Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Toxicol Appl Pharmacol. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

Toxicol Appl Pharmacol. 2019 September 01; 378: 114635. doi:10.1016/j.taap.2019.114635.

Developing novel *in vitro* methods for the risk assessment of developmental and placental toxicants in the environment

Rebecca C. Fry[a,b,c], **Jacqueline Bangma**[a], **John Szilagyi**[a], **Julia E. Rager**[a,b,c,*]

[a]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill

[b]The Institute for Environmental Health Solutions, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill

[c]Curriculum in Toxicology, The University of North Carolina at Chapel Hill

Abstract

During pregnancy, the placenta is critical for the regulation of maternal homeostasis and fetal growth and development. Exposures to environmental chemicals during pregnancy can be detrimental to the health of the placenta and therefore adversely impact maternal and fetal health. Though research on placental-derived developmental toxicity is expanding, testing is limited by the resources required for traditional test methods based on whole animal experimentation. Alternative strategies utilizing *in vitro* methods are well suited to contribute to more efficient screening of chemical toxicity and identification of biological mechanisms underlying toxicity outcomes. This review aims to summarize methods that can be used to evaluate toxicity resulting from exposures during the prenatal period, with a focus on newer *in vitro* methods centered on placental toxicity. The following key aspects are reviewed: (i) traditional test methods based on animal developmental toxicity testing, (ii) *in vitro* methods using monocultures and explant models, as well as more recently developed methods, including co-cultures, placenta-on-a-chip, and 3-dimensional (3D) cell models, (iii) endpoints that are commonly measured using *in vitro* designs, and (iv) the translation of *in vitro* methods into chemical evaluations and risk assessment applications. We conclude that findings from *in vitro* placental models can contribute to the screening of potentially hazardous chemicals, elucidation of chemical mechanism of action, incorporation into adverse outcome pathways, estimation of doses eliciting toxicity, derivation of extrapolation factors, and characterization of overall risk of adverse outcomes, representing key components of chemical regulation in the 21st century.

Keywords

Prenatal exposure; Developmental toxicity; Environmental chemicals; In vitro; Alternative methods; Pregnancy; Placenta; Risk assessment

*Corresponding Author: Julia E. Rager, jrager@unc.edu, Phone: 919-966-4410, 135 Dauer Drive, Chapel Hill, NC 27599.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1. INTRODUCTION

Exposures to environmental contaminants during pregnancy represent a growing concern worldwide. This gaining interest is a result of increasing evidence showing that the developing fetus is particularly vulnerable to prenatal exposure-induced toxicity (1–4). Because embryonic tissues and organs are differentiating and growing during prenatal periods, harmful effects can occur from prenatal exposures to certain chemicals at lower concentrations than those required to elicit effects in adults (2, 3). Studies in humans have identified potential relationships between prenatal developmental toxicity outcomes and exposure to high priority contaminants, including flame retardants, metals, phthalates, and perfluoroalkyl substances (PFAS), among others (5–9); and studies are continuing to follow-up and expand upon these findings (10, 11). Though research surrounding prenatal developmental toxicity is expanding, toxicity testing is limited by the resources required by traditional animal test methods. Alternative methods are therefore needed to more efficiently screen chemicals and also identify biological mechanisms underlying chemical-induced prenatal developmental toxicity.

The study of developmental toxicity encompasses adverse effects caused by exposure conditions during one of three periods: (i) prior to conception, (ii) during pregnancy, or (iii) during childhood (12). This review focuses on the testing of adverse effects caused by exposures during pregnancy (e.g., the prenatal) period. Studies evaluating prenatal toxicity are designed to include maternal exposures during periods of major organogenesis, providing information on potential changes on *in utero* survival, growth, and morphological development, including teratogenesis, resulting from exposure (12). Specific focus is placed on the placenta in the current review, because of its important role in prenatal exposure-induced toxicity and its promise for integration into alternative methods.

The placenta represents a critical tissue to consider in the evaluation of prenatal exposure-induced developmental toxicity. Broadly, the placenta is a structure that develops in the uterus during pregnancy, and transports oxygen and nutrients to the growing fetus, transports waste away from the fetal compartment, and secretes hormones that impact both mother and fetus (13). While the placenta can serve as a barrier for the fetus, certain exogenous substances (e.g., pharmaceuticals and environmental chemicals) can be transported across the placenta and therefore impact fetal health and development (13). Changes in cell signaling within the placenta can significantly impact fetal development. For example, fetal growth has been shown to be critically dependent on placental nutrient transport systems (14) and inflammatory cytokines (15, 16). Collectively, there is clear evidence supporting the role of the placenta in embryonic and fetal development, which can be adversely impacted by exposure to environmental toxicants.

This review summarizes toxicity testing methods that have been used to evaluate developmental toxicity resulting from exposures during the prenatal developmental period, with a focus on newer *in vitro* methods and translation into chemical evaluations. This review specifically addresses the following key aspects: (i) traditional methods based on animal developmental toxicity testing, (ii) *in vitro* methods to test placental toxicity, including emerging *in vitro* test methods, (iii) commonly measured endpoints used to

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

evaluate chemical toxicity and mechanism of action through these *in vitro* models, and (iv) the potential incorporation of these alternative placental models into chemical evaluations, with specific examples of how *in vitro* placental models may be integrated into human health risk assessments. This review therefore provides an overview of the state-of-the-science on *in vitro* placental models and suggests strategies that researchers and regulators can implement to more efficiently conduct chemical toxicity evaluations.

2. HISTORICAL ANIMAL TEST METHODS FOR PRENATAL DEVELOPMENTAL TOXICITY

Standard approaches to evaluating chemical safety have historically been based on animal testing to inform potential toxicity in humans. In general, the use of standard animal testing for chemical evaluations costs approximately $14 billion US dollars annually world-wide, requiring the expenditure of over 100 million experimental animals (17). In relation to developmental toxicity tests, a minimum of ~100 females are required per test to meet current government testing guidelines (12). These extensive costs and animal life requirements, coupled with animal welfare concerns, make it impossible to use animal models to screen for potential developmental toxicity across the hundreds of thousands of chemicals that are currently estimated to be present in the environment (18). A further limitation of using whole animal testing is that animal physiology differs from humans, and for reproductive tests there are notable differences in placental structure and function, decidualization and implantation, and *in utero* development of organs (19, 20). These limitations will be further discussed in section 2.4. Despite these shortcomings, animal testing still remains the default test method when evaluating the impact of chemical exposures on prenatal developmental toxicity.

2.1 Animal model descriptions

Animal models have historically been used to test developmental toxicity resulting from *in utero* exposures. Standard testing for prenatal developmental toxicity typically involves the use of two species of pregnant laboratory animals, commonly female mice, rats, and rabbits (21). Additional species including guinea pig, sheep and non-human primates can provide pertinent data on processes involved in placentation (22); however, these species are not widely utilized for developmental toxicity testing. For example, non-human primates placentation recapitulates human placentation relatively well, but are not often included in developmental toxicity testing due to ethical concerns and high costs (22).

During developmental toxicity testing animals are exposed to the chemical of interest throughout the period of major organogenesis, typically starting at gestation day (gd) 6 to the day prior to parturition (typically gd 20 in rats and 29 in rabbits) (21). Maternal status is evaluated during pregnancy and gross pathology is examined at the study termination, just prior to maternal term. At the same time, fetuses are harvested from the pregnant uterus and examined (21).

Several guidelines have been developed to promote consistent test methods across prenatal developmental toxicity studies. Guidelines include the U.S. Environmental Protection

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Agency (US EPA) Office of Pesticide Programs (OPP) 83-3 Pesticide Assessment Guidelines (23), the more recent US EPA Office of Pollution Prevention and Toxics (OPPTS) 870.3700 Health Effects Test Guidelines (24), and the Organization for Economic Co-operation and Development (OECD) Guideline 414 for prenatal developmental toxicity testing (25).

2.2 Animal model endpoints

During animal-based prenatal developmental toxicity studies, potential changes are evaluated both in pregnant females and developing fetuses. Maternal outcomes are evaluated during the pregnancy period up until study termination, just prior to maternal term, and include the following: body weights, clinical observations, and organ weights (e.g., uterine, liver) (12, 21). Fetal outcomes include the following: fetal weight; fetal growth retardation; external, visceral, and skeletal variations and abnormalities; and intrauterine death. The number of implantations and resorptions are also recorded (12, 21). These toxicological outcomes can then be used when deriving reference doses (RfDs) and reference concentrations (RiCs) and influence the eventual government regulation of chemical exposures (21).

Animal models have been used to evaluate developmental toxicity resulting from exposures during pregnancy across hundreds of chemicals, to date. An important database that can be used to query for and identify previously reported prenatal developmental toxicity endpoints is the US EPA's Toxicity Reference Database (ToxRefDB). ToxRefDB represents a large-scale publicly available database that captures over 30 years and $2 billion of standard animal testing results, including prenatal study results (26, 27). Pertinent to this review, the current version of ToxRefDB (vl) contains prenatal developmental toxicity results in multiple species (mostly rats and rabbits) across 672 chemicals, representing a robust resource that can be leveraged to better understand chemical-disease relationships. It is worth noting that this database is currently being updated to include expanded dose-response information and better delineation between reported outcomes that were "negative" vs. "not tested", among other updates (28). Future studies could therefore leverage this robust resource in conjunction with *in vitro* methods to inform predictive modeling approaches for prenatal developmental toxicity associated with chemicals that are lacking *in vivo* data.

2.3 Animal model strengths for the assessment of placental toxicity

As mentioned in the above text, certain animal models have unique benefits making them suitable for prenatal developmental toxicity assessments. For example, the advantages of mice and rodent models are their small size and short generation times allowing for quicker assessment of *in vivo* developmental toxicity compared to other models like non-human primates. Some animal species including guinea pig, sheep, and non-human primates have placentation processes that parallel those in humans (22). More in depth descriptions of the benefits of *in vivo* models for evaluating placental toxicity can be found in a 2016 review by Grigsby (22)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2.4 Animal model limitations for the assessment of placental toxicity

Although animal models have contributed to the current understanding of prenatal exposure-induced effects on placental and developmental toxicity outcomes, there are limitations when using these models. As previously mentioned, the time, cost, and animal lives required for whole animal testing is substantial and significantly inhibits the utility of these models to evaluate potential toxicity across large chemical domains. Furthermore, as described here and throughout this review, there are important differences between animal and human physiology, including those relevant to organismal development, placental structure and function, and placental traits at the molecular-level (19, 20, 29).

Of specific relevance to the placenta, rodent models notably have placentas that are hemochorial, in which maternal blood directly contacts the outermost membrane of the developing embryo, similar to humans (29). This similarity allows rodent toxicity responses to overlap with those observed in humans for certain applications. However, important differences exist between placenta formation in rodents vs. human. After pregnancy occurs and the placenta begins forming, the human placenta invades into the inner third of the myometrium; while invasion of the rodent placenta is restricted to the decidua (29). Because human placentas maintain a more intimate contact with the maternal circulation, chemicals can more easily transport from maternal circulation to the placenta. This structure can cause human placentas to have increased sensitivity to toxicant effects in comparison to rodent models (29). Unlike humans, rodents also develop a secondary, choriovitelline placenta, essentially an inverted yolk sac, which can provide additional protection against the placental passage of toxicants in rodent models. Important histological differences also exist between human and rodent placentas. For instance, the villous space at the maternal-fetal interface is far more open in humans than rodents, in which the villous space presents as a labyrinth of interconnected cavities. Additionally, mouse placentas also feature a zone of trophoblast giant cells bordering the maternal decidua basalis, unlike human placenta. Therefore, the use of rodent models to evaluate prenatal exposure-induced toxicity can often underestimate chemical potency (29).

Further differences exist between placentas in humans vs. animal models at the molecular-level. For example, there are certain genetic and epigenetic traits that are specific to human placentas. These traits include certain haplotypes (e.g., Killer-cell immunoglobulin-like receptor B (*KIR B*) haplotype), the expression of certain genes (e.g., sialic acid binding Ig like lectin 6 (*SIGLEC6*)), and the expression of certain miRNAs (e.g., hsa-mir-941) (29). Features at the protein-level can also be specific to the human placenta. For example, a human-specific protein isoform, the splice variant of immortalization-upregulated protein-2 (IMUP-2), has been identified as a critical responder to hypoxic conditions in placental cells and associated with preeclampsia in humans (30). These species-dependent differences support the utility of *in vitro* methods that are based on human placental models in chemical safety evaluations to protect human health outcomes.

3. IN VITRO TEST METHODS FOR PLACENTAL TOXICITY

There are several different *in vitro* approaches currently available to evaluate exposure-induced toxicity in the human placenta. These *in vitro* methods include placental trophoblast

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2-dimensional (2D) monocultures and placental explant models. More recently developed test methods include placental cell co-cultures, placenta-on-a-chip, and 3-dimensional (3D) placental cell models. This section reviews these *in vitro* models, providing a brief summary of methods that are currently available to evaluate the effects of toxicants on the human placenta *in vitro.*

3.1 Placental trophoblast monoculture models

The human placenta is comprised of various cell types including trophoblasts, stromal, and epithelial cells, all of which can be grown and tested through *in vitro* models. A wide range of methods exists for *in vitro* testing to determine the effects of chemical exposures on the placenta, with previously published reviews describing individual placental cell lines and their characteristics in detail (31, 32). For example, King and colleagues described all immortalized trophoblast cell lines published before 2000 (31), while Sullivan and colleagues characterized hormone secretion in-depth for a few primary lines as well as a number of the more heavily studied immortalized cells lines (32). The current review focuses on commonly used *in vitro* methods that incorporate trophoblastic cells as they are unique to the placenta and are vital to fetal processes, including implantation, uterine artery remodeling, and other biological and structural necessities for placental function and fetal health (33).

Placental cells that are commonly used for *in vitro* testing consist of cell lines derived from malignant tissues, primary cell lines that have been transformed, and primary cells from donors. For example, a human choriocarcinoma tumor isolated in 1968 lead to the generation of several immortalized cell lines that are now commonly used for placental toxicity testing including BeWo, JEG, JAR, and ACIM-32 cells (34). The BeWo cell line was originally established from primary isolated human choriocarcinoma tumor using decidual explants, and these cells represent the first trophoblasts to be maintained in continuous culture (35). Isolated BeWo cells in culture retain the capability to syncytialize (i.e., form syncytiotrophoblasts from the fusion of two or more cytotrophoblasts), and secrete hormones including estradiol, estrone, progesterone, human placental lactogen (hPL), and human chorionic gonadotropin (hCG) (34). JEG cells represent six cell lines, including the common JEG-3 cell line, that were also established using decidual explants and include cells that are similarly capable of secreting hormones (36). JEG-3 cells have also been used to evaluate placental cell responses to environmental chemicals (37–40). However, in contrast to BeWo cells, JEG and JAR cell lines do not syncytialize in culture (34). The ACIM-32 cell line, while created from the same original choriocarcinoma as BeWo, JEG, and JAR cells, are more removed, as ACIM lines were established by the integration of JEG and primary trophoblast cells (41).

Additional placental cell lines have been developed from primary cell lines transformed by recombinant viral vector transduction. For example, first trimester extravillous explants infected with simian virus 40 large T antigen (SV40) or the human papilloma virus (HPV) gave rise to the HTR-8/ SVneo (42, 43) and Tev-1 (44) cell lines, respectively. The HTR-8/ SVneo and TEV-1 cell lines were established to recapitulate invasive extravillous trophoblasts (44). The HTR-8/ SVneo cell line, however, is heterogeneous in terms of cell

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

population and include cells expressing villous cytotrophoblast, syncytiotrophoblast, as well as extravillous trophoblast markers (45). Other frequently employed lines include the Swan-71 cell line, derived through the immortalization of cytotrophoblasts (CTB) using purified first trimester trophoblasts and human telomerase reverse transcriptase (46). Another commonly used cell line, the TCL-1 line, was derived from choriodecidua transfected with SV40 (47).

Primary trophoblast cells are isolated directly from digestion of placenta tissue and are a commonly employed to assess chemical effects on both early and late term placenta. Early term placentas are acquired from elective termination of early stage pregnancies (48), and late term placentas are collected after full term delivery (49). The primary cells isolated from early and late term placenta offer relevant information on signaling in the placenta without the cellular changes that can sometimes result from immortalization. Example changes which have been identified in immortalized vs. primary cells include potential changes in CpG island-associated DNA methylation levels (50), up-regulation of cell cycle-associated Sanctions, and down-regulation of drug metabolizing enzymes (51), to name a few. However, a major drawback of using these primary placental cells is that they are unable to survive continued serial passages and fresh, isolated cells must be re-acquired on a regular basis.

3.2 Placental explant models

In addition to monoculture methods, *ex vivo* culture models of placental explants can be employed to investigate *in vitro* effects of chemicals on the placenta. Explant models represent cells/tissues that are originally obtained from living organisms, which then can be cultured using *in vitro* methods to evaluate tissue-level effects. Human placental explants are prepared using donated fresh placenta tissue by first removing the surrounding peripheral tissues, including the basal plate and associated boundary tissue (i.e., decidua basalis). The resulting villous placenta tissues are then cut, washed, and trimmed into individual 5-10 mm^3 pieces which are placed in cell culture media. These explants can then be maintained under standard cell culture conditions for up to 11 days, during which the *in vitro* effects of any toxicant of interest can be examined (52).

Explants are prepared from whole, native placenta, allowing these models to parallel more of the features present in the placenta in comparison to traditional trophoblast monocultures. For example, explants contain multiple cell types beyond trophoblasts that are present in the placenta and can influence toxicant response, including fetal endothelial cells, fibroblasts, and Hoffbauer cells (placental macrophages) (53). Further, explants maintain the native structure and extracellular matrix of the human placenta that are difficult to recapitulate using existing *in vitro* methods.

Explant models are also uniquely fitted to evaluate the potential impact of inter-individual differences such as maternal ethnicity, fetal gender, or disease state. For instance, isolated explants from preeclamptic placentas have been used to study the efficacy and potential toxicity of pravastatin, which is currently in clinical trial as a novel treatment for preeclampsia (54–56). Moreover, explants can be isolated from first or second trimester or term placentas to investigate how gestational age impacts sensitivity to a toxicant. For

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

example, Sieppi et al. (57) demonstrated that the xenoestrogens bisphenol A (BPA) and p-nonylphenol down-regulate the fetoprotective efflux transporter breast cancer resistance protein (BCRP) in term explants but not first trimester explants. BCRP is a key protein in placental signaling that affects the pharmacokinetics of several xenobiotics (58, 59), and paired with the aforementioned finding, these data highlight that exposure-induced placental toxicity in humans may vary depending on pregnancy stage. Taken together, placental explants provide a useful model of the human placenta in terms of cell types and macrostructure and provide the opportunity to study a range of covariates in toxicological studies.

3.3 Other emerging in vitro placental models

Organ-on-a-chip and other 3D culture methods such as organoids and paper stacks represent emerging *in vitro* alternatives that aim to recapitulate *in vivo* systems using existing cell lines. Some emerging alternative models, including organ-on-a-chip, use microfluidics-based approaches to create simulated blood flow. Other models such as organoids and 3D paper stacks are used to evaluate cells that grow in co-cultures with multiple cell types, paralleling the multiple types of cells present in an *in vivo* system.

3.3.1. Placenta-on-a-chip—Organ-on-a-chip technologies consist of differentiated stem cells or immortalized cell lines that are cultured on a flexible polymer scaffold designed to more accurately recapitulate conditions *in vivo* through the use of microfluidics, which simulate blood flow. Placenta-on-a-chip models have been designed to include trophoblasts cell lines which line a membrane that separates two microfluidics compartments that represent (1) the apical/maternal, and (2) the basolateral/fetal compartments (60, 61). This design provides a robust model to examine bi-directional nutrient and drug translocation and has been applied in the study of placental permeability to TiO_2 nanoparticles and glyburide (62, 63). In some cases, the lower compartment membrane is lined with endothelial cells to mimic the tissues which exogenous substances travel through in the placenta, through both syncytiotrophoblasts and fetal endothelial cells (61). These methods are more expensive and time-consuming in comparison to traditional tissue culture methods and require the use of specific instrumentation in their engineering and implementation.

3.3.2. 3D Spheroid and Organoid Cultures—3D culture models consist of single cell type or multiple cell types grown and tested in static conditions, rather than conditions that reply on microfluidics. Often single culture models are termed spheroids while co-culture model are referred to as organoids, though this terminology is notably in flux. While these models may not fully recapitulate the conditions of a mammalian circulatory system, there is considerable utility in using these models to study a variety of specific endpoints in a more cost-effective manner than placenta-on-a-chip methodologies. Organoid/spheroid cultures, in which cells are injected into a matrix to grow in 3D rather than monolayer, have been incorporated in placental toxicity research (64–66). Organoids have also been employed to study implantation and fetal-placental crosstalk (67, 68). There are, however, some disadvantages to using placental organoids in toxicological studies. For example,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

within *in vivo* systems, cells on the outside of the placental villi syncytialize (i.e., form syncytiotrophoblasts) while cells on the inside of the placental spheroid syncytialize (68).

3.3.3. 3D Paper Stacks—Another emerging 3D culture technique incorporates 3D paper stacks (69). These paper stacks allow for the 3D organization of either monocultures or co-cultures with a gradient through paper stacks that engineer diffusion-dominated environments similar to those found in spheroids or solid tumors (70, 71). To our knowledge, there are no published examples integrating trophoblast cells into 3D paper stacks, to date, though this technology is easily translatable to placental cell endpoints. For example, 3D paper stacks have been designed to measure cell invasion (71) and effectiveness of chemotherapeutics (72); though this technology is easily amenable to address a wide range of applications. For example, paper-based 3D cultures, in which cells are seeded in monolayer and invade through stacks of matrigel toward a stimulus, provide a cost-effective platform to assess cellular invasion and isolate invasive populations for analysis (73–75). This 3D technique could be particularly useful in assessing how toxicants impact cytotrophoblast to extravillous trophoblast differentiation and invasion. Unlike many placenta-on-a-chip platforms, these 3D paper stacks are easily assembled from equipment typically found in a lab equipped to maintain 2D cultures (69) making them more affordable and accessible than many organ-on-a-chip technologies. Together, these emerging *in vitro* methods are rapidly evolving and represent promising avenues to evaluate placental biology and responses to toxicant exposures.

3.4 Limitations of in vitro approaches

In vitro placental models have both advantages and limitations. Specific limitations include those mentioned in the above examples, including variable cellular differentiation capabilities, potential differences in biology inherent in primary vs. immortalized cell lines, and difficulties surrounding cell survival through serial passages in primary cells. More generally, *in vitro* placental models are limited in their ability to fully address impacts of chemical metabolism (76). It is also difficult to consider effects in other maternal tissues that may influence the placenta through an indirect mechanism (76). Together, it is important to consider these potential limitations when interpreting findings from *in vitro* models and placing data in the context of risk assessment.

4. IN VITRO MODEL ENDPOINTS USED TO TEST PLACENTAL TOXICITY

A variety of toxicological endpoints can be evaluated using *in vitro* test methods and used to assess chemical-induced toxicity. Some common endpoints found in the literature are discussed here and include cell death and proliferation, cell invasion, DNA damage, gene transcription, immunomodulation, nutrient uptake and transport, oxidative stress, protein expression and secretion, and epigenetic reprogramming (Figure 1). The evaluation of these endpoints is important in the context of environmental chemicals and can be used to enhance the understanding of toxicity within the placenta with relevance to prenatal exposure conditions. As this field of research continues to grow, the understanding of relationships between these more mechanistically-driven *in vitro* measures and apical endpoints observed

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in vivo will increase and further drive the accuracy of incorporating *in vitro* findings into overall chemical safety assessments.

4.1 Cell death and proliferation

The balance of cell proliferation, differentiation, and apoptosis is critical to the formation and maintenance of the placenta. The placenta must increase in size throughout pregnancy to meet the metabolic demand of the developing fetus, highlighting the need for constant cytotrophoblast cell division (33). Changes in cell death and proliferation caused by environmental chemicals are important to investigate in placental cell models, and can provide critical information towards the identification of toxicant-induced effects. For instance, the xenoestrogens BPA and p-nitrophenol have been shown to increase caspase 3 cleavage in placenta explants, indicating an increase in apoptosis associated with exposure (77, 78). This suggests trophoblasts are highly susceptible to these environmental contaminants early in development. As an example of exposure-induced changes in proliferation, the insecticide, chlorpyrifos, has been shown to decrease cell proliferation in placental explants and BeWo cells, identified through decreased immunohistochemistry staining of the mitotic protein marker, Ki67 (79). Explant models have been used to examine certain macroscopic hallmarks of trophoblast death, such as compromised villous integrity, syncytial sloughing, and fibrinoid necrosis (52). Decreased cell proliferation in the placenta could lead to or exacerbate placental diseases such as preeclampsia (76). Changes in cellular viability and proliferation can therefore induce changes in placental health and adversely impact fetal development.

4.2 Cell invasion

Invasion is a process that is carefully balanced; over-invasion and under-invasion of trophoblasts in the placenta are associated with placenta accrete and preeclampsia, respectively (80). Cellular invasion can be assessed using either immortalized cell lines or first trimester placental explants. Explants are cultured on an extracellular matrix scaffold, such as matrigel or collagen I, on a transwell insert, and invading cells can be counted in the receiving compartment (52). Similar methods can be used to evaluate invading cells using cell lines cultured on transwell inserts. The process of invasion involves molecular events similar to epithelial-mesenchymal transition, a process that is sensitive to interference by toxicants, and typically associated with cancer cell metastasis (81). Invasion can be indirectly analyzed using common markers of epithelial-mesenchymal transition such as e-cadherin and fibronectin (82).

4.3 DNA Damage

DNA damage represents a common molecular event probed for when evaluating effects of chemical exposures using *in vitro* models. When unrepaired, DNA damage can lead to mutations and genome instability, representing hallmark events underlying carcinogenesis (83). DNA damage can we evaluated for using several methodologies, including the comet, gamma H2A histone family member X (γH2AX), micronucleus, and terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assays, to name a few. DNA damage has been evaluated in placental *in vitro* models through the use of several genotoxicity-relevant assays, including use of the comet assay to evaluate genotoxicity

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and a polychlorinated dibenzo-p-dioxin/polychlorinated dibenzo-P-furan (PCDD/PCDF) mixture in JEG-3 cells (37).

4.4 Gene transcription

Changes in gene transcription (i.e., expression) also represent a commonly investigated endpoint in *in vitro* studies. If a chemical exposure changes the expression of an important gene and/or gene set, this can cause the modification of expression for the encoded protein(s), potentially resulting in functional consequences, including altered cell function and overall cell health. Gene-specific methods based on real-time quantitative PCR (QPCR) remain common to investigate changes in gene transcription. For example, this method has been used to show that acetaminophen reduces *BCRP* mRNA expression in BeWo treated cells (84), while opiate maintenance therapy drugs increased *BCRP* expression in both BeWo and JEG3 cells (85). BCRP plays a key role in maintaining the barrier function of the placenta; therefore, decreasing *BCRP* expression may hinder the placenta's ability to protect the developing fetus from potentially harmful chemicals circulating in maternal blood.

Genome-wide platforms can also be applied through high-throughput technologies such as cDNA microarrays and RNA sequencing. These techniques have already been incorporated into epidemiological investigations, for instance, in the evaluation of a human cohort exposed to varying levels of cadmium and selenium. This study found exposure-induced disruptions in placental gene expression associated with intra uterine growth restriction and newborns small for gestational age (86). An interesting research gap that warrants further investigation is understanding the extent to which *in vitro*-derived gene expression changes in the placenta compare to those observed in humans resulting from environmental exposures. Addressing this gap would better define the potential utility of using *in vitro* placental models to identify transcriptomic alterations involved in human placental toxicity.

4.5 Immunomodulation

Changes in immune cell signaling can be evaluated using monoculture systems, containing cells that may signal for immune responses to toxicological insults. Placental explants are also uniquely suited to examine immunomodulation, as these systems contain immune cells. For example, placenta explant models have been used to examine the immunomodulatory effects of toxicants by also co-treating cells with bacteria to assess the ability of the placenta to fight infections. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been evaluated in this context, wherein placenta extracts were co-treated with TCDD and *Escherichia coli* (87). This study found that TCDD increased the expression and secretion of important cytokines involved in immune cell activation, including cytochrome c oxidase subunit II (COX2) and tumor necrosis factor alpha (TNFα), and reduced the production of the anti-inflammatory cytokine, interleukin 10 (IL10) compared to treatment with *Escherichia coli* alone (87). These findings demonstrate that environmental contaminants have the potential to influence immune cell activation in response to infectious stimuli within the placenta, which can impact overall immune cell balance and function of the placenta.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

4.6 Nutrient uptake and transport

Changes in nutrient uptake and transport within the placenta can heavily influence the health of the developing fetus (88). The transfer of nutrients and waste between the maternal and fetal circulation occurs by active transport through syncytiotrophoblasts, which are fused, multinucleated cells that fortify the chorionic villi, control nutrient and waste transport between the maternal and fetal circulation, and synthesize and secrete various hormones in the placenta (89, 90). The transfer of nutrients and waste between the maternal and fetal circulation is regulated in syncytiotrophoblasts by active transport through a system of uptake and efflux transporters (91).

Toxicant-induced changes in transporter expression or function are potentially harmful to fetal development and are therefore important to evaluate in placenta *in vitro* models. Nutrient uptake in placental explants have been measured using radiolabeled substrates such as glucose, amino acids, and fatty acids (92–94). An example study demonstrated that ethanol and its metabolite, acetaldehyde, decreased the uptake of ^{14}C-methylaminoisobutyric acid and ^{3}H-taurine, respectively, in BeWo cells and first trimester explants (93). Reducing placental amino acid transport can negatively impact fetal development, and this study demonstrates a potential mechanism for fetal alcohol syndrome, a common developmental disorder that results from maternal alcohol consumption during pregnancy (95). *In vitro* techniques measuring molecular transport are therefore useful in understanding toxicant-induced changes in direct measures of placental function.

4.7 Oxidative stress

Oxidative stress is important to evaluate in the content of placental toxicity, as changes in hypoxia, reoxygenation, and subsequent oxidative stress have been implicated in adverse pregnancy outcomes including preeclampsia (96). *In vitro* placenta assays can measure exposure-induced stress by focusing on oxidative stress signals, including the expression of proteins involved in oxidative stress signaling (e.g., nuclear factor, erythroid 2 like 2 (Nrf2) and its target heme oxygenase-1 (HO)) as well as changes in the levels of reactive oxygen species (e.g., hydrogen peroxide) (97–99). An example study using placental explants found that cadmium's effect on the production of 6-keto-prostaglandin F1, a prostacyclin breakdown product, was diminished when explants were co-treated with the antioxidant, glutathione (100). These data demonstrated the role of oxidative stress in cadmium-induced toxicity (100). Similar studies could be used to further elucidate mechanisms of exposure-induced toxicity involving oxidative stress using *in vitro* models.

4.8 Protein expression and secretion

Changes in protein expression and protein excretion in response to toxicant exposure are additional endpoints of interest used in many *in vitro* studies. For example, protein expression changes have been evaluated *in vitro* to identify acetaminophen and opiate maintenance therapy drugs as modifiers of BCRP protein expression in BeWo and JEG-3 cells (84, 85). Similar to the aforementioned changes in placental *BCRP* mRNA levels, changes in BCRP protein levels can reduce the ability of the placenta to serve as a protective barrier between mother and fetus (101, 102). Changes in hormone secretion are also important to evaluate in the placenta. During pregnancy, the placenta acts as an endocrine

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

organ involved in the regulation of maternal and fetal hormone homeostasis (33). Specific hormones produced by the placenta include estrogen, progesterone, hPL, and hCG (33). Alterations in hormone secretion have been evaluated using placental *in vitro* models. For example, a study evaluating the molecular effects of BP A found that exposure reduced protein secretion of hCG in BeWo cells using immunoenzymometric assays (103). Alterations of this hormone were hypothesized to potentially increase apoptotic activity in early trimester trophoblasts with potential adverse effects on placental development. These examples showcase the utility of evaluating *in vitro* protein changes within placental cell models to better understand chemical-induced toxicity.

4.9 Epigenetic modifications

The field of epigenetics consists of molecular mechanisms that regulate gene expression profiles in a manner that does not involve changes to the underlying DNA sequence (104) and represents a field that has grown significantly in recent decades (105). Because epigenetic modifications have the potential to persist through cell division and become heritable, these changes are vital towards understanding the full extent of a toxicant's lasting effects on fetal development (106). Epigenetic mechanisms, including but limited to histone modification, DNA methylation, and microRNA (miRNA) expression (104), have yet to be fully elucidated in relation to placental-derived toxicity.

An example study evaluating chemical-induced changes in epigenetic profiles used three cell lines representing various tissue in the placenta and time points during pregnancy to evaluate the effects of BPA exposure (107). Several miRNAs were identified at significantly altered expression levels in response to BPA treatment leading to slower proliferation and higher sensitivity to DNA damage (107). In addition, JEG-3 cells exposed to cadmium have shown alterations in miRNA levels that regulate the transforming growth factor (TGF)-β pathway (38, 39). The epigenetic effects of iAs in JEG-3 cells have also recently been investigated (40). This research found that iAs exposure altered the expression levels of genes involved in glucocorticoid receptor (GR) signaling, with expression identified as associated with DNA methylation patterns, supporting an epigenetic mechanism underlying these pathway alterations (40). Generally, there are a limited number of studies examining toxicant effects on epigenetic mechanisms in the placenta, representing a critical research gap that can be addressed, in part, using *in vitro* models.

5. INCORPORATING IN VITRO PLACENTAL MODELS INTO CHEMICAL EVALUATIONS AND RISK ASSESSMENT

Placental models and their related endpoints show promise for incorporation into chemical evaluations and risk assessment. Specific steps in the human health risk assessment process include hazard identification, dose-response assessment, exposure assessment, and risk characterization (108). Here we propose that *in vitro* placental models can be incorporated into each of these steps (Figure 2), specifically through the following strategies: (i) screening of potentially hazardous chemicals (hazard identification step); (ii) elucidation of chemical mechanism of action and incorporation into adverse outcome pathways (AOPs) (hazard identification step); (iii) estimation of doses eliciting toxicity (dose-response and exposure

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

assessment steps); and (iv) characterization of the overall risk, including data-driven extrapolation factors to decrease overall uncertainty (risk characterization step). The implementation of these strategies has the potential to reduce reliance upon animal testing and increase confidence and efficiency in chemical toxicity assessments.

5.1 Use of in vitro models to identify hazardous chemicals

The potential for a chemical to induce developmental and placental toxicity can be identified through *in vitro* screening of large numbers of chemicals. The screening for potentially hazardous chemicals through *in vitro* techniques is advantageous as it represents a cost- and time-efficient strategy that can be used to prioritize which chemicals in the environment should be prioritized for further testing. This efficiency is particularly useful when one considers the vast numbers of chemicals that humans are routinely exposed to that lack toxicity data (18, 109). Data from *in vitro* models can be used collectively with other data streams in a weight-of-evidence approach to inform the identification of hazardous chemicals.

In vitro screening techniques for placental-derived toxicity can consist of a variety of methods described in part by the above sections. For example, *in vitro* placental studies have been used to screen for exposure-induced changes in hormone production and secretion (77, 110), inflammation signaling (111), and enzyme activity related to cell death pathways (112). To detail a specific example, BeWo cells have been used in combination with membrane vesicles to screen environmental chemicals for interaction with BCRP, a fetoprotective transporter that regulates the efflux of chemicals from the placenta to maternal circulation (113). This study identified the mycotoxin, zearalenone, as an inhibitor of chemical trans-placental transfer through its interaction with BCRP. This chemical has since then been confirmed as a prenatal developmental toxicant through this mechanism *in vivo* using $Bcrp^{-/-}$ mice (102). These examples clearly support the utility of *in vitro* placental models in the screening of hazardous chemicals that may impact placental biology and associated effects on prenatal developmental outcomes.

5.2 Use of in vitro models to inform mechanism of action and adverse outcome pathways (AOPs)

The use of *in vitro* placental models significantly increases the feasibility of analyzing molecular mechanisms underlying placental toxicity and potentially associated prenatal development toxicity outcomes. Some strategies to evaluate mechanisms of toxicity were previously detailed in section 4. These strategies include the investigation of cell death and proliferation, cytotrophoblast invasion, gene expression, DNA damage, immunomodulation, protein expression and secretion, nutrient uptake and transport, oxidative stress, and epigenetic reprogramming. Findings from *in vitro* models not only inform *in vivo* mechanisms of disease for tested chemicals, but can also be used to train and test *in silico* models to computationally predict mechanisms of disease for chemicals lacking data (114, 115). Integrating such mechanistic information from various models serves as an important step in the risk assessment of environmental chemicals.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The mechanism by which a chemical causes a disease outcome heavily influences how a chemical is ultimately regulated. For example, environmental chemicals that are known to cause disease outcomes through mechanisms based on mutagenicity are typically regulated based on exposure limits derived using linear low-dose extrapolation methods, wherein the chemical under evaluation is assumed to be adverse at any dose above zero (116, 117). Conversely, environmental chemicals that are known to cause disease outcomes through non-mutagenic mechanisms can be regulated based on exposure limits that are quantitatively derived using nonlinear approaches, wherein the chemical is considered to be 'safe' up until a certain exposure threshold (116, 117). Thus, chemicals that cause similar disease outcomes, yet act through different mechanisms of action, can be regulated by the setting of different chemical safety criteria.

A specific example of how mechanistic data from *in vitro* models are currently being incorporated into chemical safety assessments is through the increased integration of *in vitro* transcriptomic signatures that are predictive of *in vivo* mechanisms of action. For example, the toxicogenomics-DNA damage-inducing (TGx-DDI) signature has been established as a set of 64 biomarker genes that can be used to accurately distinguish DDI from non-DDI exposures using *in vitro* test methods (118). There is, therefore, room for the use *in vitro* models in this step relevant to risk assessment.

A growing avenue for incorporating mechanism of action into chemical evaluations is through the adverse outcome pathway (AOP) concept. AOPs represent a sequence of events that initiate as chemical interactions in cells and progress in a step-wise manner to population-level adverse responses (119). Introduced nearly a decade ago, the AOP concept has gained considerable attention in recent years as a topic of interest in scientific meetings, journal publications, and regulatory guidance documents (120). Findings from *in vitro* placental models could be used to inform important AOPs relevant to prenatal developmental toxicity outcomes, which could then influence the risk assessment of environmental chemicals. Specific examples of how AOPs could be used to influence the regulation of developmental toxicants is through the following: (i) evaluation of potential species-specific differences, (ii) overall biological plausibility of disease mechanisms, and (iii) predictions of disease outcomes that could result from chemical-induced molecular events, among others. Findings from *in vitro* placental models clearly have a place in the elucidation of chemical mechanism of action and incorporation into AOPs, representing key components of modern-day chemical regulation.

5.3 Use of in vitro models to inform doses eliciting in vivo toxicity

Characterizing the relationships between dose at which an organism/target is exposed and the resulting toxicity response(s) is vital towards understanding which exposure levels within the environment may affect developmental and placental toxicity endpoints. Data produced through *in vitro* modeling are easily amenable to dose-response evaluations. For example, an *in vitro* study found that inorganic arsenic induced dose-response changes relevant to the Nrf2 pathway in JAR cells by evaluating cell stress (i.e. production of hydrogen peroxide) and changes in the expression of Nrf2-related proteins (99). The finding that Nrf2 signaling alterations were dose-dependent reinforced the biological plausibility of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

oxidative stress-related mechanisms underlying inorganic arsenic-induced toxicity within the placenta.

Effects at low concentrations are also important to identify, as these changes may impact human health through exposure conditions that are environmentally relevant. For example, an *in vitro* study evaluated BeWo cells treated with BPA at concentrations ranging between 0.01-100 μM, and concentrations as low as 10 nM were found to inhibit BeWo cell transwell invasion and reduce the expression of genes involved in cell invasion (121). As mentioned above, these effects occurring at low concentrations are important, as placental cell invasion can adversely impact placental health, which can then impact pregnancy outcomes and potentially associated fetal development.

Once exposure concentrations that elicit bioactivity are identified through *in vitro* experimentation and dose-response modeling approaches, these concentrations can be used to estimate the equivalent doses required to elicit toxicity within animals and/or humans through *in vitro*-to-*in vivo* (IVIVE) extrapolation. IVIVE methods require the following: (i) an *in vitro* model to provide an estimate of the test chemical concentration that elicits activity, and (ii) a toxicokinetic model to relate this *in vitro*-based concentration to an *in vivo* dose that is estimated to produce an equivalent concentration in blood or other target tissues (122). Most of the IVIVE efforts based on high-throughput toxicokinetic modeling are currently focused on converting *in vitro* bioactivity concentrations to concentrations in circulating blood (123, 124). Further expansion of IVIVE and toxicokinetic modeling efforts are currently needed to better estimate tissue deposition, clearance, and toxicokinetic variability within the placenta. These *in silico* efforts can ultimately be used to effectively integrate *in vitro*-derived placental bioactivity measures into the prediction of *in vivo* doses eliciting placental and associated prenatal developmental toxicity endpoints.

5.4 Incorporating in vitro testing into risk characterization

Risk characterization is the final step involved in the human health risk assessment process and involves the integration of exposure and toxicological effects into quantitative and qualitative estimates of risk for the population(s) that the final regulation is designed to protect (116). This risk characterization step synthesizes an overall conclusion about health outcome risk related to exposures that is complete, informative, and useful for decision makers (116). Due to the depth of information needed to conduct a comprehensive risk assessment, *in vitro* data are useful towards contributing to these data requirements, with specific examples previously discussed.

An additional example supporting the use of *in vitro* data is in the derivation of data-driven extrapolation factors. Extrapolation factors are used to quantitatively derive final chemical safety criteria while taking into account potential variations in response (125). Specifically, extrapolation factors account for differences between model species used to derive toxicity information and the average human (i.e., interspecies variation, when applicable) and for variation in the human population (i.e., intraspecies variation). There is a current drive to move away from the use of established default values for inter- and intraspecies extrapolation, and move towards the use of empirically derived values based on experimental data that decrease the uncertainty in risk assessment. To elaborate, the default approach to

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

"adjust" for uncertainty in the setting of chemical safety criteria is largely based on dividing exposure concentrations by whole number estimates that are selected by default standards regardless of the type of chemical being evaluated. According to recent guidelines, *in vitro* assays can play a role in defining data-driven extrapolation factors, particularly when toxicokinetics/toxicodynamics are considered to account for target tissue dose and competing absorption, distribution, metabolism, excretion (ADME) processes (125). This movement towards data-driven extrapolation factors further supports the potential utility of *in vitro* placental models in risk assessment applications.

When incorporating findings from *in vitro* models into risk assessment, it is important to consider potential applicability domains. The term 'applicability domain' is most commonly used in toxicology when referring to a theoretical region of physicochemical space for which quantitative structure-activity relationship (QSAR) models can reliably make toxicity predictions (114, 126). The use of this term is currently evolving to include expanded computational toxicology applications, including the use of *in vitro* data to predict *in vivo* toxicity based on multifactorial model inputs, in addition to classical physicochemical/ structural properties. For *in vitro* placental models, we posit that certain classes of chemicals and/or *in vitro* endpoints can be used to more accurately predict human disease outcomes in comparison to other chemicals/endpoints. Indeed, we have found that *in vitro* bioactivity can be used to inform *in vivo* toxicity responses with a higher degree of accuracy for chemicals that fall within certain ranges of physicochemical properties (127). There was also increased *in vitro*-to-*in vivo* response concordance for certain pathways that were highly expressed and active in target tissues (127). As this field continues to develop, researchers and policymakers will be able to better identify instances when *in vitro* testing is appropriate and how findings should be interpreted in combination with *in silico* techniques to better protect human health.

6. CONCLUSION

In conclusion, this review provides an overview of *in vitro* placental models and associated endpoints that can be used to evaluate chemical-induced toxicity. The placenta is critical in embryonic and fetal development and can be adversely impacted by exposure to environmental contaminants. *In vitro* placental models that are commonly used to test the effects of toxicant exposure include trophoblast cells and explant models from humans, among others. Findings from these models can inform placental toxicity in humans as well as associated prenatal developmental toxicity outcomes associated with prenatal exposures.

Strategies that can be used to translate findings from *in vitro* placental models into chemical evaluations include those relevant to the steps in the human health risk assessment process. These strategies include the use of *in vitro* placental models to: (i) screen for hazardous chemicals, (ii) elucidate chemical mechanism of action, (iii) identify molecular events in AOPs, (iv) estimate doses eliciting toxicity through dose-response and IVIVE modeling, and (v) contribute to the overall risk characterization of adverse outcomes associated with exposure-induced placental toxicity. There is a current need to better define applicability domains for the use of *in vitro* placental models in predicting toxicity in humans through farther research. Studies in this area are currently expanding and will likely contribute to a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

growing confidence in using *in vitro* data combined with *in silico* approaches to better understand relationships between prenatal exposure to chemicals, placental toxicity, and developmental outcomes.

Acknowledgments

Funding sources

This research was supported by a grant from the National Institute of Environmental Health Sciences (P42ES005948).

REFERENCES

1. Bimbaum LS, Fenton SE. Cancer and developmental exposure to endocrine disruptors. Environ Health Perspect. 2003;111(4):389–94. Epub 2003/04/05. doi: 10.1289/ehp.5686. [PubMed: 12676588]
2. Brent RL, Tanski S, Weitzman M. A pediatric perspective on the unique vulnerability and resilience of the embryo and the child to environmental toxicants: the importance of rigorous research concerning age and agent. Pediatrics. 2004;113(4 Suppl):935–44. Epub 2004/04/03. [PubMed: 15060185]
3. Falck AJ, Mooney S, Kapoor SS, White KM, Bearer C, El Metwally D. Developmental Exposure to Environmental Toxicants. Pediatr Clin North Am. 2015;62(5):1173–97. Epub 2015/09/01. doi: 10.1016/j.pcl.2015.05.005. [PubMed: 26318946]
4. Nye MD, Fry RC, Hoyo C, Murphy SK. Investigating Epigenetic Effects of Prenatal Exposure to Toxic Metals in Newborns: Challenges and Benefits. Med Epigenet. 2014;2(1):53–9. Epub 2014/06/24. doi: 10.1159/000362336. [PubMed: 24955086]
5. Meng Q, Inoue K, Ritz B, Olsen J, Liew Z. Prenatal Exposure to Perfluoroalkyl Substances and Birth Outcomes; An Updated Analysis from the Danish National Birth Cohort. Int J Environ Res Public Health. 2018;15(9). Epub 2018/08/29. doi: 10.3390/ijerph15091832.
6. Giulivo M, Lopez de Alda M, Capri E, Barcelo D. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environmental research. 2016;151:251–64. Epub 2016/10/21. doi: 10.1016/j.envres.2016.07.011. [PubMed: 27504873]
7. Kim YR, Harden FA, Toms LM, Norman RE. Health consequences of exposure to brominated fame retardants: a systematic review. Chemosphere. 2014;106:1–19. Epub 2014/02/18. doi: 10.1016/j.chemosphere.2013.12.064. [PubMed: 24529398]
8. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 2014;13(3):330–8. Epub 2014/02/22. doi: 10.1016/S1474-4422(13)70278-3. [PubMed: 24556010]
9. Bommarito PA, Martin E, Fry RC. Effects of prenatal exposure to endocrine disruptors and toxic metals on the fetal epigenome. Epigenomics. 2017;9(3):333–50. Epub 2017/02/25. doi: 10.2217/epi-2016-0112. [PubMed: 28234024]
10. Arbuckle TE, Kubwabo C, Walker M, Davis K, Lalonde K, Kosarac I, Wen SW, Arnold DL. Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants. International journal of hygiene and environmental health. 2013;216(2):184–94. [PubMed: 22494936]
11. Monroy R, Morrison K, Teo K, Atkinson S, Kubwabo C, Stewart B, Foster WG. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environmental research. 2008;108(1):56–62. [PubMed: 18649879]
12. Tyl RW, Marr MC. Developmental toxicity testing-Methodology In: Hood RD, editor. Developmental and Reproductive Toxicology: CRC Press; 2016 p. 139–83.
13. Myren M, Mose T, Mathiesen L, Knudsen LE. The human placenta--an alternative for studying foetal exposure. Toxicology in vitro : an international journal published in association with BIBRA. 2007;21(7):1332–40. Epub 2007/07/13. doi: 10.1016/j.tiv.2007.05.011. [PubMed: 17624715]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

14. Roos S, Powell TL, Jansson T. Placental mTOR links maternal nutrient availability to fetal growth. Biochem Soc Trans. 2009;37(Pt 1)295–8. Epub 2009/01/16. doi: 10.1042/BST0370295. [PubMed: 19143650]
15. Aye IL, Lager S, Ramirez VI, Gaccioli F, Dudley DJ, Jansson T, Powell TL. Increasing maternal body mass index is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways. Biol Reprod. 2014;90(6):129 Epub 2014/04/25. doi: 10.1095/biolreprod.113.116186. [PubMed: 24759787]
16. Martin E, Smeester L, Bommarito PA, Grace MR, Boggess K, Kuban K, Karagas MR, Marsit CJ, O'Shea TM, Fry RC. Sexual epigenetic dimorphism in the human placenta: implications for susceptibility during the prenatal period. Epigenomics. 2017;9(3):267–78. Epub 2017/02/25. doi: 10.2217/epi-2016-0132. [PubMed: 28234023]
17. Hartung T Toxicology for the twenty-first century. Nature. 2009;460(7252):208–12. Epub 2009/07/10. doi: 10.1038/460208a. [PubMed: 19587762]
18. Egeghy PP, Judson R, Gangwal S, Mosher S, Smith D, Vail J, Cohen Hubal EA. The exposure data landscape for manufactured chemicals. Sci Total Environ. 2012;414:159–66. Epub 2011/11/23. doi: 10.1016/j.scitotenv.2011.10.046. [PubMed: 22104386]
19. Vuguin PM. Animal models for small for gestational age and fetal programming of adult disease. Horm Res. 2007;68(3):113–23. Epub 2007/03/14. doi: 10.1159/000100545.
20. Malassine A, Frendo JL, Evain-Brion D. A comparison of placental development and endocrine functions between the human and mouse model. Hum Reprod Update. 2003;9(6):531–9. Epub 2004/01/13. [PubMed: 14714590]
21. Kimmel CA, Makris SL. Recent developments in regulatory requirements for developmental toxicology. Toxicol Lett. 2001;120(1-3):73–82. Epub 2001/04/27. [PubMed: 11323164]
22. Grigsby PL, editor. Animal models to study placental development and function throughout normal and dysfunctional human pregnancy Seminars in reproductive medicine; 2016: Thieme Medical Publishers.
23. EPA U. Pesticide Assessment Guidelines Subdivision F Hazard Evaluation: Human and Domestic Animals. Washington, D.C.: Office of Pesticide Programs, 1984.
24. EPA U. Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study Washington, D.C1998 [cited 2018 Oct 1], Available from: https//www.regulations.gov/document?D=EPA-HQ-OPPT-2009-0156-0017.
25. OECD. OECD Guideline for Testing of Chemicals: Prenatal Development Toxicity Study 2018 [cited 2018 Sept 1], Available from: https//ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl414.pdf.
26. Knudsen TB, Martin MT, Kavlock RJ, Judson RS, Dix DJ, Singh AV. Profiling the activity of environmental chemicals in prenatal developmental toxicity studies using the U.S. EPA's ToxRefDB. Reprod Toxicol. 2009;28(2):209–19. Epub 2009/05/19. doi: 10.1016/j.reprotox.2009.03.016. [PubMed: 19446433]
27. EPA U. EPA Opens Access to Chemical Information/Searchable database on chemical hazard, exposure and toxicity data now available 2010 [cited 2018 Oct 1], Available from: https://archive.epa.gov/epapages/newsroom_archive/newsreleases/43216c4f52d46b0b85257713007cl97b.html.
28. Watford SPL, Wignall J, Shin R, Martin MT, Paul Friedman K. ToxRefDB version 2.0: Improved utility for predictive and retrospective toxicology analyses, (in prep). 2018.
29. Schmidt A, Morales-Prieto DM, Pastuschek J, Frohlich K, Markert UR. Only humans have human placentas: molecular differences between mice and humans. Journal of reproductive immunology. 2015;108:65–71. Epub 2015/03/31. doi: 10.1016/j.jri.2015.03.001. [PubMed: 25817465]
30. Jeon SY, Lee HJ, Park JM, Jung HM, Yoo JK, Lee HJ, Lee JS, Cha DH, Kim JK, Kim GJ. Increased immortalization-upregulated protein 2 (IMUP-2) by hypoxia induces apoptosis of the trophoblast and pre-eclampsia. J Cell Biochem. 2010; 110(2)522–30. Epub 2010/05/01. doi: 10.1002/jcb.22568. [PubMed: 20432246]
31. King A, Thomas L, Bischof P. Cell culture models of trophoblast II: trophoblast cell lines—a workshop report. Placenta. 2000;21:S113–S9. [PubMed: 10831135]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

32. Sullivan M Endocrine cell lines from the placenta. Molecular and cellular endocrinology. 2004;228(1-2):103–19. [PubMed: 15541575]
33. Kay H, Nelson DM, Wang Y. The placenta: from development to disease: John Wiley & Sons; 2011.
34. Wolfe MW. Culture and transfection of human choriocarcinoma cells Placenta and Trophoblast: Springer; 2006 p. 229–39.
35. Pattillo RA, Gey GO. The establishment of a cell line of human hormone-synthesizing trophoblastic cells in vitro. Cancer research. 1968;28(7):1231–6. [PubMed: 4299001]
36. Kohler POB, William E. Isolation of hormone-producing clonal lines of human choriocarcinoma. The Journal of Clinical Endocrinology & Metabolism. 1971;32(5):683–7. [PubMed: 5103722]
37. Augustowska K, Magnowska Z, Kapiszewska M, Gregoraszezuk EL. Is the natural PCDD/PCDF mixture toxic for human placental JEG-3 cell line? The action of the toxicants on hormonal profile, CYP1A1 activity, DNA damage and cell apoptosis. Human & experimental toxicology. 2007;26(5):407–17. [PubMed: 17623765]
38. Brooks SA, Fry RC. Cadmium inhibits placental trophoblast cell migration via miRNA regulation of the transforming growth factor beta (TGF-beta) pathway. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2017;109(Pt 1):721–6. Epub 2017/08/05. doi: 10.1016/j.fct.2017.07.059. [PubMed: 28774740]
39. Brooks SA, Martin E, Smeester L, Grace MR, Boggess K, Fry RC. miRNAs as common regulators of the transforming growth factor (TGF)-β pathway in the preeclamptic placenta and cadmium-treated trophoblasts: Links between the environment, the epigenome and preeclampsia. Food and Chemical Toxicology. 2016;98:50–7. [PubMed: 27375191]
40. Meakin CJ, Martin EM, Szilagyi JT, Nylander-French LA, Fry RC. Inorganic Arsenic as an Endocrine Disruptor: Modulation of the Glucocorticoid Receptor Pathway in Placental Cells via CpG Methylation. Chem Res Toxicol. 2019;32(3):493–9. Epub 2019/02/13. doi: 10.1021/acs.chemrestox.8b00352. [PubMed: 30746931]
41. Frank H-G, Gunawan B, Ebeling-Stark I, Schulten H-J, Funayama H, Cremer U, Huppertz B, Gaus G, Kaufinann P, Füzesi L. Cytogenetic and DNA-fingerprint characterization of choriocarcinoma cell lines and a trophoblast/choriocarcinoma cell hybrid. Cancer genetics and cytogenetics. 2000; 116(1):16–22. [PubMed: 10616526]
42. Graham CH, Hawley TS, Hawley RC, MacDougall JR, Kerbel RS, Khoo N, Lala PK. Establishment and characterization of first trimester human trophoblast cells with extended lifespan. Experimental cell research. 1993;206(2):204–11. [PubMed: 7684692]
43. Takao T, Asanoma K, Kato K, Fukushima K, Tsunematsu R, Hirakawa T, Matsumura S, Seki H, Takeda S, Wake N. Isolation and Characterization of Human Trophoblast Side-Population (SP) Cells in Primary Villous Cytotrophoblasts and HTR-8/SVneo Cell Line. PloS one. 2011;6(7):e21990. doi: 10.1371/journal.pone.0021990. [PubMed: 21760941]
44. Feng HC, Choy MY, Deng W, Wong HL, Lau WM, Cheung A, Ngan H, Tsao SW. Establishment and characterization of a human first-trimester extravillous trophoblast cell line (TEV-1). Journal of the Society for Gynecologic Investigation. 2005;12(4):e21–32. [PubMed: 15866109]
45. Abou-Kheir W, Barrak J, Hadadeh O, Daoud G. HTR-8/SVneo cell line contains a mixed population of cells. Placenta. 2017;50:1–7. [PubMed: 28161053]
46. Straszewski-Chavez SL, Abrahams VM, Alvero AB, Aldo PB, Ma Y, Guller S, Romero R, Mor G. The isolation and characterization of a novel telomerase immortalized first trimester trophoblast cell line, Swan 71. Placenta. 2009;30(11):939–48. [PubMed: 19766308]
47. Lewis M, Clements M, Takeda S, Kirby P, Seki H, Lonsdale L, Sullivan M, Elder M, White J. Partial characterization of an immortalized human trophoblast cell-line, TCL-1, which possesses aCSF-1 autocrine loop. Placenta. 1996;17(2):137–46. [PubMed: 8730883]
48. Stenqvist AC, Chen T, Hedlund M, Dimova T, Nagaeva O, Kjellberg L, Innala E, Mincheva-Nilsson L. An efficient optimized method for isolation of villous trophoblast cells from human early pregnancy placenta suitable for functional and molecular studies. American Journal of Reproductive Immunology. 2008;60(l):33–42. [PubMed: 18593436]
49. Jolibois LS Jr, Burow ME, Swan KF, George WJ, Anderson MB, Henson MC. Effects of cadmium on cell viability, trophoblastic development, and expression of low density lipoprotein receptor

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

transcripts in cultured human placental cells. Reproductive Toxicology. 1999;13(6):473–80. [PubMed: 10613395]

50. Novakovic B, Gordon L, Wong NC, Moffett A, Manuelpillai U, Craig JM, Sharkey A, Saffery R. Wide-ranging DNA methylation differences of primary trophoblast cell populations and derived cell lines: implications and opportunities for understanding trophoblast function. Molecular human reproduction. 2011;17(6):344–53. [PubMed: 21289002]
51. Pan C, Kumar C, Bohl S, Klingmueller U, Mann M. Comparative Proteomic Phenotyping of Cell Lines and Primary Cells to Assess Preservation of Cell Type-specific Functions. Molecular & Cellular Proteomics. 2009;8(3):443–50. doi: 10.1074/mcp.M800258-MCP200. [PubMed: 18952599]
52. Miller RK, Genbacev O, Turner MA, Aplin JD, Caniggia I, Huppertz B. Human placental explants in culture: approaches and assessments. Placenta. 2005;26(6):439–48. Epub 2005/06/14. doi: 10.1016/j.placenta.2004.10.002. [PubMed: 15950058]
53. Caruso M, Evangelista M, Parolini O. Human term placental cells: phenotype, properties and new avenues in regenerative medicine. International journal of molecular and cellular medicine. 2012;l(2):64–74.
54. Brownfoot FC, Tong S, Hannan NJ, Binder NK, Walker SP, Cannon P, Hastie R, Onda K, Kaitu'u-Lino TJ. Effects of Pravastatin on Human Placenta, Endothelium, and Women With Severe Preeclampsia. Hypertension (Dallas, Tex : 1979). 2015;66(3):687–97; discussion 445. Epub 2015/07/30. doi: 10.1161/hypertensionaha.115.05445.
55. Gangooly S, Muttukrishna S, Jauniaux E. In-vitro study of the effect of anti-hypertensive drugs on placental hormones and angiogenic proteins synthesis in pre-eclampsia. PloS one. 2014;9(9):e107644 Epub 2014/09/25. doi: 10.1371/journal.pone.0107644. [PubMed: 25251016]
56. Ramma W, Ahmed A. Therapeutic potential of statins and the induction of heme oxygenase-1 in preeclampsia. Journal of reproductive immunology. 2014;101-102:153–60. Epub 2014/02/08. doi: 10.1016/j.jri.2013.12.120. [PubMed: 24503248]
57. Sieppi E, Vahakangas K, Rautio A, Ietta F, Paulesu L, Myllynen P. The xenoestrogens, bisphenol A and para-nonylphenol, decrease the expression of the ABCG2 transporter protein in human term placental explant cultures. Mol Cell Endocrinol. 2016;429:41–9. Epub 2016/04/03. doi: 10.1016/j.mce.2016.03.034. [PubMed: 27036933]
58. Young AM, Allen CE, Audus KL. Efflux transporters of the human placenta. Advanced drug delivery reviews. 2003;55(1):125–32. [PubMed: 12535577]
59. Huls M, Russel FG, Masereeuw R. The role of ATP binding cassette transporters in tissue defense and organ regeneration. Journal of Pharmacology and Experimental Therapeutics. 2009;328(1):3–9. [PubMed: 18791064]
60. Blundell C, Tess ER, Schanzer AS, Coutifaris C, Su EJ, Parry S, Huh D. A microphysiological model of the human placental barrier. Lab on a chip. 2016;16(16):3065–73. Epub 2016/05/28. doi: 10.1039/c61c00259e. [PubMed: 27229450]
61. Lee JS, Romero R, Han YM, Kim HC, Kim CJ, Hong JS, Huh D. Placenta-on-a-chip: a novel platform to study the biology of the human placenta. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016;29(7):1046–54. Epub 2015/06/16. doi: 10.3109/14767058.2015.1038518.
62. Blundell C, Yi YS, Ma L, Tess ER, Farrell MJ, Georgescu A, Aleksunes LM, Huh D. Placental Drug Transport-on-a-Chip: A Microengineered In Vitro Model of Transporter-Mediated Drug Efflux in the Human Placental Barrier. Advanced healthcare materials. 2018;7(2). Epub 2017/11/10. doi: 10.1002/adhm.201700786.
63. Yin F, Zhu Y, Zhang M, Yu H, Chen W, Qin J. A 3D human placenta-on-a-chip model to probe nanoparticle exposure at the placental barrier. Toxicology in vitro : an international journal published in association with BIBRA. 2018;54:105–13. Epub 2018/09/25. doi: 10.1016/j.tiv.2018.08.014. [PubMed: 30248392]
64. Ma T, Li Y, Yang ST, Kniss DA. Tissue engineering human placenta trophoblast cells in 3-D fibrous matrix: spatial effects on cell proliferation and function. Biotechnology progress. 1999;15(4):715–24. Epub 1999/08/12. doi: 10.1021/bp990072y. [PubMed: 10441363]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

65. McConkey CA, Delorme-Axford E, Nickerson CA, Kim KS, Sadovsky Y, Boyle JP, Coyne CB. A three-dimensional culture system recapitulates placental syncytiotrophoblast development and microbial resistance. Science advances. 2016;2(3):e1501462 Epub 2016/03/15. doi: 10.1126/sciadv.1501462. [PubMed: 26973875]
66. Muoth C, Wichser A, Monopoli M, Correia M, Ehrlich N, Loeschner K, Gallud A, Kucki M, Diener L, Manser P, Jochum W, Wick P, Buerki-Thurnherr T. A 3D co-culture microtissue model of the human placenta for nanotoxicity assessment. Nanoscale. 2016;8(39):17322–32. Epub 2016/10/08. doi: 10.1039/c6nr06749b. [PubMed: 27714104]
67. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, McWhinnie A, Esposito L, Fernando R, Skelton H. Trophoblast organoids as a model for maternal-fetal interactions during human placentation. Nature. 2018:1.
68. Wang H, Pilla F, Anderson S, Martinez-Escribano S, Herrer I, Moreno-Moya JM, Musti S, Bocca S, Oehninger S, Horcajadas JA. A novel model of human implantation: 3D endometriumlike culture system to study attachment of human trophoblast (Jar) cell spheroids. Mol Hum Reprod. 2012;18(l):33–43. Epub 2011/10/13. doi: 10.1093/molehr/gar064. [PubMed: 21989169]
69. Kenney RM, Lloyd CC, Whitman NA, Lockett MR. 3D cellular invasion platforms: how do paper-based cultures stack up? Chemical Communications. 2017;53(53):7194–210. [PubMed: 28621775]
70. Boyce MW, Kenney RM, Truong AS, Lockett MR. Quantifying oxygen in paper-based cell cultures with luminescent thin film sensors. Analytical and bioanalytical chemistry. 2016;408(11):2985–92. [PubMed: 26667655]
71. Truong AS, Lochbaum CA, Boyce MW, Lockett MR. Tracking the invasion of small numbers of cells in paper-based assays with quantitative PCR. Analytical chemistry. 2015;87(22):11263–70. [PubMed: 26507077]
72. Boyce MW, LaBonia GJ, Hummon AB, Lockett MR. Assessing chemotherapeutic effectiveness using a paper-based tumor model. The Analyst. 2017;142(15):2819–27. [PubMed: 28702529]
73. Kenney RM, Boyce MW, Truong AS, Bagnell CR, Lockett MR. Real-time imaging of cancer cell chemotaxis in paper-based scaffolds. The Analyst. 2016;141(2):661–8. Epub 2015/11/10. doi: 10.1039/c5an01787d. [PubMed: 26548584]
74. Mosadegh B, Lockett MR, Minn KT, Simon KA, Gilbert K, Hillier S, Newsome D, Li H, Hall AB, Boucher DM, Eustace BK, Whitesides GM. A paper-based invasion assay: assessing chemotaxis of cancer cells in gradients of oxygen. Biomaterials. 2015;52:262–71. Epub 2015/03/31. doi: 10.1016/j.biomaterials.2015.02.012. [PubMed: 25818432]
75. Truong AS, Lockett MR. Oxygen as a chemoattractant: confirming cellular hypoxia in paper-based invasion assays. The Analyst. 2016;141(12):3874–82. Epub 2016/05/04. doi: 10.1039/c6an00630b. [PubMed: 27138213]
76. Orendi K, Kivity V, Sammar M, Grimpel Y, Gonen R, Meiri H, Lubzens E, Huppertz B. Placental and trophoblastic in vitro models to study preventive and therapeutic agents for preeclampsia. Placenta. 2011;32 Suppl:S49–54. Epub 2011/01/25. doi: 10.1016/j.placenta.2010.11.023. [PubMed: 21257083]
77. Bechi N, Ietta F, Romagnoli R, Focardi S, Corsi I, Buffi C, Paulesu L. Estrogen-like response to p-nonylphenol in human first trimester placenta and BeWo choriocarcinoma cells. Toxicological sciences : an official journal of the Society of Toxicology. 2006;93(1):75–81. Epub 2006/06/23. doi: 10.1093/toxsci/kfl043. [PubMed: 16790488]
78. Morck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F, Rytting E, Mathiesen L, Paulesu L, Knudsen LE. Placental transport and in vitro effects of Bisphenol A. Reprod Toxicol. 2010;30(1):131–7. Epub 2010/03/11. doi: 10.1016/j.reprotox.2010.02.007. [PubMed: 20214975]
79. Ridano ME, Racca AC, Flores-Martin JB, Fretes R, Bandeira C, Reyna L, Bevilacqua E, Genti-Raimondi S, Panzetta-Dutari G. Impact of chlorpyrifos on human villous trophoblasts and chorionic villi. Toxicology and applied pharmacology. 2017;329:26–39. [PubMed: 28549829]
80. Zhu J-Y, Pang Z-J, Yu Y-H. Regulation of trophoblast invasion: the role of matrix metalloproteinases. Reviews in obstetrics & gynecology. 2012;5(3-4):e137–e43. [PubMed: 23483768]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

81. DaSilva-Arnold S, James JL, Al-Khan A, Zamudio S, Illsley NP. Differentiation of first trimester cytotrophoblast to extravillous trophoblast involves an epithelial-mesenchymal transition. Placenta. 2015;36(12):1412–8. Epub 2015/11/08. doi: 10.1016/j.placenta.2015.10.013. [PubMed: 26545962]
82. Epstein Shochet G, Tartakover-Matalon S, Drucker L, Pasmanik-Chor M, Pomeranz M, Fishman A, Lishner M. Placenta-breast cancer cell interactions promote cancer cell epithelial mesenchymal transition via TGFbeta/JNK pathway. Clinical & experimental metastasis. 2014;31(8):961–75. Epub 2014/10/16. doi: 10.1007/s10585-014-9683-0. [PubMed: 25316285]
83. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. Epub 2011/03/08. doi: 10.1016/j.cell.2011.02.013. [PubMed: 21376230]
84. Blazquez AG, Briz O, Gonzalez-Sanchez E, Perez MJ, Ghanem CI, Marin JJ. The effect of acetaminophen on the expression of BCRP in trophoblast cells impairs the placental barrier to bile acids during maternal cholestasis. Toxicology and applied pharmacology. 2014;277(1):77–85. [PubMed: 24631341]
85. Neradugomma NK, Liao MZ, Mao Q. Buprenorphine, norbuprenorphine, R-methadone, and S-methadone upregulate BCRP/ABCG2 expression by activating Aryl hydrocarbon receptor in human placental trophoblasts. Molecular pharmacology. 2017;91(3):237–49. [PubMed: 27974484]
86. Everson TM, Kappil M, Hao K, Jackson BP, Punshon T, Karagas MR, Chen J, Marsit CJ. Maternal exposure to selenium and cadmium, fetal growth, and placental expression of steroidogenic and apoptotic genes. Environmental research. 2017;158:233–44. [PubMed: 28662449]
87. Peltier MR, Arita Y, Klimova NG, Gurzenda EM, Koo HC, Murthy A, Lerner V, Hanna N. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) enhances placental inflammation. Journal of reproductive immunology. 2013;98(1-2):10–20. Epub 2013/05/07. doi: 10.1016/j.jri.2013.02.005. [PubMed: 23642494]
88. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. International journal of molecular sciences. 2014;15(9):16153–85. doi: 10.3390/ijms150916153. [PubMed: 25222554]
89. Costa MA. The endocrine function of human placenta: an overview. Reproductive biomedicine online. 2016;32(l):14–43. Epub 2015/12/01. doi: 10.1016/j.rbmo.2015.10.005. [PubMed: 26615903]
90. Jones HN, Powell TL, Jansson T. Regulation of Placental Nutrient Transport – A Review. Placenta. 2007;28(8):763–74. doi: 10.1016/j.placenta.2007.05.002. [PubMed: 17582493]
91. Staud F, Cerveny L, Ceckova M. Pharmacotherapy in pregnancy; effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. Journal of drug targeting. 2012;20(9):736–63. Epub 2012/09/22. doi: 10.3109/1061186x.2012.716847. [PubMed: 22994411]
92. Landau D, Haghiac M, Minium J, Skomorovska-Prokvolit Y, Calabuig-Navarro V, O'Tiemey-Ginn P. Activation of AMPK in Human Placental Explants Impairs Mitochondrial Function and Cellular Metabolism. Reproductive sciences (Thousand Oaks, Calif). 2018:1933719118776803 Epub 2018/05/24. doi: 10.1177/1933719118776803.
93. Lui S, Jones RL, Robinson NJ, Greenwood SL, Aplin JD, Tower CL. Detrimental effects of ethanol and its metabolite acetaldehyde, on first trimester human placental cell turnover and function. PloS one. 2014;9(2):e87328 Epub 2014/02/08. doi: 10.1371/journal.pone.0087328. [PubMed: 24503565]
94. Mitchell MD, Osepchook CC, Leung KC, McMahon CD, Bass JJ. Myostatin is a human placental product that regulates glucose uptake. The Journal of clinical endocrinology and metabolism. 2006;91(4):1434–7. Epub 2006/02/09. doi: 10.1210/jc.2005-2361. [PubMed: 16464946]
95. Washburn SE, Sawant OB, Lunde ER, Wu G, Cudd TA. Acute alcohol exposure, acidemia or glutamine administration impacts amino acid homeostasis in ovine maternal and fetal plasma. Amino acids. 2013;45(3):543–54. Epub 2013/01/12. doi: 10.1007/s00726-012-1453-1. [PubMed: 23315157]
96. Hung TH, Burton GJ. Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. Taiwan J Obstet Gynecol. 2006;45(3):189–200. Epub 2006/12/19. doi: 10.1016/S1028-4559(09)60224-2. [PubMed: 17175463]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

97. Adebambo OA, Ray PD, Shea D, Fry RC. Toxicological responses of environmental mixtures: Environmental metal mixtures display synergistic induction of metal-responsive and oxidative stress genes in placental cells. Toxicology and applied pharmacology. 2015;289(3):534–41. [PubMed: 26472158]
98. Adebambo OA, Shea D, Fry RC. Cadmium disrupts signaling of the hypoxia-inducible (HIF) and transforming growth factor (TGF-beta) pathways in placental JEG-3 trophoblast cells via reactive oxygen species. Toxicol Appl Pharmacol. 2018;342:108–15. Epub 2018/02/07. doi: 10.1016/j.taap.2018.01.010. [PubMed: 29408318]
99. Massrieh W, Derjuga A, Blank V. Induction of endogenous Nrf2/small maf heterodimers by arsenic-mediated stress in placental choriocarcinoma cells. Antioxidants & redox signaling. 2006;8(1-2):53–9. [PubMed: 16487037]
100. Eisenmann CJ, Miller RK. Cadmium and glutathione: effect on human placental thromboxane and prostacyclin production. Reprod Toxicol. 1995;9(1):41–8. [PubMed: 8520130]
101. Neradugomma NK, Liao MZ, Mao Q. Buprenorphine, Norbuprenorphine, R-Methadone, and S-Methadone Upregulate BCRP/ABCG2 Expression by Activating Aryl Hydrocarbon Receptor in Human Placental Trophoblasts. Molecular pharmacology. 2017;91(3):237–49. Epub 2016/12/16. doi: 10.1124/mol.116.107367. [PubMed: 27974484]
102. Szilagyi JT, Gorczyca L, Brinker A, Buckley B, Laskin JD, Aleksunes LM. Placental BCRP/ABCG2 transporter prevents fetal exposure to the estrogenic mycotoxin zearalenone. Toxicological sciences : an official journal of the Society of Toxicology. 2018 Epub 2018/12/24. doi: 10.1093/toxsci/kfy303.
103. Mørck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F, Rytting E, Mathiesen L, Paulesu L, Knudsen LE. Placental transport and in vitro effects of Bisphenol A. Reproductive toxicology. 2010;30(1):131–7. [PubMed: 20214975]
104. Inbar-Feigenberg M, Choufani S, Butcher DT, Roifman M, Weksberg R. Basic concepts of epigenetics. Fertility and sterility. 2013;99(3):607–15. [PubMed: 23357459]
105. Liu H, Li S, Wang X, Zhu J, Wei Y, Wang Y, Wen Y, Wang L, Huang Y, Zhang B. DNA methylation dynamics: identification and functional annotation. Briefings in functional genomics. 2016;15(6):470–84. [PubMed: 27515490]
106. Dolinoy DC, Das R, Weidman JR, Jirtle RL. Metastable epialleles, imprinting, and the fetal origins of adult diseases. Pediatric research. 2007;61(5 Part 2):30R.
107. Avissar-Whiting M, Veiga KR, Uhl KM, Maccani MA, Gagne LA, Moen EL, Marsit CJ. Bisphenol A exposure leads to specific microRNA alterations in placental cells. Reproductive toxicology. 2010;29(4):401–6. [PubMed: 20417706]
108. EPA US. Human Health Risk Assessment 2018 [cited 2018 Oct 1]. Available from: https://www.epa.gov/risk/human-health-risk-assessment.
109. Judson R, Richard A, Dix DJ, Houck K, Martin M, Kavlock R, Dellarco V, Henry T, Holderman T, Sayre P, Tan S, Carpenter T, Smith E. The toxicity data landscape for environmental chemicals. Environ Health Perspect. 2009;117(5):685–95. Epub 2009/05/30. doi: 10.1289/ehp.0800168. [PubMed: 19479008]
110. Vinggaard A, Hnida C, Breinholt V, Larsen JC. Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro. Toxicology in vitro. 2000;14(3):227–34. [PubMed: 10806373]
111. Wang B, Parobchak N, Martin A, Rosen M, Yu LJ, Nguyen M, Gololobova K, Rosen T. Screening a small molecule library to identify inhibitors of NF-κB inducing kinase and pro-labor genes in human placenta. Scientific reports. 2018;8(1):1657. doi: 10.1038/s41598-018-20147-0. [PubMed: 29374256]
112. Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini G-E. Major pesticides are more toxic to human cells than their declared active principles. BioMed research international. 2014;2014.
113. Xiao J, Wang Q, Bircsak KM, Wen X, Aleksunes LM. In vitro screening of environmental chemicals identifies zearalenone as a novel substrate of the placental BCRP/ABCG2 transporter. Toxicology research. 2015;4(3):695–706. [PubMed: 26052432]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

114. Raies AB, Bajic VB. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley Interdiscip Rev Comput Mol Sci. 2016;6(2):147–72. Epub 2016/04/12. doi: 10.1002/wcms.1240. [PubMed: 27066112]

115. Ciallella HL, Zhu H. Advancing Computational Toxicology in the Big Data Era by Artificial Intelligence: Data-Driven and Mechanism-Driven Modeling for Chemical Toxicity. Chem Res Toxicol. 2019;32(4):536–47. Epub 2019/03/26. doi: 10.1021/acs.chemrestox.8b00393. [PubMed: 30907586]

116. EPA US. Framework for Human Health Risk Assessment to Inform Decision Making. Risk Assessment Forum, 2014 Contract No.: EPA/100/R-14/001 4 2014.

117. EPA US. Guidelines for Carcinogenic Risk Assessment. Washington, D.C.: Risk Assessment Forum, 2005 Contract No.: EPA/630/P-03/001F.

118. Cho E, Buick JK, Williams A, Chen R, Li HH, Corton JC, Fomace AJ Jr., Aubrecht J, Yauk CL. Assessment of the performance of the TGx-DDI biomarker to detect DNA damage-inducing agents using quantitative RT-PCR in TK6 cells. Environ Mol Mutagen. 2019;60(2):122–33. Epub 2018/11/30. doi: 10.1002/em.22257. [PubMed: 30488505]

119. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem. 2010;29(3):730–41. Epub 2010/09/08. doi: 10.1002/etc.34. [PubMed: 20821501]

120. OECD. Revised Guidance Document on Developing and Assessing Adverse Outcome Pathways 2017 [cited 2018 Jun 4], Available from: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en.

121. Wang ZY, Lu J, Zhang YZ, Zhang M, Liu T, Qu XL. Effect of Bisphenol A on invasion ability of human trophoblastic cell line BeWo. International journal of clinical and experimental pathology. 2015;8(11)44355–64.

122. Bell SM, Chang X, Wambaugh JF, Allen DG, Bartels M, Brouwer KLR, Casey WM, Choksi N, Ferguson SS, Fraczkiewicz G, Jarabek AM, Ke A, Lumen A, Lynn SG, Paini A, Price PS, Ring C, Simon TW, Sipes NS, Sprankle CS, Strickland J, Troutman J, Wetmore BA, Kleinstreuer NC. In vitro to in vivo extrapolation for high throughput prioritization and decision making. Toxicology in vitro : an international journal published in association with BIBRA. 2018;47:213–27. Epub 2017/12/06. doi: 10.1016/j.tiv.2017.11.016. [PubMed: 29203341]

123. Ring CL, Pearce RG, Setzer RW, Wetmore BA, Wambaugh JF. Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability. Environ Int. 2017;106:105–18. Epub 2017/06/20. doi: 10.1016/j.envint.2017.06.004. [PubMed: 28628784]

124. Pearce RG, Setzer RW, Strope CL, Wambaugh JF, Sipes NS. httk: R Package for High-Throughput Toxicokinetics. J Stat Softw. 2017;79(4):1–26. Epub 2017/07/17. doi: 10.18637/jss.v079.i04. [PubMed: 30220889]

125. EPA US. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. Washington, D.C.: Office of the Science Advisor. Risk Assessment Forum., 2014.

126. Marzo M, Roncaglioni A, Kulkarni S, Barton-Maclaren TS, Benfenati E. In Silico Model for Developmental Toxicity: How to Use QSAR Models and Interpret Their Results. Methods Mol Biol. 2016;1425:139–61. Epub 2016/06/18. doi: 10.1007/978-1-4939-3609-0_8. [PubMed: 27311466]

127. Klaren WD, Ring C, Harris MA, Thompson CM, Borghoff S, Sipes NS, Hsieh JH, Auerbach SS, Rager JE. Identifying Attributes that Influence In Vitro-to-In Vivo Concordance by Comparing In Vitro Tox21 Bioactivity versus In Vivo DrugMatrix Transcriptomic Responses across 130 Chemicals. Toxicological sciences : an official journal of the Society of Toxicology. 2018 Epub 2018/09/12. doi: 10.1093/toxsci/kfy220.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Highlights

- The placenta is a critical tissue to evaluate for prenatal development toxicity testing
- *In vitro* placental models can screen hazardous chemicals
- Mechanism of action can be informed by *in vitro* placental models
- Doses eliciting prenatal toxicity can be estimated with *in vitro/in silico* methods
- *In vitro* approaches can be used in the modernization of chemical risk assessment

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

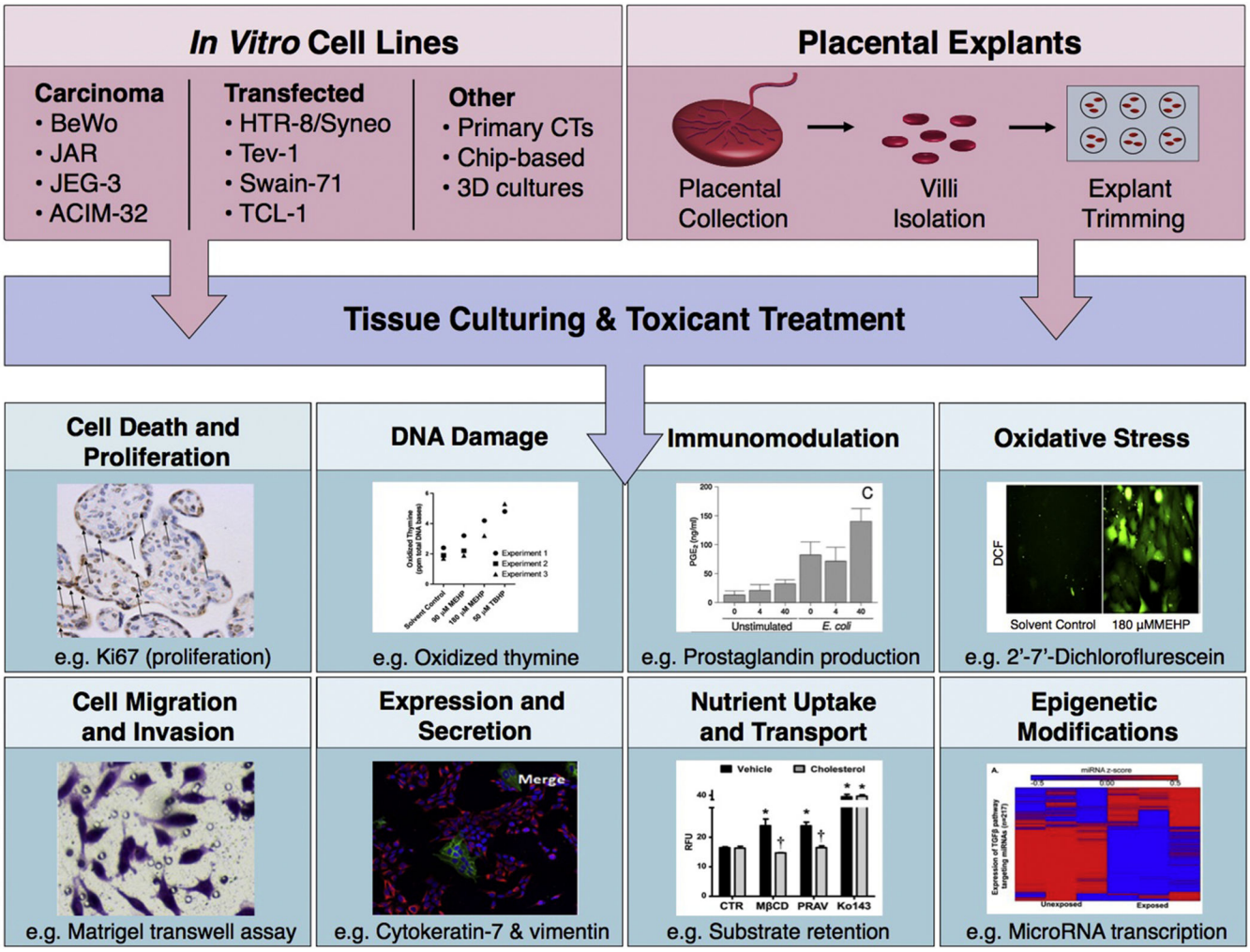

Figure 1. Selected endpoints that can be measured using *in vitro* test methods to evaluate chemical-induced toxicity in the placenta.

Some common endpoints evaluated using *in vitro* models are illustrated here. These endpoints can be assessed using *in vitro* placenta cell lines, placental cell co-cultures, placenta-on-a-chip, and 3-dimensional (3D) placental cell models, among others. Formal descriptions of the models and endpoints presented here are provided in Sections 3 and 4 of this review, respectively. Subheadings of model endpoints here correspond to those in Section 4 wherein experimental approaches and applications of each are detailed.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

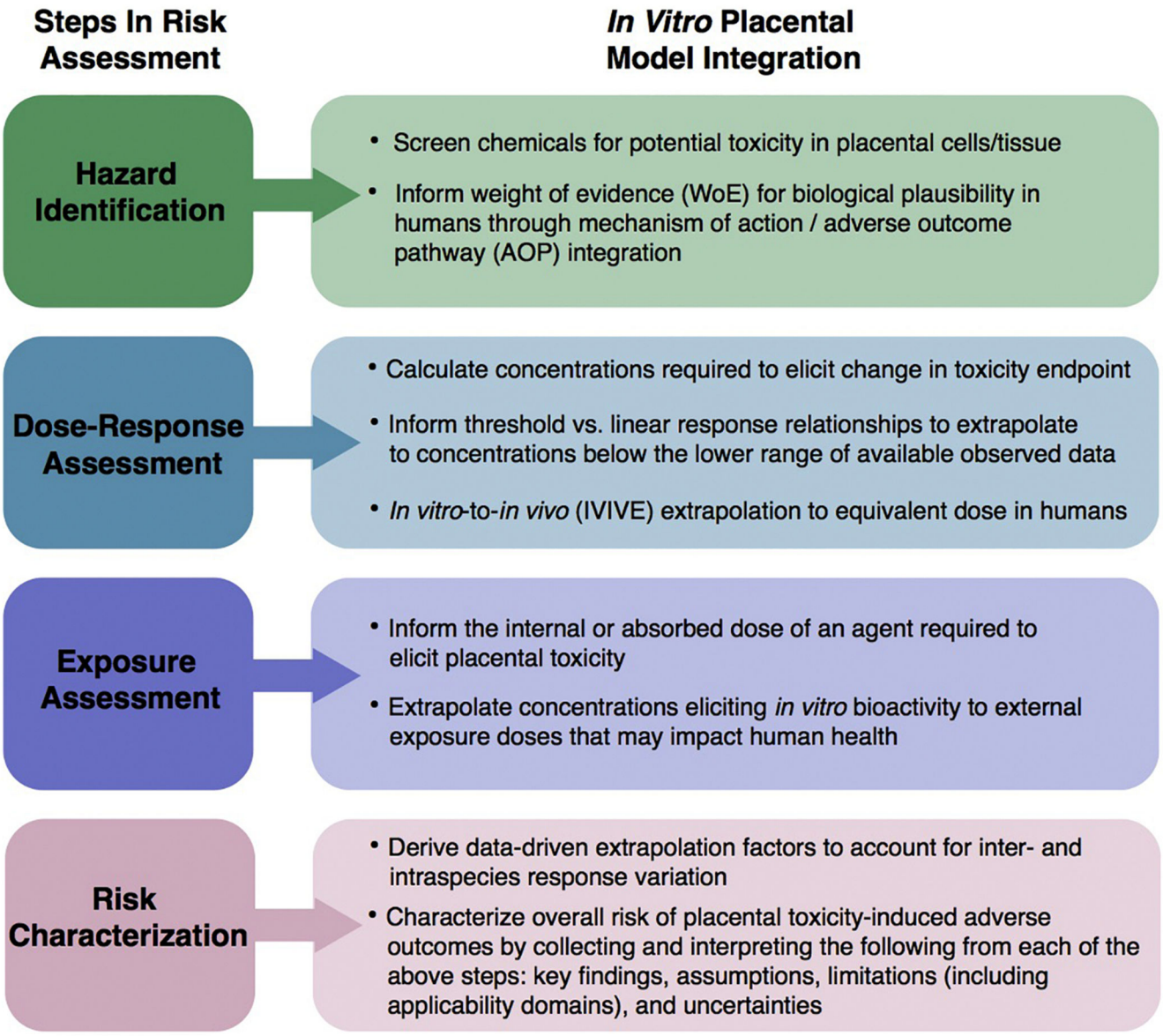

Figure 2. An overview of strategies that can be used to integrate *in vitro* placental models into the human health risk assessment process.

These strategies are aimed at the overall goal of reducing reliance upon animal testing and increasing confidence and efficiency in chemical safety evaluations.