50		4	Effects on miR expression: NO	
			24	Effects on viability: NO, ROS production: YES	
			48	Effects on viability: NO, miR expression: NO	
	100		4	Effects on miR expression: SOME, cell cycle- and oxidative stress-related mRNA: SOME	
			24	Effects on viability: NO, ROS production: YES, cell cycle- and oxidative stress-related mRNA: YES	
			48	Effects on viability: NO, miR expression: NO, cell cycle- and oxidative stress-related mRNA: YES	
	180		4	Effects on miR expression: YES, cell cycle- and oxidative stress-related mRNA: YES	
			24	Effects on viability: YES, ROS production: YES, cell cycle- and oxidative stress-related mRNA: YES	
			48	Effects on viability: YES, miR expression: YES, cell cycle- and oxidative stress-related mRNA: YES	

Table 4. (continued)

Cell Model	μM Phthalates	Phthalate	Treatment Length (h)	Outcomes	Ref.
1° cytotropho-blasts	1, 10, 50, 150, 300, 500	MEHP	24	Effect on cell survival: YES (300 and 500 μM)	Wang *et al.* (2016)
	1, 10, 50	MEHP	?	Effects on CRH and COX-2 expression: NO	
	100, 150	MEHP	24	Effects on CRH and COX-2 mRNA/prot: YES, related nuclear factor protein and localization at CRH and COX-2 promoters: YES, potentially through NIK nuclear factor	
JEG-3 microsomes	100	Di-phthalates	12	Effects on aromatase activity: by DCHP and BBOP: YES, by DMP, DEP, DPrP, DBP, DPP, DHP, DEHP, DNOP DINP: NO	Xu, *et al.* (2016)
				Effects on progesterone production: by DPrP, DBP, DPP, DCHP, BBOP, DHP (DEHP, DNOP, DINP with 8Br-cAMP stimulation): YES, by DMP, DEP, DEHP, DNOP, DINP: NO	
				Effects on estradiol production: by DCHP, BBOP: YES, by DPrP, DBP, DPP, DHP, DMP, DEP, DEHP, DNOP, DINP: NO	
		Mono-phthalates		Effects on aromatase activity: by MMP, MBP, MEHP: NO	
				Effects on progesterone production: by MBP, MEHP: YES, by MMP: NO	
				Effects on estradiol production: by MMP, MBP, MEHP: NO	
HTR-8/Svneo	1	MEHP	24	Effects on viability or necrosis: NO, invasion: NO, invasion-related protein/mRNA expression/activity: NO/NO/NO	Gao *et al.* (2017)
	10			Effects on viability or necrosis: NO, invasion: YES, invasion-related protein/mRNA expression/activity: YES/NO/NO	
	100			Effects on viability or necrosis: NO, invasion: YES, invasion-related protein/mRNA expression/activity: YES/NO/YES	
				PPAR-γ inhibitors reversed: MEHP-induced decrease in invasion and invasion-related protein activity.	
	200			Effects on viability or necrosis: NO, invasion: YES, invasion-related protein/mRNA expression/activity: YES/NO/YES	
JEG-3	0.01–500	DMP	24	Effects on cytotoxicity: NO, ROS production: NO, aromatase activity: YES (at $>$50 μM); treatment concentrations were above those in media after 24 h	Perez-Albaladejo *et al.* (2017)
		DBP		Effects on cytotoxicity: YES (at 466 μM), ROS production: NO, aromatase activity (YES: 104 μM); treatment concentrations were above those in media after 24 h	
		BBP		Effects on cytotoxicity NO, ROS production: NO, aromatase activity (YES: 167 μM); treatment concentrations were above those in media after 24 h	
		DEHP		Effects on cytotoxicity NO, ROS production: NO, aromatase activity: NO; treatment concentrations were above those in media after 24 h	

Prot, protein; DEHP, di(2-ethylhexyl)phthalate; EHA, 2-ethylhexanoic acid; MEHP, mono-(2-ethylhexyl) phthalate.

SV40-transformed placental cell lines showed that BPA altered 25 miRs in 3 A cells and 60 miRs in HTR-8 cells, without significant changes in TCL-1 cells (Avissar-Whiting *et al.*, 2010). Authors suggested that these contrasting responses to BPA are likely due to stage-specific differences between cell lines, as HTR-8 cells are derived from first trimester extravillous cells from the termination of a normal pregnancy and 3 A cells from first trimester villous cells, whereas TCL-1 cells come from third trimester villous cells. Although this is a possible explanation, the maintenance and growth of these cells often varies depending on the supplier and even from lab-to-lab, so additional systematic studies may be needed to confirm these findings in primary cell lines.

The mechanisms by which BPA targets the placenta are still largely unknown, but there is some evidence that different cells may uniquely interact with specific toxicants. The estrogen-related receptor gamma (ERRγ1), a receptor that binds BPA with high affinity, has been suggested to drive the preferential accumulation of BPA within the placenta (Takeda *et al.*, 2009). In one study, the baseline mRNA expression of *ERRγ1* was higher in JEG-3 and human isolated extravillous cytotrophoblasts than in BeWo cells. Importantly, the expression of *ERRγ1* was 20–25 and 50-fold higher in first and third trimester placentas, respectively, when compared with JEG-3 cells. Other ERRγ subtypes (*ERRγ2* and *ERRγ3*) were not expressed at all in JEG-3 or BeWo cells, but were expressed in first-trimester placentas. Furthermore, in JEG-3, but not BeWo cells, BPA concentration-dependently decreased a marker of DNA synthesis rate partly through ERRγ (Morice *et al.*, 2011). These studies in a variety of cell models suggest that, as with well-known cell models of cancer initiation, progression, and metastasis, future placental studies must select cell models based on their genetic and developmental characteristics. For example, immortalized cells from first trimester placentas (eg, HTR-8, BeWo, JEG-3) may be better suited for studies related to invasion and vascularization, whereas third trimester immortalized cells (eg, TCL-1) or primary cells from term placentas may be best when assessing effects on placental nutrient transfer or hormonal signaling.

BPA Concentration and Treatment Length

BPA's nonmonotonicity and mechanism-of-action require careful selection of both treatment concentrations and timing. For example, in human placental chorionic villi explants, BPA increased a proinflammatory cytokine associated with immune adaptations in pregnancy after 24 h of treatment, but the effect was absent at 48 h (Mannelli *et al.*, 2014). In primary cytotrophoblasts from term placentas of uncomplicated pregnancies treated with 0.0002 to 0.2 μg/ml BPA, apoptosis was induced by 0.02–200 μg/ml BPA, but not by the 2 lower concentrations, whereas necrosis and tumor necrosis factor α (*TNF-α*) mRNA and protein were induced by the lower BPA concentrations. Conversely, several higher concentrations of BPA had no effect on *TNF-α* mRNA, decreased TNF-α protein, and decreased necrosis (Benachour and Aris, 2009). In primary cultures of human placental trophoblasts, 11-β-dehydrogenase isozyme 2 (11β-HSD2) activity, protein content, and gene expression were also concentration-dependently increased by BPA (increased by 0.25–2.0 μg/ml but not 0.1 μg/ml BPA) (Rajakumar *et al.*, 2015). Another study in placental explants showed that in term but not first trimester placental villus cultures, 48-h treatment with 1 nM but not 100 nM BPA decreased the ABCG2 protein, which transports a variety of compounds in human placental syncytiotrophoblasts (Sieppi *et al.*, 2016), highlighting the importance of both chemical concentration and developmental timing.

Similarly, in immortalized cells, the mRNA and protein expression of CRH in JEG-3 cells was increased by 25 and 50 μM BPA, but not by 1 or 5 μM BPA (Huang *et al.*, 2012). In addition, in JEG-3 cells, BPA had both time- and concentration-dependent effects on aromatase activity, increasing activity at some concentrations and treatment windows and decreasing activity at others (Nativelle-Serpentini *et al.*, 2003). In BeWo cells, BPA increased cell proliferation at 1 and 10 μM, whereas other concentrations (0.01, 0.1, and 100 μM) had no impact on proliferation, and the highest concentration (1000 μM) decreased proliferation. These outcomes were accompanied by dose-specific effects on E-cadherin mRNA and protein, but relatively consistent effects across doses on invasion and markers of invasion (MMP-9, MMP-2, TIMP-1, and TIMP-2 protein) (Wang *et al.*, 2015). Similarly, in HTR-8/SVneo cells, BPA had both concentration- and time-specific effects on cell migration and concentration-specific effects on markers of invasion (MMP-9 and TIMP-3 protein) (Lan *et al.*, 2017). Taken together, these studies confirm the concentration- and time-dependent nature of BPA's effects on downstream targets and highlight the importance of selecting physiologically relevant concentrations and treatment lengths for investigating potential mechanisms of action of BPA using *in vitro* models. Specifically, many of these studies support the nonmonotonicity of BPA—where effects on some pathways were only observed at lower or higher concentrations, whereas other pathways were targeted by the lowest and highest, but not middle BPA concentrations. This suggests that while both low and high concentrations of BPA impact placental pathways, BPA's mechanisms of action may differ at the 2 ends of the dose-response curve. These data also reinforce the need for extensive dose-response curves that first: include the expected range of exposure in humans and second: allow for the interpretation of BPA concentration-specific mechanisms-of-action within the placenta.

Phthalate Concentration and Treatment Length

Numerous *in vitro* studies have assessed the effects of MEHP, the major DEHP metabolite, on placental cellular endpoints. For example, CRH and COX-2 regulate parturition in humans and have been linked with preterm birth, and in human placental cytotrophoblasts, only higher concentrations of MEHP (100 μM and 150 μM) increased the expression of these proteins (Wang *et al.*, 2016). In HTR-8/SVneo cells treated with a range of MEHP concentrations, only the highest concentration (180 μM) increased ROS production, oxidative DNA damage, and apoptosis, whereas both 90 and 180 μM concentrations induced the mRNA expression of prostaglandin-endoperoxide synthase 2 (*PTGS2/COX-2*) (Tetz *et al.*, 2013). In THP-1 cells (human monocyte cells derived from an infant with acute monocytic leukemia), this group later showed that only the highest concentration (180 μM, after 24 h of treatment, not 2–8 h) induced prostaglandin E2 release (Tetz *et al.*, 2015). A more recent study in HTR-8/SVneo cells showed that invasion was decreased and TIMP-1 protein increased by 10, 100, and 200 μM MEHP, but not by the lowest concentration (1 μM), whereas MMP-9 activity was only decreased at 100 and 200 μM MEHP (Gao *et al.*, 2017). In addition, in HTR-8/SVneo cells, 50, 100, or 180 μM MEHP induced ROS production, but the most pronounced effects were at the highest concentration and primarily after 72 h. In another study, 50 μM MEHP had no effect on miR-16 (which authors discussed is altered in pregnancy pathologies), whereas 100 μM MEHP increased miR-16 at 4 and 48 h, and 180 μM increased miR-16 at all timepoints (4, 8, 24, 48 h) (Meruvu *et al.*, 2015). The same group later showed that intracellular ROS production in HTR-8/SVneo

cells was increased by a range of MEHP concentrations, but the expression of several miRs was only increased at higher concentrations (100 or 180 μM), with the exception of miR-17-5p (Meruvu *et al.*, 2016). These concentrations correspond to 30 and 54 μg/ml MEHP (respectively), whereas pregnant women have been shown to have much lower median plasma concentrations of 0.68 ± 0.85 μg/ml (Latini *et al.*, 2003), and ranging from 0.17 to 6.74 μg/ml (Li *et al.*, 2013). These studies suggest that most MEHP concentrations affect transcription and translation, but that cellular phenotypic changes are typically only observed at higher MEHP concentrations. It is possible that the expected chronic low-dose MEHP exposure in humans can be modeled in cells with short-term treatment at higher concentrations. However, it is more likely that as with BPA, MEHP's mechanisms-of-action differ at the extremes of the concentration curve. Therefore, in order to understand the mechanisms by which MEHP affects the placenta, more studies are warranted assessing a variety of endpoints using broader and more human-relevant concentration curves.

As previously described, pregnant women are exposed daily to an array of phthalates. However, few cell studies have assessed outcomes in response to DEHP metabolites other than MEHP. However, 2 *in vitro* studies treated rat HRP-1 placental cells with DEHP or 2 of its metabolites, MEHP and 2-ethylhexanoic acid (EHA). DEHP and both metabolites had concentration- and time-specific effects on the expression of placental fatty acid uptake and metabolism-related genes. All 3 also increased the uptake rates of several essential fatty acids, whereas only MEHP and EHA increased arachidonic acid transport, and only DEHP increased DHA transport (Xu *et al.*, 2005). In a follow-up study in HRP-1 cells, DEHP, MEHP, and EHA also had unique effects on the overall contents of several lipid classes (Xu *et al.*, 2006).

Another current limitation in the field is that only 2 *in vitro* studies thus far have investigated phthalates other than DEHP and its metabolites. One assessed the structure-activity relationship of 100 μM of 11 diphthalates and 3 monophthalates on progesterone and estradiol production in JEG-3 cells. Estradiol production was only decreased by DCHP and BBOP, whereas 5 diphthalates decreased progesterone production, with no effects of monophthalates on either hormone (Table 4) (Xu *et al.*, 2016), suggesting that the ability of phthalates to inhibit aromatase and 3β-hydroxysteroid dehydrogenase 1 (3β-HSD1), and to decrease estradiol and progesterone production is highly dependent on their chemical structure. Another study treated JEG-3 cells with 0.01–500 μM DMP, DBP, BBP, and DEHP. None of the phthalates affected ROS production, but DMP increased aromatase activity at concentrations >50 μM, DBP and BBP decreased aromatase activity at IC50 = 104 and 167 μM (respectively), whereas DEHP had no effect on aromatase activity (Perez-Albaladejo *et al.*, 2017). Phthalate mixtures used in other experimental models have shown their distinct phenotypic effects compared with DEHP alone (Zhou *et al.*, 2017a,b). Such approaches in placental studies will be valuable for establishing these chemicals' mechanisms of action when present as complex mixtures, as they are in humans.

CONCLUSIONS AND FUTURE DIRECTIONS

Placental Actions of BPA and Phthalates

As summarized in Tables 1–4, studies assessing effects of BPA or phthalates on placental outcomes in experimental animal and cell models have focused on hormones, epigenetics, inflammation/oxidative stress, cellular damage, and nutrient transfer.

BPA and phthalates are known endocrine disruptors, and there is substantial evidence that BPA can disrupt placental hormones, their receptors, or regulatory enzymes, whereas studies related to the effects of phthalates on placental hormones are more limited. In addition to hormones, placental nutrient transfer capacity is the best-characterized measure of placental efficiency (Burton and Fowden, 2012). Therefore, more studies are warranted in all models to investigate the effects of BPA and phthalates on placental energy metabolism, and fetal nutrient supply.

Although there is great interest in the role of epigenetics in placental development, additional data are needed related to the ability of BPA and phthalates to disrupt placental epigenetic signaling in experimental models. Currently, evidence for the ability of BPA or phthalates to induce placental inflammation or oxidative stress comes primarily from cell models; given that oxidative stress and inflammation are proposed to mediate associations of BPA or phthalate exposure with pregnancy outcomes (Ferguson *et al.*, 2017; Veiga-Lopez *et al.*, 2015; Watkins *et al.*, 2015), more studies in animal and cell models are warranted to investigate these mechanisms. Finally, there is substantial evidence from experimental models that BPA and phthalates cause frank damage to placental vasculature, structure, and function. However, these findings should be substantiated in animal models using concentrations of both chemicals that are in line with human exposures.

Summary of Animal and Cell Models

Given the challenges in human placental sampling (**Figure 2**), various experimental models have been employed to address mechanisms of placental disruption by BPA and phthalates. However, these studies vary in their design and measured outcomes, and few accurately reflect human exposures to these chemical. Therefore, both animal and cell models would benefit from use of more extensive BPA/phthalate doses and concentrations (**Figure 2**). Furthermore, exposures in animal models should ideally begin prior to decidualization, and treatment in cell models should be timed appropriately, with more extensive time ranges. Assessing sex-specific outcomes in animal models is also critical, as is the selection of precisely applicable cell models. In the case of phthalates, future animal and cell studies should assess exposure to a wider variety of phthalates (beyond DEHP and its metabolites), and at more relevant doses, with the ultimate goal of modeling more complex exposures to the mixtures of phthalates that occur in humans. Finally, given broad outcome selection in animal and cell models, a useful approach moving forward would be to design mechanistic animal and cell studies that parallel placental endpoints altered in humans in response to BPA and phthalates. Such approaches will be indispensable in helping to unravel the mechanisms involved in placental toxicity and the placenta-mediated effects of EDCs on fetal development.

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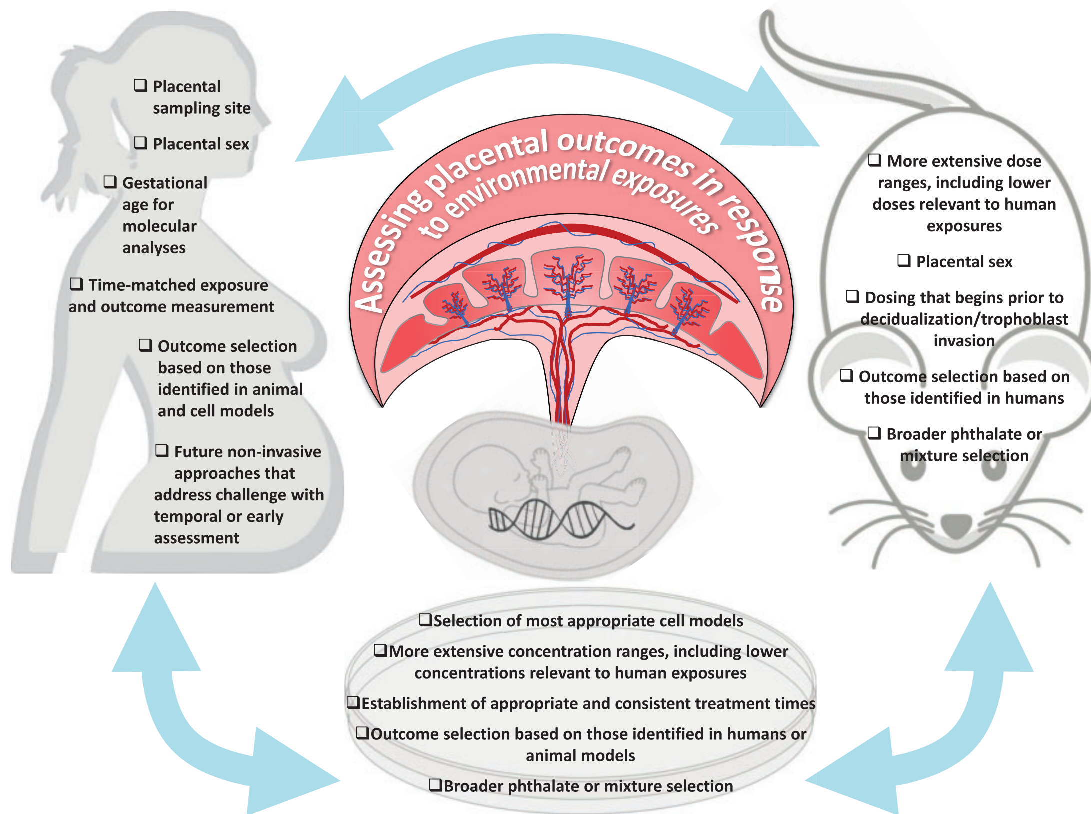

Figure 2. An integrated approach between human, animal, and cell studies will be needed to determine the precise effects of environmental exposures on the placenta. To accomplish this, as illustrated in the figure, numerous factors should be carefully considered when establishing animal and *in vitro* models, and using findings from animal or *in vitro* studies to inform questions in humans. Furthermore, these factors should be taken into account when drawing conclusions regarding mechanisms of action of BPA and phthalates from the currently available literature.

the grantee and do not necessarily represent the official views of the US EPA. Further, the US EPA does not endorse the purchase of any commercial products or services mentioned in the publication. The authors declare no financial conflict of interest.

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