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Using *in vitro* data to derive acceptable exposure levels: A case study on PBDE developmental neurotoxicity

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ABSTRACT

Background: Current acceptable chemical exposure levels (e.g., tolerable daily intake) are mainly based on animal experiments, which are costly, time-consuming, considered non-ethical by many, and may poorly predict adverse outcomes in humans.
Objective: To evaluate a method using human *in vitro* data and biological modeling to calculate an acceptable exposure level through a case study on 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) developmental neurotoxicity (DNT).
Methods: We reviewed the literature on *in vitro* assays studying BDE-47-induced DNT. Using the most sensitive endpoint, we derived a point of departure using a mass-balance *in vitro* disposition model and benchmark dose modeling for a 5% response (BMC_{05}) in cells. We subsequently used a pharmacokinetic model of gestation and lactation to estimate administered equivalent doses leading to four different metrics of child brain concentration (i.e., average prenatal, average postnatal, average overall, and maximum concentration) equal to the point of departure. The administered equivalent doses were translated into tolerable daily intakes using uncertainty factors. Finally, we calculated biomonitoring equivalents for maternal serum and compared them to published epidemiological studies of DNT.
Results: We calculated a BMC_{05} of 164 μg/kg of cells for BDE-47 induced alteration of differentiation in neural progenitor cells. We estimated administered equivalent doses of 0.925–3.767 μg/kg/day in mothers, and tolerable daily intakes of 0.009–0.038 μg/kg/day (composite uncertainty factor: 100). The lowest derived biomonitoring equivalent was 19.75 ng/g lipids, which was consistent with reported median (0.9–23 ng/g lipids) and geometric mean (7.02–26.9 ng/g lipids) maternal serum concentrations from epidemiological studies.
Conclusion: This case study supports using *in vitro* data and biological modeling as a viable alternative to animal testing to derive acceptable exposure levels.

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1. Introduction

Currently, over 85,000 chemicals are on the market, with more entering every year (Krimsky, 2017). However, health hazard information is lacking for most commercial chemicals. Whole animal models are considered the traditional "gold standard" to investigate deleterious endpoints and determine points of departure (PODs) for risk assessment and regulatory decision-making. Unfortunately, the use of animal models comes with multiple limitations. *In vivo* animal testing requires a large number of laboratory animals to assess a single chemical and is very costly and time-consuming, and causes ethical concerns (Hartung, 2017; Schmidt, 2009). Providing necessary safety assessments for all these chemicals within an acceptable time frame is impractical, leaving many chemicals data-poor.

The scientific community has long been aware of the uncertainties associated with and the limited predictive power of animal models, as evident from post-market withdrawal of numerous drugs in the pharmaceutical sector (Blaauboer, 2010; Hartung, 2013) and lack of concordance in the chemical sector, with 60 % concordance for reproductive toxicity testing (Hartung, 2009). To shift away from an animal-centered paradigm of testing, the influential National Research Council (2007) report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*, recommended using data from *in vitro*, human cell-based assays as initial substrates for chemical safety assessments. In addition, legislative mandates such as the European Union cosmetic animal testing ban (Sreedhar et al., 2020), and amendments to the U.S. Toxic Substance Control Act (Rayasam et al., 2022) and to the Canadian Environmental Protection Act ("Strengthening Environmental Protection for a Healthier Canada Act," 2023) now aim to replace, reduce, and refine the use of vertebrate animals in chemical safety testing. With this global shift, interest in the field of *in silico* predictive tools offered in conjunction with *in vitro* testing has increased to bypass the use of animal models. However, there are still obstacles to overcome in order to increase confidence in *in silico* models for regulatory decision-making purposes.

Efforts to phase out animal testing include recent advances in the use of new approach methodologies (NAMs) using *in vitro* and *in silico* methods to bypass the use of animal models for risk-based prioritization of chemicals (Schmeisser et al., 2023). A study exploring the use of *in vitro* bioactivity demonstrated that the PODs of 448 substances derived through high-throughput *in vitro* bioactivity were mostly below to those determined through animal/traditional approaches (Friedman et al., 2020). The applicability of this method was then successfully demonstrated through a collaboration between Health Canada and the US EPA (Beal et al., 2022). In total, 5,801 chemicals were profiled, and 95 % were shown to have conservative bioactivity PODs when compared to those derived through traditional methods. Though some have evaluated this approach against animal data (Honda et al., 2019), few efforts have been made to validate this approach against human data.

Another way to evaluate the use of *in vitro* data and biological modeling for human health risk assessment is to compare guidance values derived using this approach to data from epidemiological studies reporting associations between exposure and adverse health outcomes. Evaluation through case studies on chemicals with extensive *in vitro* and epidemiological data will increase confidence in the approach and potentially facilitate regulatory uptake. For example, the developmental neurotoxicity of polybrominated diphenyl ethers (PBDEs) has been documented in both *in vitro* studies and numerous longitudinal birth cohorts, and could be used as a case chemical to evaluate the approach (Verner et al., 2011).

PBDEs are flame retardants that have been used in the manufacture of a variety of consumer products, like electronics and upholstered furniture (Buttke et al., 2013). Studies demonstrating the toxicity of PBDEs led many countries including Canada and the United States to regulate their manufacture, sale, and use. Despite these regulations, PBDEs are still present in multiple products dating back before the bans, and it has been estimated that products containing PBDEs will remain in use for years to come (Abbasi et al., 2015). PBDEs can leach out of products and become available for absorption in humans; biomonitoring surveys have shown that a large portion of the general North American population have detectable plasma levels of predominant PBDE congeners (Arbuckle et al., 2013; Fisher et al., 2016; Foster et al., 2011; Oulhote et al., 2018). Many congeners have biological half-lives exceeding one year (Geyer et al., 2004; Song et al., 2016; Trudel et al., 2011; Wong et al., 2013), which means that even after exposure has ceased, accumulated body burdens take years to decrease.

PBDEs have been shown to cross the placenta during pregnancy and reach the developing fetus (Aylward et al., 2014), as well as to transfer into breast milk during lactation (Marchitti et al., 2013). In addition to lactational exposure, children are extensively exposed through the ingestion of dust containing PBDEs (Sjödin et al., 2008). These exposures occur at a time of rapid growth and development of the central nervous system (Rice & Barone, 2000) and the immune system (Dietert, 2015). Many studies have documented PBDE levels during childhood; where children's levels were compared to levels in adults, they were generally higher (Toms et al., 2008; Toms et al., 2009). Multiple epidemiological studies evaluated the long-term effects of prenatal exposure to PBDE congeners, namely 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), on neurodevelopment (Azar et al., 2021; Braun et al., 2017; Chen et al., 2014; Chevrier et al., 2016). Although not all PBDE congeners were associated with all evaluated neurodevelopmental endpoints, the evidence seems sufficient to support an association between prenatal PBDE exposure and altered neurodevelopment in children in observational studies (Gibson et al., 2018; Messer, 2010).

In this paper, our objective was to evaluate an approach using *in vitro* data for human health risk assessment through a case study on BDE-47 developmental neurotoxicity. Specifically, we aimed to i) derive tolerable daily intake and biomonitoring equivalent values through using human *in vitro* data and biological modeling, and to ii) compare derived biomonitoring equivalent values with concentrations measured in mothers participating in longitudinal birth cohort studies of prenatal PBDE exposure and neurodevelopmental outcomes.

2. Methods

2.1. Methods overview

We created a flowchart (Fig. 1) to aid in the visualization of the methodology. Briefly, a literature review was undertaken to compile available *in vitro* assays utilizing human cells with endpoints relating to BDE-47 and neurodevelopmental toxicity. Although we recognize that mass-balance modeling should ideally be performed for each experiment prior to critical study selection, model inputs (e.g., description of serum contents in medium, dissolved organic matter concentration) are most often not provided. Here, we decided to first select a study with the lowest nominal POD based on phenotypic response. Once a study was selected, a nominal (i.e., initial medium concentration) POD was determined using benchmark dose modeling. This nominal POD was then converted into an intracellular concentration through mass-balance modeling. We assumed this POD in cells reflects the internal POD at the effective site. To determine the administered equivalent dose, i.e., the maternal intake leading to the internal POD, we employed a pharmacokinetic model in a reverse dosimetric fashion. Finally, uncertainty factors were applied to derive the biomonitoring equivalent and tolerable daily intake values.

2.2. Compilation of available in vitro dose–response data

We compiled *in vitro* dose–response data for this study using the PubMed database. Keywords were "PBDE", "neurodevelopmental toxicity", "developmental neurotoxicity", "human", "*in vitro*", and "neuroblastoma". Given that we were only interested in *in vitro* data based on human cells in line with the perspective of animal-free human

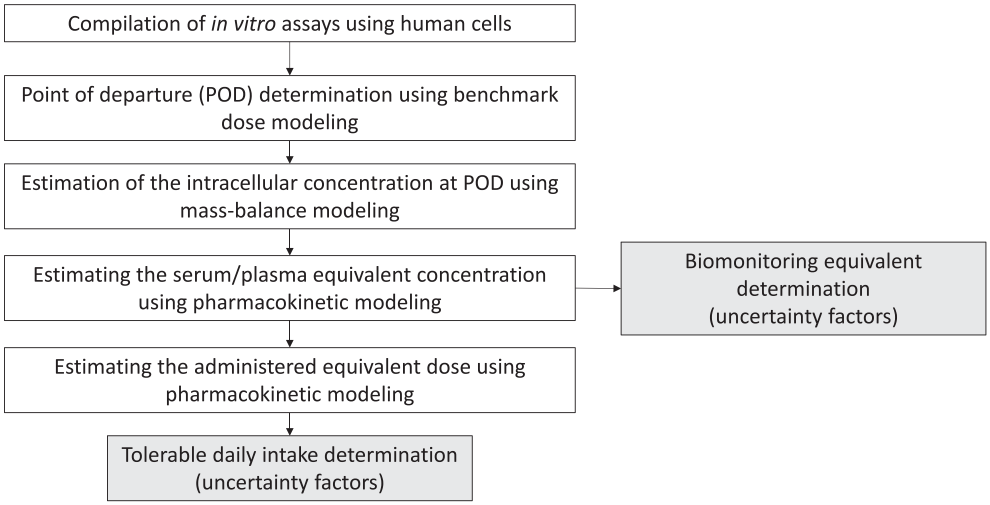

Fig. 1. Methods Flowchart for the determination of tolerable daily intake and biomonitoring equivalent values using *in vitro* data and biological modeling.

health risk assessment, we excluded any animal-based data. Furthermore, as our study focuses on neurodevelopmental toxicity, we only included data based on cells relating to neurodevelopment, such as neuroblastoma SK-N-SH and SK-N-MC cell lines, human 132–1 N1 astrocytoma cells, primary fetal neural human neural progenitor cells (hNPCs), and human embryonic stem cells (hESC). Due to the shortage of studies satisfying all our criteria, no limitation was placed on publication dates. Compiled data is presented in Table 1.

2.3. Determination of an in vitro point of departure

For our study, we performed our calculations using the data of Klose et al. (2022), which demonstrated significant phenotypic responses with the lowest doses of BDE-47 (LOAEC 0.03 μM). This LOAEC was lower than that for other phenotypic responses in the literature, which is consistent with a recently published report where oligodendrocyte differentiation was the most sensitive endpoint for multiple chemicals in a battery of *in vitro* assays of neurotoxicity (Masjosthusmann et al., 2020). For this case study, we elected not to use genotypic responses as a POD because we assumed this response may be an early response that may not necessarily result in a deleterious phenotypic manifestation. Also, additional analyses would have been necessary to confirm that the transcriptomic changes observed in these *in vitro* studies are reliable to derive a POD for human health risk assessment (Johnson et al., 2022).

Raw data from the Klose et al. (2022) study was used to determine a POD (available in Supplementary Material). Briefly, 3D human primary neural progenitor cells (hNPC)-based neurospheres with a diameter of 0.3 mm were exposed to 0 μM, 0.03 μM, 0.08 μM, and 0.25 μM of BDE-47. Exposure occurred over 5 days in flat-bottom 96-well plates, coated with Poly-D-Lysine and laminin. 100 μL of media per well was constituted of DMEM and Hams F12 (3:1) supplemented with 1 % N2 and 1 % penicillin and streptomycin. No serum was added to solution. Half of the medium volume was replaced on day three (added medium with the same BDE-47 concentrations mentioned above).

2.4. BMDS modeling and comparison with ToxCast data

To determine a POD, we used U.S. EPA's benchmark dose software (https://www.epa.gov/bmds). Briefly, constraints chosen for the program were a frequentist analysis type, and a benchmark response (BMR) for a relative deviation of 5 % compared to control, as recommended by Health Canada and in the literature (Haber et al., 2018). Model selection was then determined by the BMDS program which recommended viable benchmark concentration (BMC_{05}) according to the global goodness-of-fit p-value > 0.1, lowest AIC, BMC:BMCL < 3, and lowest BMCL. We visually inspected model fit to assess goodness-of-fit.

We compared the POD to the ToxCast data on BDE-47 compiled on the CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/). The CompTox Chemicals Dashboard provides a graphical representation of the concentrations associated with 50 % of the maximum bioactivity (AC_{50}) in ToxCast assays. It also includes the cytotoxicity limit (μM) (labeled as lower bound in the graph) and median cytotoxicity concentration.

2.5. Chemical disposition in the in vitro system

To estimate cellular levels of BDE-47 associated with the media concentration-based BMC_{05} within the system described by Klose et al. (2022), we first used the IV-MBM DP v1.0 dynamic mass-balance model (Bloch et al., 2022). This mass-balance model estimates the distribution of the chemical within the different compartments of the *in vitro* environment, and calculates the concentration in air, albumin, lipids, dissolved organic matter, free in solution, and in cells. Subsequently, the model calculates the enrichment factor through the formula:

$$\boldsymbol{EF} = \frac{\boldsymbol{C_{cells}}}{\boldsymbol{C_{nominal}}} \quad (1)$$

Where EF represents the enrichment factor, C_{cells} is the cellular concentration estimated by the mass-balance model, and $C_{nominal}$ is the nominal concentration. Then, to determine the intracellular concentration of BDE-47 capable of soliciting a 5 % response (point of departure in cells [POD_{cells}]), we devised the following formula:

$$\boldsymbol{POD_{cells}} = \boldsymbol{BMC_{0.05}} \times \boldsymbol{MM} \times \boldsymbol{EF} \quad (2)$$

Where POD_{cells} is the intracellular concentration (μg/kg) in hNPCs based on the nominal $BMC_{0.05}$ (μM) derived using BMDS, the MM is the molar mass of BDE-47 (μg/μmol), and the EF is the enrichment factor estimated using the mass-balance model. The unit conversion in this equation, which assumes that cells weigh 1 kg per L, is a necessary step for the subsequent calculations using our toxicokinetic model. For more information regarding the parameterization of the mass-balance model, refer to the Supplementary Material.

2.6. Toxicokinetic modeling

To estimate tolerable daily intake and biomonitoring equivalent values, we used a toxicokinetic model of maternal, gestational and lactational exposure to persistent organic pollutants (Verner et al., 2013). The toxicokinetic model used in this study is a two-compartment

Table 1
Compilation of *in vitro* data on bde-47 using human-based cells and with neurodevelopmental toxicity as the adverse endpoint.

Reference	Experimental model	End-point and potential PODs
He et al. (2008)	SH-SY5Y Neuroblastoma	Reactive oxygen species • NOAEC = 1 μg/mL (2.06 μM) • LOAEC = 2 μg/mL (4.12 μM) Apoptotic cells • NOAEC = 2 μg/mL (4.12 μM) • LOAEC = 4 μg/mL (8.23 μM) DNA damage • LOAEC = 1 μg/mL (2.06 μM) • Nuclear division index • NOAEC = 1 μg/mL (2.06 μM) • LOAEC = 2 μg/mL (4.12 μM) LDH leakage • NOAEC = 2 μg/mL (4.12 μM) • LOAEC = 4 μg/mL (8.23 μM) Cell viability • LOAEC = 1 μg/mL (2.06 μM)
Gao et al. (2008)	SH-SY5Y Neuroblastoma	Olive tail moment and increased DNA in the tail • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Gao et al. (2009)	SH-SY5Y Neuroblastoma	Reactive oxygen species • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Gao et al. (2009)	SH-SY5Y Neuroblastoma	DNA damage • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Schreiber et al. (2010)	Primary fetal neural progenitor cells (hNPCs)	hNPCs migration inhibited • NOAEC = 0.1 μM (0.05 μg/mL) • LOAEC = 1 μM (0.49 μg/mL) • hNPC differentiation inhibited • NOAEC = 0.1 μM (0.05 μg/mL) • LOAEC = 1 μM (0.49 μg/mL) • Proliferation (no effect over time)
Tagliaferri et al. (2010)	SK-N-MC Neuroblastoma	Cell viability • NOAEC = 2.5 μM (1.22 μg/mL) • LOAEC = 5 μM (2.43 μg/mL) • BMD_{10} = 3.6 μM (1.75 μg/mL) • $BMDL_{10}$ = 3.1 μM (1.50 μg/mL)
He et al. (2010)	SH-SY5Y Neuroblastoma	Cell viability • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL) LDH leakage • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Pellacani et al. (2012)	SK-N-MC Neuroblastoma	DNA damage: No significance detected at 5, 10, or 20 μM
Jiang et al. (2012)	SH-SY5Y Neuroblastoma	Reactive oxygen species generation • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Zhang et al. (2013)	SH-SY5Y Neuroblastoma	Apoptosis • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Gassmann et al. (2014)	hNPCs	Ca^{2+} homeostasis (calcium increase) • NOAEC = 2 μM (0.97 μg/mL) • LOAEC = 20 μM (9.72 μg/mL)
Tian et al. (2016)	SH-SY5Y Neuroblastoma	Migration • LOAEC = 0.1 μM (0.05 μg/mL)
Zhang et al. (2016)	SH-SY5Y Neuroblastoma	Ca^{2+} homeostasis • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL) Apoptosis • NOAEC = 5 μM (2.43 μg/mL) • LOAEC = 10 μM (4.86 μg/mL)
Zhang et al. (2017)	SH-SY5Y Neuroblastoma	Increased autophagosomes • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Harrill et al. (2018)	Human Neuroprogenitor Cells (hNP1)	Proliferation • EC_{30} = 28.2 μM (13.68 μg/mL) Viability • EC_{30} = 28.0 μM (13.58 μg/mL)
	Human Neurons (hN2)	Neurite initiation • EC_{30} = 7.0 μM (3.4 μg/mL) Viability • EC_{30} = 2.6 μM (1.26 μg/mL)
Chen et al. (2019)	Human embryonic stem cells (hESC)	Neural precursor cells decreased viability and increased LDH activity • NOAEC = 10 μM (4.86 μg/mL) • LOAEC = 25 μM (12.15 μg/mL) hNPC proliferation • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 10 μM (4.86 μg/mL) • hNPC neurosphere expansion • NOAEC = 1 μM (0.49 μg/mL) • LOAEC = 5 μM (2.43 μg/mL)
Liang et al. (2019)	hESC	Gene expression (related to neurodevelopment) • PAX6 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • NES LOAEC = 10e-3 μM (4.86e-3 μg/mL) • TFAP2A LOAEC = 10e-3 μM (4.86e-3 μg/mL) • SOX1 NOAEC = 10e-3 μM (4.86e-3 μg/mL) LOAEC = 10e-2 μM • SOX2 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • SOX3 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • CNTN2 NOAEC = 10e-2 μM LOAEC = 1 μM (0.49 μg/mL) • SLIT1 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • LRRC4C LOAEC = 10e-3 μM (4.86e-3 μg/mL) • RELN (no significance) • CBLN1 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • CHRNB4 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • ZIC1 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • ZIC3 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • HES3 (no significance) • RFX4 (no significance) • IGFBP3 LOAEC = 10e-3 μM (4.86e-3 μg/mL) • DLX5 LOAEC = 10e-3 μM (4.86e-3 μg/mL)
Klose et al. (2022)	hNPC	Oligodendrocyte differentiation • LOAEC = 0.03 μM (1.46e-2 μg/mL)

EC_{30}: Effective concentration value resulting in an absolute 30% change from control; LOAEC: Lowest observed adverse effect concentration; NOAEC: No observed adverse effect concentration.

model of the lipids in the mother and the child, as it is assumed that the highly lipophilic PBDEs will predominantly distribute to body lipids. As Fig. 2 demonstrates, the model assumes the mother is exposed to the chemical by ingestion, while inhalation and dermal exposures are negligible. Partition coefficients specific to BDE-47 were used to calculate the transfer of chemical during pregnancy and breastfeeding.

For all simulations, the maternal age at delivery was set to 31.4 years with a prepregnancy body weight of 78.1 kg. The child was parameterized to be born with a birthweight of 3.5 kg. The half-life of BDE-47 was set to 1.19 years (10424.4 h) (Sjödin et al., 2020), the partition coefficient for cord plasma lipids to maternal plasma lipids was 0.84 (Zheng et al., 2017), and the partition coefficient for breastmilk lipids to maternal plasma lipids was 1.46 (Marchitti et al., 2013). Because the concentrations in the toxicokinetic model are expressed on a lipid basis (assuming homogenous distribution in body lipids, including brain lipids), we determined the wet weight concentration of BDE-47 (μg/kg)

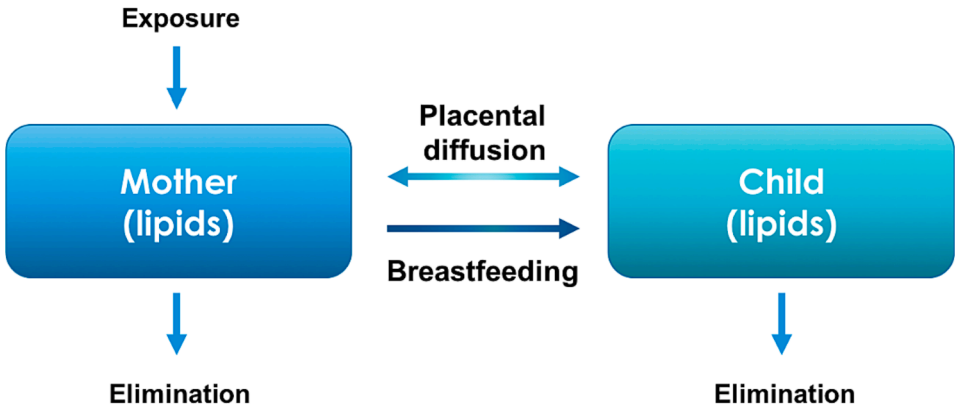

Fig. 2. Conceptual representation of the Toxicokinetic model (Verner et al., 2013).

in the child brain (consistent with estimated cell concentration in the *in vitro* system) using the following formula:

$$C_{brain}(\mu g/kg) = C_{child}(\mu g/kg lipids) \times Fl_{brain} \quad (3)$$

Where C_{child} is the lipid adjusted concentration of BDE-47 in the child compartment and Fl_{brain} is the fraction of lipids in the newborn child's brain, which was set to 0.026 (White et al., 1991).

The brain develops both prenatally and postnatally, including in terms of differentiation (critical effect identified herein) (Rice and Barone, 2000). To account for the potential windows of vulnerability to BDE-47 neurotoxic effects, we determined exposure metrics for average prenatal, average postnatal (0–12 months), average overall (conception-12 months), and maximum (Cmax) concentrations.

2.7. Toxicokinetic model evaluation

Although the toxicokinetic model used herein has been evaluated against measured plasma concentrations in children for many persistent organic pollutants (e.g., polychlorinated biphenyls, hexachlorobenzene), it hasn't been evaluated against PBDE concentrations. To evaluate the accuracy of the toxicokinetic model, we investigated the relationship between maternal concentrations and children's concentrations by comparing simulated levels to longitudinal measurements from the Markers of Austism Risk in Babies – Learning Early Signs (MARBLES) study (Hertz-Picciotto et al., 2018). In this study, blood samples were obtained from a subset of mothers during their first (n = 14), second (n = 24), and third (n = 46) trimester, and at delivery (n = 17). Children's blood samples were drawn at 12 (n = 12), 24 (n = 42) and 36 months (n = 44). Sample preparation and analysis of PBDEs in plasma was performed as detailed in Lin et al. (2013). We calculated child:mother concentration ratios for each dyad, using maternal concentration at delivery when available, or maternal concentrations earlier during pregnancy when concentrations at delivery were unavailable. Because the focus of this study is the first year of life, we evaluated simulated child:mother concentration ratios measured at 12 months in the MARBLES study. The original data/sample collection was approved by the University of California-Davis institutional review board. PBDE measurement in maternal and child plasma samples, and data analyses for the current study were approved by the Université de Montréal institutional review board (CERES-16–095-D). Participants provided written informed consent before collection of any data/sample.

2.8. Estimating biomonitoring equivalent values

We derived biomonitoring equivalent values for maternal plasma BDE-47 during pregnancy using an *in vitro* POD based on the guidelines of (Hays et al., 2008). Due to the impracticality of collecting biological samples from the developing fetus and child, maternal plasma BDE-47 at conception (representing women of childbearing age in biomonitoring surveys) was chosen as the most practical matrix and biomarker to determine a biomonitoring equivalent. Therefore, we used the toxicokinetic model to estimate maternal plasma concentrations at conception associated with children's brain BDE-47 concentration matching the *in vitro*-derived POD_{cells}.

In the guidelines for the derivation of biomonitoring equivalents (Hays et al., 2008), there are suggestions to apply certain portions of the uncertainty factors before and/or after the application of the toxicokinetic model. However, to harmonize biomonitoring equivalent and tolerable daily intake calculations, we used the uncertainty factors at the end of our calculations. Given the linearity of the toxicokinetic model (no saturable process), the results were not affected by whether the uncertainty factors are applied before or after toxicokinetic modeling (results not shown). For the biomonitoring equivalents, we used uncertainty factors of 10 for subchronic-chronic extrapolation to correct for the short duration of the *in vitro* experiment, and 10 for interindividual variability (composite factor of 100). We decided to use the full uncertainty factor of 10 for interindividual variability because the biomonitoring values are derived for maternal plasma BDE-47 concentrations at conception, and the concentrations in the target tissue (fetus/child) may vary for a given maternal concentration based on parameters like gestational weight gain, milk:plasma partitioning and breast milk consumption.

2.9. Calculating a tolerable daily intake

We calculated a tolerable daily intake based on the *in vitro* POD_{cells}. We used the toxicokinetic model presented above to estimate a lifetime maternal daily dose (μg/kg/d) associated with a fetal/child brain concentration equal to the POD_{cells}. The lifetime maternal daily dose was subsequently divided by uncertainty factors: 10 for subchronic-chronic extrapolation, and 10 for interindividual variability (composite factor of 100).

2.10. Compilation of exposure levels in epidemiologic studies and comparison with estimated biomonitoring equivalent values

We compiled epidemiological studies investigating associations between BDE-47 maternal exposure and signs of developmental neurotoxicity through PubMed. Key words used were "polybrominated diphenyl ethers", "BDE-47", "neurodevelopment", "cognitive", "behavior", and "psychomotor". Criteria for selection was limited to human studies for which exposure was measured in serum or plasma of pregnant women or children under two years of age. Measurements of neurodevelopment were cognitive, behavioral, and/or psychomotor in children, with results presented for BDE-47. Distributions of BDE-47

concentrations were extracted for available matrices. Concentration thresholds (e.g., analyses based on exposure quantiles) were explored and taken into consideration where available. Biomonitoring equivalents derived in this study were compared with distributions of BDE-47 concentrations in epidemiological studies and thresholds where available.

3. Results

3.1. Determination of a BMC_{05} and comparison with ToxCast data

When performing benchmark dose modeling, two viable benchmark concentrations were determined by the BMDS program: one was the Hill frequentist restricted model, while the other was the Frequentist polynomial degree 2 unrestricted model.

As seen in Table 2, both models displayed good visual goodness-of-fit with global goodness-of-fit p-values > 0.1. In the case of the AIC, the Hill model yielded the lowest AIC among all the models. Both models generated acceptable BMC:BMCL ratios < 3. However, the BMDS program determined that the Frequentist Polynomial degree 2 model produced the most viable BMC since it had the lowest BMCL. Therefore, we utilized the nominal BMC_{05} of 0.004477 μM as a POD for this study (see Fig. 3).

ToxCast data compiled on the CompTox Chemicals Dashboard showed a cytotoxicity median of 37.06 μM and a lower bound of 6.586 μM (see Fig. 4). The median and lower bound concentrations associated with cytotoxicity were 4 and 3 orders of magnitude greater than our chosen POD, respectively. The lowest AC_{50}s were related to neurodevelopment, with a range of 0.013 μM to 0.092 μM, all generated in the study by Shafer et al. (2019). In this study, Shafer et al. (2019) analyzed bioactivity in a neural formation assay for a set of chemicals which includes BDE-47. Of note, this range is consistent with the LOAEC produced by Klose et al. (2022), although a direct comparison would require working out the *in vitro* disposition in the assays by Shafer et al. (2019). Our POD was about three times lower than the lowest AC_{50}.

3.2. Determination of an in vitro point of departure

The timeframe of the experiments conducted by Klose et al. (2022) was 120 h. To determine the cellular enrichment factor at 120 h, we parametrized the IV-MBM DP v1.0 dynamic mass-balance model (Bloch et al., 2022) according to the *in vitro* environment described by Klose et al. (2022). At day 3, 50 % of the medium was replaced with fresh medium solution containing the nominal concentration of BDE-47 resulting in an increased enrichment factor after 48 h. Therefore, at 120 h, the enrichment factor was predicted to be 75.4 (see Fig. 5).

Given BDE-47 molecular weight of 485.79 g/mol, a calculated enrichment factor of 75.4, and assuming 1 L of hNPCs weighs 1 kg, we estimated the POD_{cells} to be 164 μg/kg of cells. This represents the concentrations in cells in the *in vitro* system at the BMC_{05}.

Table 2
BMDS viable model characteristics.

	Hill (restricted)	Frequentist polynomial degree 2 (unrestricted)
BMC_{05} (μM)	0.024979	0.004477
$BMCL_{05}$ (μM)	0.024142	0.003147
Goodness of fit	*Good*	*Good*
Test 4 p-value	0.5990853	0.1149718
AIC	10.99592698	13.20406437
BMC:BMCL ratio	1.03	1.422
BMDS recommendation	Viable - Alternate	Viable - Recommended

3.3. Toxicokinetic model evaluation

The median child/mother BDE-47 ratio calculated from the MARBLES data (Hertz-Picciotto et al., 2018) was 2.5 ng/g lipids with a range of 1.2 to 5.0 ng/g lipids. As shown in Fig. 6, the child/mother BDE-47 ratio simulated with the toxicokinetic model at 12 months (3.6) is higher than the median (2.5) but falls within the range of data gathered by the MARBLES study. It is important to note that our model estimates BDE-47 for a child breastfed for 12 months, while the data of the MARBLES study is gathered from children breastfed for varying amounts of time, or not at all.

3.4. Determining tolerable daily intake and biomonitoring equivalent values

As previously mentioned, we used a toxicokinetic model and calibrated maternal daily doses to match four metrics of child brain concentration (see simulation example in Fig. 7); we derived four administered equivalent doses (i.e., maternal doses) leading to a BDE-47 brain concentration of 164 μg/kg i) on average for the prenatal period (administered equivalent dose: 3.767 μg/kg/d), ii) on average for the postnatal period (administered equivalent dose: 1.201 μg/kg/d), iii) on average for the overall period from conception to 12 months postnatally (administered equivalent dose: 1.706 μg/kg/d), and iv) as the maximum concentration (administered equivalent dose:0.925 μg/kg/d). The fact the administered equivalent dose is lowest when based on the maximum concentration metric may seem counterintuitive. The maximum concentration in the child brain in our simulations occurred at the end of the breastfeeding period, when the child reaches 12 months of age (see Fig. 7). To reach a maximum concentration of 164 μg/kg in the child brain, we estimated that mothers need to be exposed to 0.0925 μg/kg/d. For the child brain to reach 164 μg/kg on average over a period of time, the maternal dose needs to be higher (1.706 ug/kg/d). For a graphical representation of concentration profiles in the child brain, maternal compartment and child compartment, see Figure S6 in the Supplementary Material.

Next, we applied uncertainty factors to calculate tolerable daily intakes related to these four maternal daily doses. Using a composite uncertainty factor of 100, the tolerable daily intakes ranged between 0.009 and 0.038 μg/kg/d (Table 3).

3.5. Compilation of exposure levels in epidemiologic studies and comparison with estimated biomonitoring equivalent values

A graphic summary of a compilation of measured BDE-47 in maternal and child blood found in epidemiological studies alongside biomonitoring equivalents are presented in Fig. 8. Our collection includes data from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) (Eskenazi et al., 2013; Sagiv et al., 2015), the Health Outcomes and Measures of the Environment (HOME) (Braun et al., 2017; Chen et al., 2014; Hartley et al., 2022; Vuong, Yolton, et al., 2017; Vuong et al., 2016), the Groningen Infant Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens (GIC) (Roze et al., 2009), the World Trade Center Cohort (Cowell et al., 2015; Herbstman et al., 2010), the Menorca Birth Cohort (part of the INMA project) (Gascon et al., 2011), the Shanghai-Minhang Birth Cohort Study (Ji et al., 2019), Laizhou Wan (LW) Birth Cohort (Ding et al., 2015), and the Maternal-Infant Research on Environmental Chemicals (MIREC) cohort (Azar et al., 2021; Oulhote et al., 2018). The biomonitoring equivalents based on the maximum concentration and average postnatal (19.75 and 25.29 ng/g lipids) (Table 2) were close to medians from the epidemiological studies (0.19–20.2 ng/g lipids). The complete compilation including respective sample sizes, biological matrices studied, and geometric means can be found in the Supplementary Material.

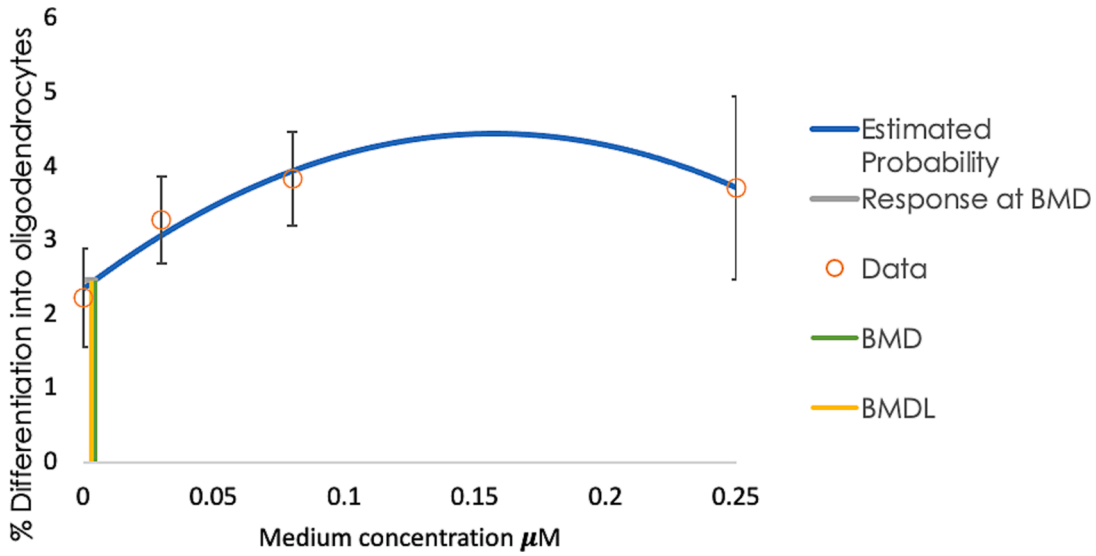

Fig. 3. Benchmark dose modeling graph generated by BMDS 3.1.2 for the data of percent of differentiation into oligodendrocytes. The model selected (modeled response in blue) was the frequentist polynomial degree 2 model. The Benchmark response was set to 5%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

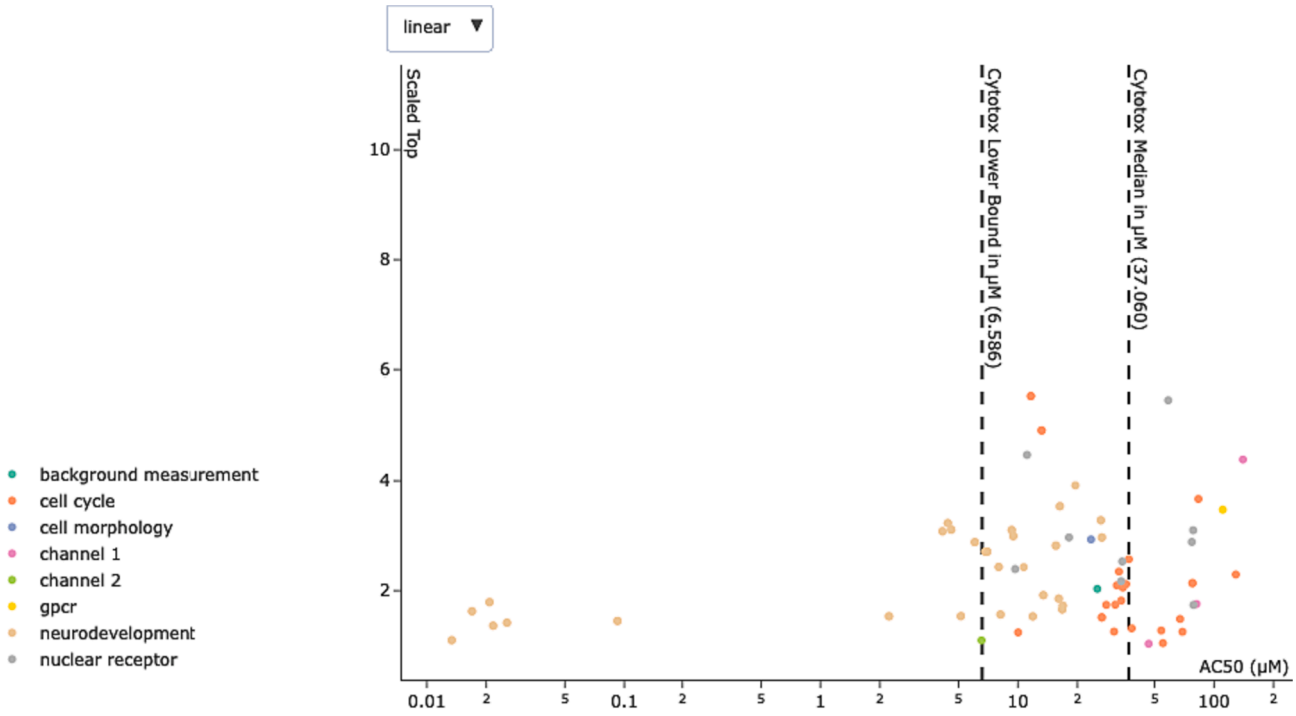

Fig. 4. CompTox Chemicals Dashboard generated graph of ToxCast AC_{50} bioactivity Data for BDE-47. Lower bound and median Cytotoxicity were 6.586 and 37.060 μM, respectively. (https://comptox.epa.gov/dashboard/chemical/invitrodb/DTXSID3030056; retrieved may 12, 2023).

4. Discussion

Over the last few years, regulatory agencies aimed to adopt NAMs for chemical risk assessment. To date, *in vitro*-derived data has been employed in screening for chemical prioritization (Beal et al., 2022; Friedman et al., 2020). Here, we aimed to evaluate a new way to employ human *in vitro* data and biological modeling to estimate tolerable daily intake and biomonitoring equivalent values using the case study of BDE-47. We compared our calculated tolerable daily intakes with existing values, and biomonitoring equivalents with concentrations measured in epidemiological studies investigating associations between early-life BDE-47 exposure and neurodevelopmental toxicity.

As seen in the flowchart of the methods section (Fig. 1), our first step was the determination of an *in vitro* POD with an endpoint relating to neurodevelopment. In the case of BDE-47, multiple *in vitro* endpoints exist, but it is difficult to determine which endpoint may be directly related to the neurodevelopmental assessments conducted in epidemiological studies. For the purpose of this study, we determined oligodendrocyte differentiation by Klose et al. (2022) as the most sensitive endpoint with a toxic effect established at the lowest tested concentration of 0.03 μM. By comparison, the lowest AC_{50} displayed on the CompTox Chemicals Dashboard was a concentration of 0.013 μM from

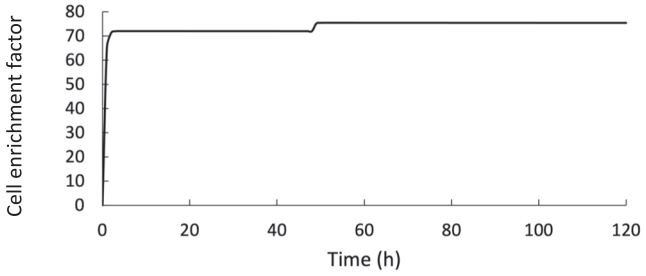

Fig. 5. Dynamic profile of the cell enrichment factor over time, as predicted by the IV MBM L4 model (Bloch et al., 2022).

an assay using rat-derived cortical tissue cells and targeting neurodevelopment. This lends support to the selection of a POD in the nM range, although a mass-balance assessment of *in vitro* disposition in the rat cell assay would need to be performed to compare PODs at the cellular concentration level.

We employed benchmark dose modeling to define the BMC_{05} denoting a 5 % change in response. As mentioned before, multiple rationales exist to resolve which percent change in response to investigate, as well as whether to use the lower bound concentration of the BMC for the following calculations (Haber et al., 2018). For the purpose of our study, we chose to adopt the rationale of Health Canada, which recommends the use of the BMD resulting in a 5 % change in response, which is estimated to be within the range of the $BMDL_{10}$ resulting in a 10 % change in response (Haber et al., 2018). Two viable models were presented by the BMDS program, both with agreeable goodness of fit, and global goodness of fit p-values > 0.1; however, the Hill model exhibited the lowest AIC, denoting the best model fit. Nonetheless, we

Fig. 6. Ratio of simulated child BDE-47 concentration (ng/g lipids) to maternal BDE-47 concentration (ng/g lipids) at delivery. The data point at 1 year represents the median BDE-47 concentration ratio from the MARBLES study, and the error bars represent the minimum and maximum concentration ratios.

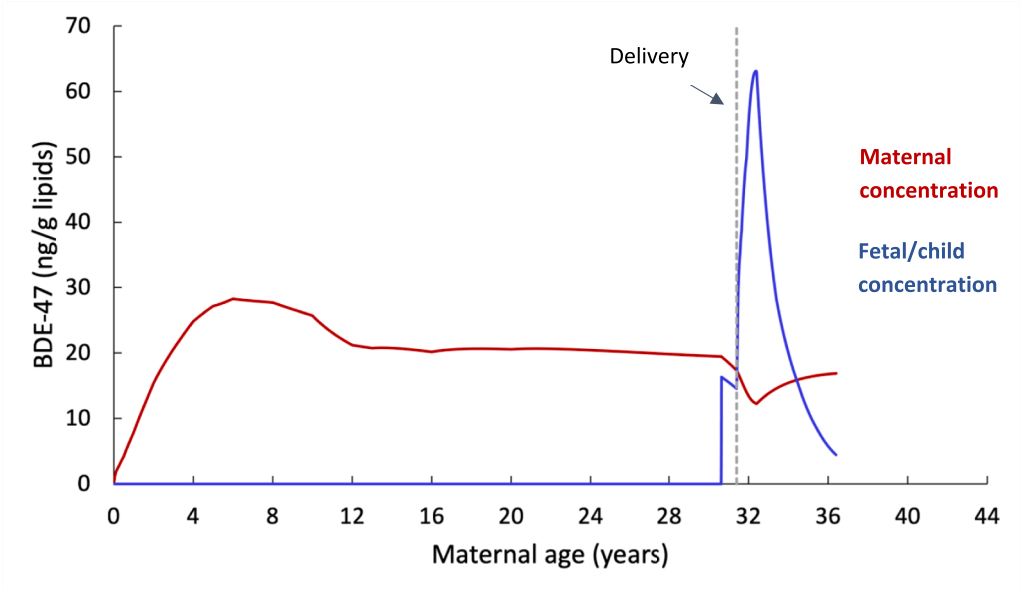

Fig. 7. Graph generated by the toxicokinetic model displaying BDE-47 concentrations (μg/kg or ng/g lipids) in the fetus/child and maternal serum as a function of maternal age. Based on An estimated TDI 0.00925 μg/kg/day.

Table 3
Calculated administered equivalent dose, tolerable daily intake and biomonitoring equivalent values matching metrics of concentration in child brain (164 μg/kg).

Metric of fetal/child exposure	Administered equivalent dose[a] (μg/kg/day)	Tolerable daily intake[b] (μg/kg/day)	Biomonitoring equivalent[c] (ng/g lipid)
Average prenatal	3.767	0.038	79.324
Average postnatal (0 to 12 months)	1.201	0.012	25.290
Average overall (conception to 12 months)	1.706	0.017	35.924
Maximum concentration (C_{max})	0.925	0.009	19.748

[a]The administered equivalent doses are the maternal daily doses (ug/kg/day) necessary to reach an average prenatal, average postnatal, average overall or maximum child brain concentration of 164 μg/kg.
[b]The tolerable daily intakes are the administered equivalent doses divided by uncertainty factors. Here, we used uncertainty factors of 10 for subchronic-chronic extrapolation, and 10 for interindividual variability (composite factor of 100).
[c]The biomonitoring equivalents represent the simulated maternal plasma concentration (ng/g lipids) at conception (in women exposed to the administered equivalent dose over their lifetime) divided by the same uncertainty factors used for tolerable daily intake calculation (composite factor of 100).

chose the Frequentist Polynomial degree two model as it yielded a BMC one order of magnitude lower than the Hill model and therefore would be more conservative. We recently showed that choice of biostatistical model for BMC determination can impact compound classification for DNT (Keßel et al., 2023). Another rationale by the US EPA (EPA, 2012) recommends the use of the $BMDL_{05}$ (5 % change in response) as it was often close to the NOAEC (Haber et al., 2018). On the other hand, guidance by the European Food Safety Authority (EFSA Scientific Committee et al., 2022) recommends the use of a BMR of 1 standard deviation for a BMC with more biologically relevant qualities. However, when we performed additional analyses using the BMR of 1 standard deviation, the range of biomonitoring equivalents produced a range of 113 to 461 ng/g lipids, which was an order of magnitude above the range where adverse associations were measured in epidemiological studies (see Supplementary Material). Of note, guidance on BMR selection mentioned above was established for *in vivo* data. Given how influential BMR selection is, *in vitro*-specific guidance should be established for NAM-based risk assessment.

Once the BMC_{05} was determined, we used *in vitro* biokinetics to estimate the effective intracellular concentration capable of eliciting the change in oligodendrocyte differentiation. Traditionally, investigators of *in vitro* studies have developed concentration–response relationships using the nominal concentrations (i.e., initial medium concentration) applied to the *in vitro* environment and the corresponding examined effect. However, studies show it is necessary to observe the chemical biokinetics of the *in vitro* environment when investigating a concentration–response relationship (Bell et al., 2018; Blaauboer, 2010; Groothuis et al., 2015; Zhang et al., 2018). Depending on the characteristics of the chemical and the whole of the *in vitro* environment, the investigated chemical may bind non-specifically to the vessel and constituents within solution, partition into the headspace or evaporate out of the system. Thus, exposed cells may receive chemical doses far below or above the original nominal concentration (Armitage et al., 2014; Groothuis et al., 2015). We used a dynamic mass-balance model developed by our team (Bloch et al., 2022) to consider the effects of the *in vitro* environment on chemical disposition, and determined an enrichment factor of 75.4. This enrichment factor is slightly above the experimentally determined enrichment factor of 60 by Schreiber et al. (2010), which studied the accumulation of BDE-47 in hNPCs over 7 days with a 50 % exchange of media every two days using a similar *in vitro* environment. Given the important shift in the concentration–response curve due to BDE-47 biokinetics in the *in vitro* system, these results support the use of a mass-balance model to determine the intracellular concentration when provided with the nominal concentration and settings of the *in vitro* environment. We postulate that the use of a mass-balance model constitutes a necessary step in the consolidation of *in vitro* data and extrapolation to guidance values in the context of risk assessment.

Following the estimation of a point of departure in cells (POD_{cell}), we used a toxicokinetic model in a reverse dosimetric fashion to calculate tolerable daily intake and biomonitoring equivalent values. The estimated tolerable daily intakes from this study (0.009–0.038 μg/kg/day) were more conservative than the reference dose determined by the U.S. Environmental Protection Agency (0.1 μg/kg/day) (EPA, 2008). This reference dose was derived from a neurobehavioral study by Eriksson et al. (2001) where three groups of mice were administered a single dose (0, 0.7, and 10.5 mg/kg) of BDE-47 at postnatal day 10. The study described the exposed mice as displaying permanent aberrations in

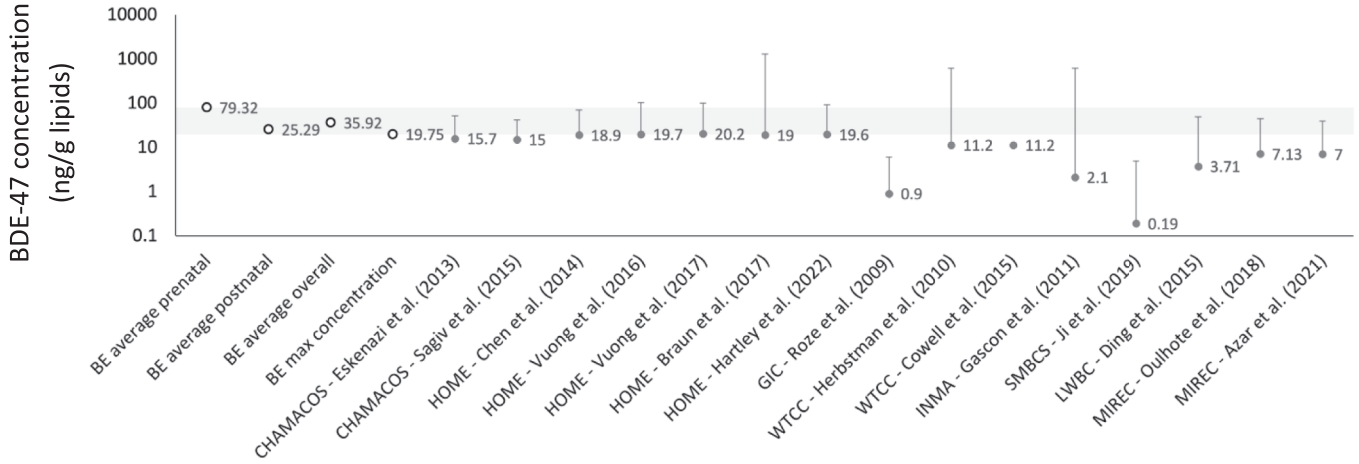

Fig. 8. Comparison of biomonitoring equivalents (BEs; ng/g lipids) with epidemiological studies. Circles denote medians compiled from Epidemiological studies with error bars representing the 90th percentile for Eskenazi et al. (2013), (Sagiv et al., 2015), and Chen et al. (2014); and the 95th percentile for Vuong et al. (2016), Vuong, Braun, et al. (2017), Hartley et al. (2022), Ding et al. (2015), Oulhote et al. (2018), and Azar et al. (2021). For Braun et al. (2017), Roze et al. (2009), Herbstman et al. (2010), Gascon et al. (2011), Ji et al. (2019), the error bars represent the maximum concentration. Open circles represent biomonitoring equivalents from our study. The grey area specifies the range of our estimated biomonitoring equivalents (17.52–71.35 ng/g lipids).

spontaneous behavior. In their assessment, the U.S. Environmental Protection Agency derived a $BMDL_{1sd}$ (0.35 mg/kg) and divided it by a composite uncertainty factor of 3000 to calculate their reference dose. The uncertainty factor included a factor of 3 for extrapolating from a single exposure to a lifetime exposure, a factor of 10 for interspecies extrapolation (mice to humans), a factor of 10 for interindividual variability, and a factor of 10 due to database uncertainties. The difference between the reference dose and the tolerable daily intakes calculated herein could be partially explained by the experimental model used to derive a POD. The use of the mouse model may not allow detecting subtle neurotoxic effects such as changes in intelligence or emotional control, which may occur at lower doses than those tested in the mouse study. Here, we used a sensitive endpoint of oligodendrocyte differentiation, which lead to a relatively conservative POD_{cell}. The difference could also be due to the choice of a 5 % benchmark response; the tolerable daily intakes derived using a benchmark response of one standard deviation were similar to the reference dose (see Supplementary Material). Overall, our study suggests that the use of human-based *in vitro* derived data may lead to a more conservative POD and tolerable daily intake than traditional methods, but this conservatism is highly dependent on methodological choices.

To determine where the estimated biomonitoring equivalents were in relation to epidemiologically measured maternal concentrations associated with neurodevelopmental toxicity, we compiled data from published epidemiological studies and their associated endpoints. Overall, our derived biomonitoring equivalents were close or slightly above the range of medians found in epidemiological studies reporting associations between early-life exposure to BDE-47 and neurodevelopmental outcomes (Fig. 8). We also evaluated available epidemiological studies for a threshold dose leading to adverse effects. However, most studies evaluated associations between exposure to BDE-47 and neurodevelopmental endpoints using linear regression models, an approach that does not lead to the identification of a threshold. Ji et al. (2019) did evaluate neurodevelopment by tertiles of BDE-47 levels, but we were unable to find a clear threshold. The use of benchmark dose modeling in epidemiological studies could facilitate the identification of thresholds (Grandjean & Budtz-Jørgensen, 2013; Kullar et al., 2019), which could be easier to use in the context of risk assessment.

Our approach has multiple limitations. Given that the approach is relatively new, there is no consensus on the use of uncertainty factors for *in vitro-in vivo* extrapolation. We included the interindividual uncertainty factor of 10, and the subchronic to chronic uncertainty factor of 10; however, because the experiment is based on human cells, we determined that it was unnecessary to include an uncertainty factor for *in vitro* to human. Exposure in *in vitro* studies is generally much shorter than exposures in humans for which acceptable exposure levels are derived. Unless *in vitro* studies specifically evaluate the impact of exposure duration on effects, it is difficult to quantitatively incorporate this factor in the *in vitro-in vivo* extrapolation. Where possible, uncertainty factors should be replaced with chemical-specific adjustment factors. Another limitation is the fact that we evaluated only one BDE congener, whereas humans are exposed to multiple congeners at the time. Effects may be additive or work together synergistically to cause a myriad of effects. However, in the HOME study on maternal plasma PBDEs and neurodevelopment, the associations with IQ and behavior were similar for the sum of four PBDE congeners (BDE-47, −99, −100, −153) and BDE-47 (Chen et al., 2014). Furthermore, the association between BDE-47 exposure and neurodevelopmental outcomes can vary by sex. Multiple epidemiological studies noted associations between ΣPBDE concentrations and different long-term effects depending on sex, noting differences in executive function and social behaviors (Hartley et al., 2022; Ji et al., 2019; Sagiv et al., 2015). For instance, Sagiv et al. (2015) noted an association with parent-reported decreased attention and executive function for girls, while Hartley et al. (2022) noted associations with decreased social skills and increased problem behaviors in boys. The neural progenitor cells used for the experiments in Klose et al. (2022) were received from three individuals, all male, which did not show significant inter-individual differences in their responses to BDE-47. If there might have been sex-differences we cannot say at this point as no cells from female individuals were used. It is possible that if the study were performed on hNPCs from single donors of different sex, we could have observed effects at lower levels. For example, we have observed sex-specific effects for other compounds in endpoints of the neurosphere assay (unpublished data). Whether the dynamic component of the uncertainty factor used herein is sufficiently protective to account for variability as well as both male and female vulnerabilities is unknown. Finally, our mass-balance model used to estimate intracellular concentration has yet to be thoroughly evaluated against experimental data from repeated dosing assays.

As the human health risk assessment field moves toward using *in vitro* data and biological modeling (Schmeisser et al., 2023), a dialogue must be undertaken between risk assessors, biological modelers, and *in vitro* scientists. Namely, the present study highlights the need for increased transparency regarding many method-related details in estimating the distribution of chemicals in the *in vitro* system. Information on material and procedures (e.g., media refreshing) is often lacking, limiting the ability to predict cellular concentrations using mass-balance models. Another critical element is the exposure duration's impact on *in vitro* endpoints. In the current study, we extrapolated from five days of neural progenitor cells exposure to months/years of exposure in humans. Measuring endpoints for different concentrations, but also different exposure durations, could help strengthen extrapolations.

5. Conclusion

Overall, this approach led to biomonitoring equivalents that were similar to median plasma BDE-47 concentrations in epidemiological studies reporting on neurodevelopmental endpoints such as behavioral problems and cognition. Interestingly, our method yielded a lower tolerable daily intake compared to the reference dose derived from traditional methods of animal modeling. Whereas a single case study cannot fully lend support to the overall approach using human cell-derived *in vitro* data and biological modeling for risk assessment, it adds to previous efforts in the field and sets the stage for subsequent case studies. The accumulation of case studies will help increase confidence in the use of NAMs and shed light on uncertainties in *in vitro*-based human health risk assessment.

CRediT authorship contribution statement

Sherri Bloch: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Laura Lévêque:** Data curation, Writing – review & editing. **Irva Hertz-Picciotto:** Resources, Writing – review & editing. **Birgit Puschner:** Resources, Writing – review & editing. **Ellen Fritsche:** Resources, Writing – review & editing. **Jördis Klose:** Resources, Writing – review & editing. **Nynke I. Kramer:** Writing – review & editing. **Maryse F. Bouchard:** Writing – review & editing, Funding acquisition. **P. Charukeshi Chandrasekera:** Funding acquisition, Writing – review & editing. **Marc-André Verner:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108411.

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