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Principles of quantitative in vitro to in vivo extrapolation (QIVIVE) - As example applied to the DNT IVB

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The Guidance Document is accompanied by an Excel file that describes the available gestation and lactational PBK models available at the following link:
https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/assessment-of-chemicals/annex-b-overview-of-gestational-and-lactational-physiologically-based-kinetic-models.xlsx

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Foreword

Quantitative in vitro to in vivo extrapolation (QIVIVE) refers to the process of converting a concentration from an in vitro assay to an external exposure level. This conversion often involves using a physiologically based kinetic (PBK) model to estimate a plausible exposure level from a target tissue or plasma concentration equivalent to the in vitro concentration. Alternatively, other quantitative tools or data that can establish the relationship between external exposure level and internal concentration may be used instead of a PBK model. The exposure level predicted using PBK models or other methods can then be compared to relevant exposure levels, such as estimated human exposures or animal-derived points of departure, depending on the intended purpose.

The aim of this document is to provide an overview of QIVIVE principles, particularly through the application of PBK modelling, to facilitate the incorporation of data from the Developmental Neurotoxicity In Vitro Battery (DNT IVB) into chemical assessment. This document should be read alongside the **OECD Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In Vitro Battery (IVB)**[1] as a supplement, providing general background on QIVIVE.

This document was developed as part of an activity by the OECD Expert Group on DNT IVB. The activity started in September 2022, and was co-led by Cecilia Tan (US EPA, US), Jochem Louisse (EFSA, IT), Alicia Paini (EFSA, IT), and the OECD secretariat. The document was drafted in close collaboration with experts contributing input on various aspects of QIVIVE. The experts included Kathrin Bothe (Bayer, DE), Xiaoqing Chang (Inotiv, US), Iain Gardner (Certara, UK), Sunil Kulkarni (HC, Canada), Dennis Mueller (Bayer, DE), and Steven Webb (Syngenta, UK). Additionally, Pavani Gonnabathula (CDC, US) provided support to compile the list of available gestational and lactational PBK models (Annex B) published in open literature. The Expert Group on DNT IVB reviewed the document in September 2023, and the WPHA reviewed it in October/November 2024 and March/April 2025. Comments received from the experts were discussed, and the document was revised accordingly. The document received approval by the WPHA at the June 2025 meeting. It is published under the responsibility of the OECD Chemicals and Biotechnology Committee.

[1] ENV/CBC/MONO(2023)13

Table of contents

Executive summary

The document provides an overview of quantitative in vitro to in vivo extrapolation (QIVIVE) principles, through the application of physiologically based kinetic (PBK) modelling, to facilitate the incorporation of data from the Developmental Neurotoxicity In Vitro Battery (DNT IVB) into chemical assessment. The document should be read alongside the OECD Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In Vitro Testing Battery (OECD, 2023).

The document outlines the principles and steps involved in applying QIVIVE to interpret DNT IVB data for chemical safety assessment. It discusses the process of converting a concentration from an in vitro assay to an external exposure level, often using PBK models to estimate a plausible external exposure level associated with a target tissue or plasma concentration equivalent to the in vitro concentration. It is not intended as technical guidance for conducting and reviewing QIVIVE analysis, but rather to provide an understanding of the process and its application.

General recommendations for users of this document

Consider reverse and/or forward dosimetry based on convenience and study objective.

Communicate and justify the chosen in vitro PODs within the framework of the analysis, as it is context- and purpose-dependent.

Consider the potential discrepancy between nominal concentration and biologically effective concentration (e.g., affected by migration to plastics, binding to media, stability, intracellular accumulation).

Identify an in vivo dose metric that reflects the biologically effective dose and align it with the in vitro concentration of interest.

Model blood-brain barrier, placental transfer, and lactational transfer only when sufficiently supported by data.

Use maternal total or unbound (if available) plasma concentration as a practical and conservative surrogate dose metric for DNT to overcome the challenges associated with measuring or predicting the unbound developing brain concentration.

Apply a tiered PBK modelling approach based on data availability and the intended regulatory purpose.

Select PBK models for QIVIVE analysis of DNT IVB data based on the appropriate in vivo dose metric to be used as the output (e.g., juvenile, fetus, nursing infant exposure).

Progress from lower to higher tier models only if added complexity provides more precision or reduces uncertainty.

The conclusions emphasise the need for a fit-for-purpose approach, suggesting that QIVIVE and PBK modelling may not be necessary for all regulatory applications where DNT IVB data is used. The document also provides examples from literature to illustrate key concepts and facilitate discussion.

In summary, the document aims to provide an understanding of QIVIVE principles and their application to DNT IVB data, offering insights into the process of converting in vitro concentrations to in vivo exposures and the importance of selecting appropriate models and metrics for chemical safety assessment.

1 Introduction

This document provides an overview of the concept of quantitative in vitro to in vivo extrapolation (QIVIVE) within the context of the OECD *Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In Vitro Testing Battery* (OECD, 2023). QIVIVE refers to the process of converting an in vitro concentration of interest into an equivalent in vivo exposure (Moxon et al., 2020; Chang et al., 2022a). This process entails selecting an in vitro point of departure (POD)[2], determining its corresponding in vivo target tissue or plasma/blood concentration, and converting that in vivo concentration to an external dose using a physiologically based kinetic (PBK) model or other tools.

It is important to note that this document is not intended as a technical guide for conducting and reviewing a QIVIVE analysis. It does not provide guidance on selecting an in vitro POD, determining an in vivo dose metric, constructing and evaluating a PBK model, or extrapolating in vitro bioactivities to in vivo adverse outcomes. It is also not a guidance for interpreting results from a QIVIVE analysis of DNT in vitro battery (IVB) data. Rather, this document outlines key principles and steps involved in applying QIVIVE to interpret DNT IVB data for chemical safety assessment. Additionally, a tiered PBK modelling approach is proposed, emphasising that the selection of a specific tier should be guided by data availability for model parameterisation and evaluation, the intended purpose of conducting QIVIVE, and the level of uncertainty acceptable for the context of use. Examples from the literature are highlighted to illustrate key concepts and facilitate discussion.

This document should be read alongside the *Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In Vitro Testing Battery* (OECD, 2023). The DNT document describes the assays that comprise the battery, provides criteria that allow evaluation of the relevance of the data to DNT, and assists in the determination of the degree of certainty in any positive or negative findings. To that end, the sections on “Criteria for Individual Assay Evaluation” and “Evaluation of the DNT IVB for chemical testing” from the OECD Initial Recommendation should be consulted together with the available Integrated Approaches to Testing and Assessment (IATA) case studies specific to DNT (Appendix D of the DNT document).

[2] POD can also be read as reference point (RP). Throughout the document POD is used.

2 QIVIVE approaches and considerations

The QIVIVE process involves correlating an in vitro dose of interest to an in vivo internal dose metric, which is then extrapolated to an external dose using quantitative tools, most commonly a PBK model. Extrapolation between external and internal doses can be approached through either reverse dosimetry or forward dosimetry. Reverse dosimetry is an approach that starts with an internal concentration (such as peak plasma concentration, average plasma concentration) and works backward to estimate the likely external dose that would be required to produce that internal concentration. One reverse dosimetry approach (**Figure 1A**) equates an in vitro POD to an in vivo internal concentration, which is then extrapolated into an external dose (e.g., Verwei et al., 2006; Wetmore et al., 2015). Another approach, using reverse dosimetry (**Figure 1A**), converts the entire in vitro concentration-bioactivity response data into external dose-bioactivity response relationship, from which an external POD is derived (e.g., Louisse et al., 2010; Strikwold et al., 2013). While these approaches differ in where the POD is defined, both share the same underlying principles.

Forward dosimetry is an approach that starts with a specific or a range of external doses to predict the resulting internal concentrations. The predicted internal concentrations are then compared to in vitro PODs (e.g., Maass et al., 2023) (**Figure 1B**). The key distinction between reverse and forward dosimetry approaches is the point of comparison: reverse dosimetry estimates external concentrations and compared them to exposure levels of interest, while forward dosimetry evaluates internal concentrations against in vitro benchmarks. The choice between reverse and forward dosimetry depends on convenience and study objective. For instance, forward dosimetry is particularly efficient when multiple in vitro PODs are compared to one internal concentration that corresponds to a single exposure of interest. In such cases, only one model simulation is needed to allow straightforward comparison at the internal concentration level, avoiding the need for multiple simulations to translate each in vitro POD into an external dose. In sum, the principles underlying forward and reverse dosimetry are fundamentally similar, only differing in the starting point of the analysis and in the specific outputs to inform safety assessment.

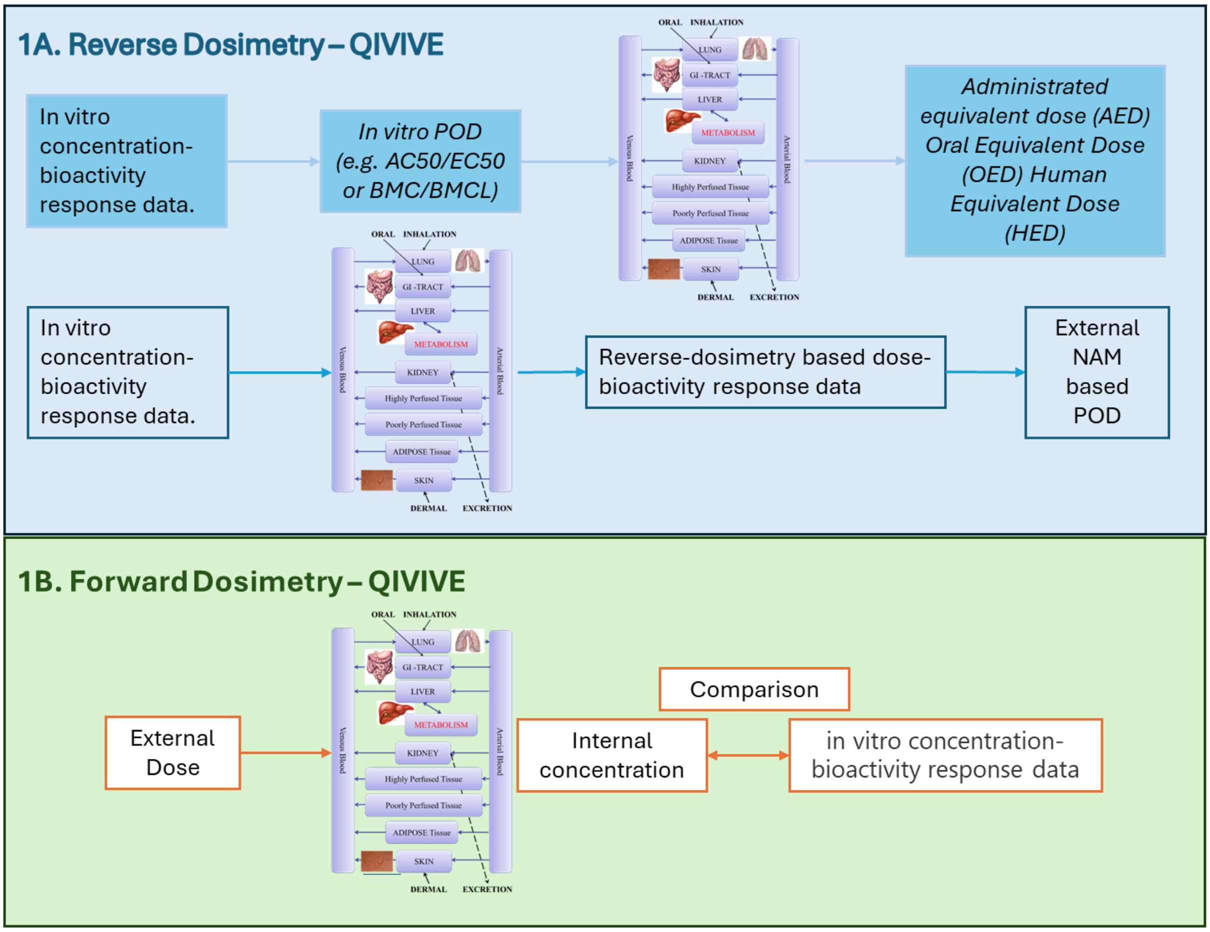

Figure 1A. Schematic presentation of reverse dosimetry QIVIVE approaches in which an *in vitro* POD is translated to an external dose using PBK modelling; or *in vitro* concentration-bioactivity response data are translated into *in vivo* dose-bioactivity response data using PBK modelling, from which an external (NAM-based) POD can be derived. 1B. Schematic presentation of forward dosimetry QIVIVE approach in which a PBK model is used to predict the internal concentrations resulting from a specific or a range of external doses. The predicted internal concentration(s) can then be compared to the *in vitro* concentration-bioactivity response data.

3 Selection of an in vitro POD

The assays within the DNT IVB are designed to evaluate chemical effects on key neurodevelopmental processes, such as proliferation and neurite outgrowth, which occur at various developmental windows and in different brain regions. The underlying hypothesis is that these processes are highly conserved, and as such, a chemical affecting one or more of these assays has the potential to elicit similar effects in vivo regardless of the specific timing or brain region involved. However, in vitro activities do not necessarily predict adverse outcomes in vivo (OECD, 2023 paragraph 19), so that a consensus of the preferred in vitro PODs has not yet been formalised (OECD, 2023 paragraphs 48-50).

One way to derive an in vitro POD is the benchmark concentration (BMC) approach, which involves fitting a concentration-response curve to all data points and identifying the BMC as the concentration associated with a predefined Benchmark Response (BMR). Other commonly used in vitro PODs include the half-maximal activity concentration (AC50) and activity concentration at cutoff (ACC). In high throughput toxicity testing, the AC50 is frequently used as a measure of in vitro potency (Judson et al., 2016; Karmaus et al., 2016) for ranking and prioritising chemicals for further testing (Paul Friedman et al., 2016). When comparing the bioactivities across multiple chemicals within a given assay, the ACC is considered a more appropriate metric because it is standardised to a consistent response threshold within each assay (Fay et al., 2019).

The initial step of the QIVIVE is to select the in vitro POD. Some assays of the DNT IVB have been developed to categorise test results into hits and non-hits (and in some cases borderline hits) and to indicate whether hits are considered specific or non-specific related to cytotoxicity. In vitro PODs (e.g. BMCs or EC5, EC10, EC50) are subsequently set based on expert judgement (e.g., related to a meaningful extent of reduction) and statistical considerations (e.g., all positive controls showed a certain reduction) (paragraph 50, OECD, 2023) regarding how to interpret the information. No specific PODs are currently recommended for QIVIVE, and providing such recommendations falls outside the scope of this document. POD selection should be context- and purpose-dependent, and users are expected to communicate and justify their chosen PODs within the framework of their analysis. In some cases, no-effect concentrations can be used for QIVIVE. For example, if no response is detected up to the highest concentration tested in the in vitro assay(s), estimating the corresponding external concentration through QIVIVE using that highest test concentration may provide useful insights for the assessment.

Another crucial aspect to consider when selecting an in vitro POD is the potential discrepancy between the nominal concentration and the biologically effective concentration (typically free or intracellular concentration) within the test system. Such discrepancy can arise from various factors, such as non-specific migration to plastics, binding to media constituents, binding to membrane lipid and protein of cells, intracellular lysosomal trapping, evaporation, metabolism, and intracellular accumulation (Groothuis et al., 2019; Schreiber et al., 2010; Armitage et al., 2021). In vitro distribution studies can help quantify the impact of these factors. Additionally, computational distribution models have been developed to estimate free and intracellular concentrations from nominal values (Armitage et al., 2014; Fischer et al., 2019; Proença et al., 2021). However, such models are currently not available for the DNT IVB. Developing system-specific models would require evaluation against empirical data to ensure reliability, as well as detailed assay information, such as plate type, construction material, medium composition, cell number, and protein and lipid content of the cells. Some of the assay information is summarised in Annex A.

4 Selection of an in vivo dose metric

Another critical decision point in the QIVIVE process is identifying an in vivo concentration at the target tissue (or called internal dose [concentration] metric), or its suitable surrogate, that closely corresponds to the in vitro concentration of interest. Ideally, an in vivo dose metric reflects the biologically active form of the chemical (toxicophore: parent and/or metabolite(s)), its level (e.g., concentration or amount), duration (e.g., acute or daily, lifetime or a particular developmental window), intensity (e.g., peak or average), and biological matrix (e.g., tissue or plasma concentration) (US EPA, 2006). For a given chemical, the toxicophore may not always be known. However, it can potentially be identified by testing both the parent chemical and any known major metabolites in DNT IVB. In cases where major metabolites are unknown, an integrated approach combining in silico predictions with in vitro methods can be used to identify likely metabolites with significant predicted formation (Leonard et al., 2018). Commonly used dose metrics include average concentration, peak concentration, and average concentration at steady state (or area under the curve; [AUC]).

In the context of DNT, a relevant dose metric is likely the unbound concentration of a chemical crossing the blood-brain barrier (BBB) and reaching the developing brain during critical developmental windows (based on Pelkonen et al., 2008). However, measuring brain concentrations is not feasible in humans and remains challenging in animal studies. The scarcity of empirical data adds to the challenge of accurately predicting the brain concentration, which can be influenced by multiple factors such as plasma and tissue protein and lipid binding, BBB permeability, active transport, metabolism, and exposure routes (Kulkarni et al., 2016). While PBK models can often reasonably predict tissue concentrations using tissue:plasma partition coefficients, using brain:plasma partition coefficient may lead to overestimation of brain exposure due to the lack of consideration of the BBB. Additionally, unlike most tissue partitioning, which is often assumed to be similar across species, brain partitioning can vary significantly across species, especially for chemicals that are substrates of influx and efflux transporters (Sato et al., 2021). Several predictive models are currently available to estimate BBB permeability: some provide qualitative predictions (i.e., permeable vs. non-permeable) (Kumar et al., 2022; Huang et al., 2024), others estimate the unbound brain-to-plasma concentration ratio using in vitro transport activity data for known transporter substrates (Sato et al., 2021), and some extrapolate BBB permeability from rats to humans for compounds that cross the BBB primarily via passive diffusion (Mehta et al., 2024).

The BBB is a vital component of the neurovascular unit; it maintains a tightly controlled microenvironment necessary for proper neuronal function (Goasdoue et al., 2017). Acting as a highly selective barrier between the systemic circulation and the brain parenchyma, the BBB regulates the influx of nutrients, ions, and other essential compounds while facilitating the efflux of waste products or preventing harmful substances from entering the brain. This regulation is achieved through tight junctions between brain endothelial cells and specialised transporters on the luminal and abluminal membranes (Abbott et al., 2006; Rhea et al., 2019). Additionally, the BBB serves as a metabolic barrier, utilising intracellular and extracellular enzymes to degrade or modify certain molecules, further limiting their access to the brain (Chaulagain et al., 2023).

The BBB becomes functional immediately after its formation to provide essential protection during early embryonic development (Abbott et al., 2010). This early functionality is evidenced by the expression of tight junction proteins, such as occludin and claudin-5, in the developing human brain as early as 12 weeks of gestation (Virgintino et al., 2000). These proteins are essential for sealing the spaces between endothelial cells, regulating paracellular permeability, and maintaining the structural integrity of the BBB. The presence of these proteins in the fetal BBB regulates and protects the brain in the initial stages of development. Also, members of the ATP-binding cassette family of transporter proteins, expressed in the endothelial cells of the BBB have been reported to play important roles in protecting the brain against chemical exposure (Abbott et al., 2010). The expression of these transporters is also present in fetal stages (Eng et al., 2022), but differences in expression and possible age-related variations may affect the extent of protection (Koehn et al., 2019).

Due to these protective mechanisms, it is estimated that most small-molecule drugs and all large molecules have limited translocation across the BBB (Pardridge, 2005). Designing drugs to successfully penetrate the BBB requires careful balance of physicochemical and pharmacokinetic properties, such as lipophilicity, molecular weight, hydrogen bonding capacity, charge, metabolic stability, and plasma protein binding (Pajouhesh and Lenz, 2005). These design considerations further illustrate the inherent difficulty of estimating the extent to which a chemical can cross the BBB. Additionally, very few environmental chemicals have been documented to reach higher concentrations in the brain than in plasma in animal studies, with nicotine and mercury being two notable examples (Craig et al., 2014; Burbacher et al., 2005). Given the protective nature of the BBB and the challenges associated with measuring or predicting the unbound brain concentration, the unbound plasma concentration can serve as a practical and conservative surrogate dose metric. This approach assumes equilibrium between plasma and brain without explicitly accounting for the restrictive effects of BBB (Pelkonen et al., 2008; Kalvass and Maurer, 2002).

Furthermore, estimating plasma concentrations in fetuses or nursing infants resulting from secondary exposure through the mother presents additional challenges beyond those of estimating maternal plasma concentrations. These difficulties stem from limited or nonexistent data on chemical-specific transfer across the placenta and into breast milk. Even when animal data exist, cross-species differences in placental and maternal tissue physiology and permeability are poorly understood. To better understand fetal exposure, it is essential to consider the unique role of the placenta in mediating chemical transfer between mother and fetus. The placenta supplies nutrients and oxygen to the fetus via the umbilical cord and serves as a selective membrane barrier separating maternal and fetus blood (Kotta-Loizou et al., 2024). It also facilitates gas exchange and produces hormones that are vital to fetus growth and development. Although the placenta can offer protection to some chemical exposures, certain substances can still cross the placental barriers, as has, for example, been reported for polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs) and some pesticides (Matovu et al., 2020; Fernandez-Cruz et al., 2020).

Transport across the placenta can occur through passive diffusion across the trophoblast cytoplasm or mediated by various transporters, such as P-glycoprotein, multidrug resistance-associated proteins, breast cancer-resistant protein, organic anion transporters, and organic cation transporters. Passive diffusion occurs bidirectionally between mother and fetus and is governed by the physicochemical properties of the chemical, such as lipophilicity, polarity, and molecular weight (Syme et al., 2012). Additional factors such as plasma protein binding, which can alter over the course of gestation, and clearance mechanisms in both the mother and fetus, also influence the equilibrium between maternal and fetal concentrations. However, limited data are available to quantitatively link these physicochemical and biological factors to placental transfer, and rapid physiological changes in both mother and fetus during gestation further complicate these relationships, making it challenging to accurately predict a chemical’s placental transability (Kotta-Loizou et al., 2024).

The placenta is not only a passive barrier, but it can also metabolise chemicals, sometimes forming bioactive metabolites that may affect both the mother and fetus. Given the complexity of these mechanisms, assessing placental transfer and metabolism would benefit from a multifaceted approach, including in vitro assays (such as using primary trophoblast cells, placental cell lines, and explants), ex vivo perfusion methods, in vivo animal studies, and computational models such as PBK models to complement empirical data (Kotta-Loizou et al., 2024). Each method has its own strengths and limitations. For example, the ex vivo human placental perfusion approach is widely regarded as the most reliable approach for assessing placental transfer and metabolism. However, it relies on full-term placenta, which does not reflect the characteristics of placenta at earlier stages due to structural and enzymatic changes during gestation (Sferruzzi-Perri et al., 2022). Thus, accurately predicting fetal plasma concentrations will likely require extensive data across gestational stages.

After an appropriate in vivo dose metric is selected, the next step is to establish a quantitative relationship between the in vivo dose metric and the external dose, often using a PBK model. In some cases, a full PBK model may not be needed and data from animal studies or other sources may be used directly. Incorporating the DNT IVB data into regulatory assessments should follow a fit for purpose approach, so QIVIVE and PBK modelling may not be necessary for all applications. In some cases, alternative combined testing approaches may be suitable for specific context of use. For example, Li and colleagues (2015; 2016) demonstrated that integrating data from the ES-D3 cell differentiation assay with the BeWo transport model improves the prediction of relative in vivo developmental toxicity potencies by considering both toxicodynamic and toxicokinetic factors. This screening approach combines in vitro toxicodynamic data (the ES-D3 cell differentiation assay evaluates how chemicals inhibit spontaneous differentiation of mouse embryonic stem cells) with in vitro toxicokinetic data (the BeWo transport model measures the chemical translocation rate across placental cells).

5 Tiered physiologically based kinetic modelling framework

A PBK model consists of a set of mathematical equations that collectively describe the absorption, distribution, metabolism, and excretion (ADME) characteristics of a chemical within an organism. A PBK model incorporates physiological and anatomical parameters (e.g., cardiac output, tissue volumes, tissue blood flows, and may also capture physiological changes during gestational and lactational periods) along with chemical-specific parameters (e.g., absorption constants, plasma protein binding, tissue:plasma partition coefficients, metabolism and elimination constants). Together, these inputs allow for the simulation of a chemical's ADME following exposure through one or multiple routes. Recommendations for parameterising a PBK model using in vitro and in silico approaches (OECD 331, 2021) or in vivo data (e.g., OECD TG417, 2010) are available in multiple guidance documents (e.g., US EPA 2006, WHO 2010). These resources also provide guidance on the characterisation, application, and documentation of PBK models intended for use in risk assessment. Furthermore, these documents provide recommendations for the evaluation of PBK models, considering key elements such as model purpose, model structure, mathematical representation, parameter estimation, computer implementation, predictive capacity, and analyses of sensitivity, variability, and uncertainty (US EPA 2006, WHO 2010, OECD 331, 2021).

When selecting a model structure, parsimony should serve as the guiding principle (US EPA, 2006). A parsimonious model includes only the essential parameters needed to adequately represent the physiology of the target species, the chemical's key ADME characteristics, and relevant exposure scenarios. A model should be as complex as necessary to achieve its intended purpose, but no more. Introducing unnecessary complexity, in the absence of reliable data or a robust biological basis to parameterise or evaluate a model, can lead to unreliable predictions and diminish the model's utility (Kilkenny et al., 2018). It can inadvertently instil a false sense of confidence, giving the impression that the model can predict a broader range of outcomes or offer greater precision beyond its validated performance.

For DNT effects, initial exposure may occur in the mother, with effects manifesting in the fetus or nursing infant through secondary exposure. Alternatively, juveniles may be directly exposed, such as through hand-to-mouth behaviour, potentially leading to DNT outcomes. When selecting a PBK model for QIVIVE analysis of DNT IVB data, a critical consideration is determining the appropriate in vivo dose metric as the output. For scenarios involving direct juvenile exposure, a PBK model parameterised for juvenile physiology and age-specific ADME characteristics can be used to predict plasma concentrations in this population. In maternal exposure scenarios, free or even total maternal plasma concentration may serve as a conservative surrogate for the free plasma concentration in the fetus or nursing infants (Chang et al., 2022b). However, when data are available to properly parameterise the gestational and/or lactational component, an investigator may choose to incorporate these components to predict internal concentrations in fetus or nursing infant. To address these varying needs, a tiered framework is proposed to guide the selection of PBK model structure, with increasing complexity and precision applied only as needed (**Figure 2**).

The tiers of models range from high-throughput models to more detailed gestational and lactational models that include PBK models of the fetus or infants. Lower-tier models prioritise simplicity and practicality, providing rapid and often conservative insights. In contrast, higher-tier models are more resource-intensive and are supported by more extensive data or knowledge, resulting in greater precision and reduced uncertainty. Higher-tier models do not inherently perform better than lower-tier ones in all situations. Adding complexity to the PBK model by moving from Tier 0 to Tier 1 or 2 should only be undertaken if the more complex model is necessary for the intended purpose and supported by reliable data. Additionally, the higher-tier model should provide a more precise answer or lower uncertainty compared to the lower tier model. For example, if critical inputs like placental transfer or fetal clearance are highly uncertain, a gestational model predicting fetal plasma concentrations does not offer an advantage over a lower tier model such as a simpler non-pregnant model that predicts maternal concentrations, which could be more easily validated.

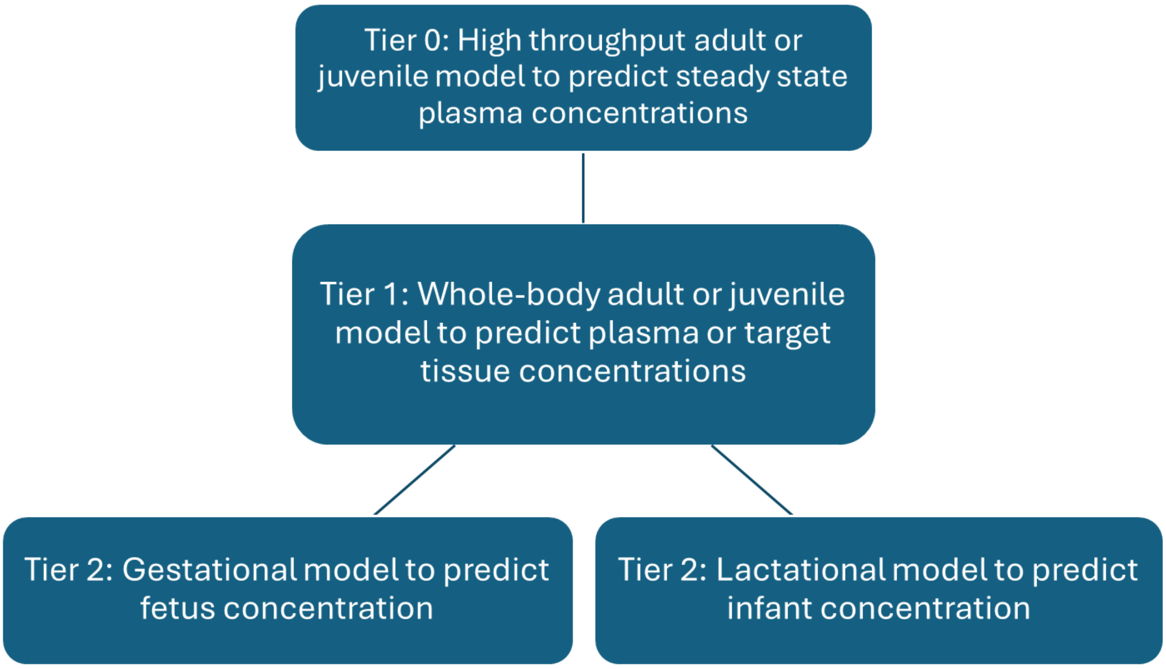

Figure 2. Tiered PBK modelling framework. Progression from a lower to a higher tier should only be undertaken if the added complexity gives more precision or reduces the uncertainty.

Model selection within this framework should also be guided by the intended application. For example, if the margin between the predicted internal concentration (from forward dosimetry) and the in vitro POD, or between the predicted external dose (from reverse dosimetry) and a specific external dose of interest, is sufficiently high, lower-tier models may be appropriate. In such cases, the range of possible model predictions is unlikely to influence the overall conclusion of the application. Conversely, if the margin at a particular tier is not sufficiently high and greater precision is needed, additional data will be required, whether to support a transition to a higher tier or to refine the current model. It is beyond the scope of this document to define what constitutes an acceptable margin, as this should be determined within the context of use. For regulatory applications, these criteria should be established within the regulatory jurisdictions based on their specific processes and requirements. Additionally, when evaluating the margin, two sources of uncertainty should be characterised: first, the uncertainty associated with the selected external dose, which can be based on exposure scenarios, established toxicological reference values, or PODs from animal studies; and second, the uncertainty associated with the predicted internal concentrations, which depends on the data used for model parameterisation. Furthermore, in the QIVIVE, uncertainty in the in vitro POD is to be considered.

Tier 0 models are generic, simplified models with a limited number of compartments. These models rely on high-throughput in vitro data and structure-derived physicochemical properties to predict steady state concentration in maternal or juvenile plasma, following exposure to a chemical. Typically implemented for chemical screening and prioritisation purposes, Tier 0 models offer a simple representation of the system, assuming linear kinetics to estimate steady state plasma concentrations (Rotroff et al., 2010; Wetmore et al., 2012; 2015; Wambaugh et al., 2015; 2018). Studies comparing Tier 0 model predictions with in vivo data have shown that these models generally overestimate steady state plasma concentrations, which is consistent with their intended role as conservative screening tools. Tier 1 models introduce additional compartments or complexity informed by ADME or hazard-related knowledge to address specific needs, such as tracking both parent compound and metabolite disposition, capturing nonlinear ADME behaviours, or predicting target tissue concentrations. To support the enhanced capabilities, Tier 1 models require additional data both to parameterise key physiological and kinetic processes and to evaluate the performance of these models. In many cases, maternal plasma concentrations predicted using Tier 0 or Tier 1 models are likely sufficient to serve as conservative surrogates for fetal or infant plasma concentrations. For example, an analysis of 112 studies examining maternal and cord blood levels of over 130 chemicals, including brominated flame retardants, polychlorinated biphenyls, polychlorinated dibenzodioxins and dibenzofurans, organochlorine pesticides, perfluorinated compounds, polyaromatic hydrocarbons, metals, and tobacco smoke components, showed that cord blood concentrations were generally comparable to or lower than maternal blood levels (Aylward et al., 2014). Exceptions were observed for specific brominated flame retardants, polycyclic aromatic hydrocarbons, and some metals. While these data reflect chemical concentration ratios at or near the time of delivery and may not represent earlier stages of pregnancy, human data are not available to determine whether the partitioning between maternal and fetal blood varies with developmental age or placental maturation.

Tier 2 models build on Tier 1 by incorporating gestational or lactational components. Gestational models are designed to predict plasma concentration in the foetus, while lactational models predict concentrations in breastmilk and subsequent plasma concentrations in nursing infants. It is important to reiterate that higher-tier models are not inherently superior or necessary for all applications. These models require more detailed and complex data, such as concentration-time profiles obtained from laboratory animals, to ensure reliable predictions, which are often difficult, if not impossible, to obtain. When data are limited, Tier 0 or Tier 1 models are often preferable, as they involve fewer parameters and are generally sufficient for predicting plasma concentrations in exposed individuals. In contrast, Tier 2 models, which incorporate gestational and lactational components, are widely recognised as highly challenging to develop. A screening of a PBK model database containing 7,541 models for 1,150 unique chemicals (Thompson et al., 2021) found that fewer than 300 include gestational components, covering approximately 160 unique chemicals. Only 16 models were found to include a lactational component. The existing database (Thompson et al., 2021) includes models published only up to 2020, so an additional PubMed search was conducted to identify gestational and lactational models published between 2020 and 2023. The combined list, totalling approximately 300 models, is provided in the annex (Annex B). Inclusion in this list does not imply model endorsement, validation, or approval by the OECD or other regulatory bodies, nor does it suggest that these modelling approaches should be adopted.

Numerous knowledge gaps limit the development of gestational and lactational models (Lu et al., 2012; Thepaut et al., 2023; Chaphekar et al., 2021; Jones et al., 2023; Nauwelaerts et al., 2021). For gestational models, challenges arise from the scarcity of pregnancy-specific and fetal data. Critical processes, such as enzyme ontogeny, active transport mechanisms, and other biochemical factors during fetal development, remain poorly characterised. The dynamic and complex physiological changes during pregnancy further complicate accurate modelling. Additionally, most animal studies prioritise toxicity outcomes over chemical concentrations, resulting in limited data to support model development and evaluation. Even when in vivo data are available, extrapolating to humans can be problematic due to species-specific differences, such as variations in placental structure and function, and the timing of nervous system development (Carter, 2007; Schmidt et al., 2015). In vitro tools (e.g., the BeWo cell transfer model) or ex vivo placenta model can provide insights into placental transfer (Li et al., 2013; Mathiesen et al., 2014), but their application in PBK model parameterisation has been limited.

Developing lactational models is equally challenging due to limited reliable data on maternal parameters, particularly the milk-to-plasma ratio, especially for chemicals that interact with transporters. Both in vitro cell culture methods (Athavale et al., 2013, Zhang et al., 2022) and in silico models (Atkinson and Begg, 1990; Yang et al., 2022; Pansari et al., 2022; Abduljalil et al., 2018, 2021) exist to estimate the milk-to-plasma ratio, and certain physicochemical properties have been associated with reduced chemical transfer into breast milk. These properties include high polarity, large molecular weight, strong plasma protein binding, short half-life, and low lipophilicity (Sachs et al., 2013). Simulating exposure in nursing infants adds further complexity, requiring the use of milk concentration as input to a neonatal PBK model that incorporates infant physiology and kinetics (Chou et al., 2021, Abduljalil et al., 2021). Additional uncertainties are also introduced, including variations in milk production rates and infant feeding patterns.

Both Tier 1 and Tier 2 models may include predictions of brain concentrations in juveniles (Kapraun, 2019, 2022). These models may incorporate additional compartments and parameters relevant to brain uptake and distribution, such as brain permeability, partitioning, or transporter activity. As previously discussed, using brain:plasma partition coefficients within a flow-limited compartment may not account for BBB, so it is expected to provide a conservative estimate of the free concentration in brain. When plasma and brain concentrations are measured in paired dam and foetus samples, these in vivo data can provide insights into whether maternal plasma concentrations can serve as a conservative surrogate for foetal plasma or brain concentrations (Sakamoto et al., 2018; Clewell et al., 2008). However, such studies are rare, with few references available in literature. In most cases, predicting brain concentrations is rarely necessary due to the restrictive nature of the BBB, which limits the passage of many chemicals into the developing brain. More importantly, the scarcity of data needed to parameterise such a model significantly reduces the confidence in the accuracy and reliability of these predictions.

It is important to emphasise that this tiered modelling framework is specifically designed for QIVIVE analysis of DNT IVB data. Regardless of the chosen PBK model, regulatory fitness of the PBK model should be evaluated according to the OECD PBK guidance, including the theoretical basis, parameterisation, validation, uncertainty and sensitivity analysis to consider them fit for QIVIVE (OECD 2021). In scenarios where precise predictions of internal concentrations and subsequent DNT potential in the fetus and nursing infant are required, QIVIVE and DNT IVB data alone may not suffice. In such cases, rather than relying solely on higher tier models for QIVIVE, additional data sources may be necessary to achieve the desired level of accuracy for the DNT assessment.

6 DNT QIVIVE examples

Although the literature includes numerous examples demonstrating the application of QIVIVE approaches for general developmental toxicity (e.g., Verwei et al., 2006; Louisse et al., 2010; Strikwold et al., 2013; Louisse et al., 2015; Li et al., 2017; Wang et al., 2021; Chang et al., 2022b), there are relatively few examples specific to DNT. Among the limited examples identified, two involved the application of QIVIVE to assays included in the DNT IVB (Dobreniecki et al., 2022; Maass et al., 2023), whereas the other two applied QIVIVE to data from alternative in vitro DNT assays (Algharably et al. 2023; Johansson et al., 2024). The examples provided in this document are drawn from open literature and are included solely for informational purposes. Their inclusion does not imply endorsement, validation, or acceptance of the PBK models or overall approach presented in the studies by the OECD or other regulatory agencies. Additionally, these examples should not be interpreted as definitive guidance or recommendations for standard practice.

Dobreniecki et al. (2022) presented a weight of evidence approach, in which the requirement for an additional guideline DNT study for L-glufosinate (L-GLF) was waived. The decision considered several critical factors, including findings from selected assays of the DNT IVB, QIVIVE, established animal POD for L-GLF, and existing in vivo data for both L-GLF and DL-form glufosinate (DL-GLF). A guideline DNT study for DL-GLF has previously established an offspring no-observed-adverse-effect-level (NOAEL), which is similar to the offspring NOAEL reported from a non-guideline DNT study conducted for L-GLF. Both DL-glufosinate and L-glufosinate were tested in selected DNT IVB assays, including neural network development and formation, as well as neurite outgrowth. Neither compound showed effects in these assays, except that both increased the mean firing rate of mature networks. Using a high-throughput model, QIVIVE was performed to estimate administered equivalent doses (AEDs), for rats and humans, representing the external doses required to achieve a steady-state plasma concentration equivalent to the in vitro effect concentration in the relevant DNT IVB assays. For rats, the AED of L-GLF was found to be 3 times higher than the in vivo NOAEL of DL-GLF in rats. For humans, the AED of L-GLF was approximately the same as the NOAEL for DL-GLF in rats. Additionally, the in vivo animal POD for L-GLF was 30-fold lower than the calculated rat AED, further supporting the decision. This example illustrates how DNT IVB data and QIVIVE can support regulatory decisions in a weight-of-evidence context, potentially reducing the need for additional animal studies.

Maass et al. (2023) investigated the potential impact of DNT IVB data on the current human health risk assessment framework, which traditionally relies on extrapolating from in vivo animal PODs. Using a gestational PBK model, they predicted human fetal brain concentrations under repeated exposure scenarios related to a given health-based guidance value of deltamethrin. To address key uncertainties, several critical parameters, such as blood-brain and blood-placenta partitioning, were varied. The predicted fetal brain concentrations were then compared to nominal effect concentrations observed in the DNT IVB assays. These comparisons allowed for the evaluation of the resulting internal margins of exposure to provide insights into how DNT IVB data could complement traditional risk assessment methods.

Algharably et al. (2023) conducted a study that began with BMD modelling, using in vitro concentration-effect data from biomarkers that assess neuronal cell development and function following exposure to chlorpyrifos. The upper bound of the BMD, corresponding to fetal brain concentrations, was then extrapolated to maternal doses using a gestational PBK model. These predicted external doses were compared to maternal doses estimated from maternal blood concentrations measured in epidemiological studies, applying the same gestational model. This example demonstrates how QIVIVE allows for a comparison between in vitro bioactivity data and exposure biomarkers measured in epidemiological studies, providing insights into the relationship between biological activity and real-world human exposure.

Johansson et al. (2024) used a gestational PBK model to estimate external doses from in vitro PODs based on human neuroblastoma SH-SY5Y cells exposed to acrylamide, assuming equivalence to fetal plasma concentrations. The external doses derived from the QIVIVE analysis were then compared to reported estimated daily intake doses for a pregnant population and non-pregnant high-intake consumers to assess margins of exposure. This example demonstrates the capability of QIVIVE to support risk assessment by providing an early indication of potential health concerns across different populations.

7 References

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8 Glossary

Biologically effective concentration/ Target concentration
The biologically effective concentration refers to the concentration of a substance that is responsible for producing a (toxicological) effect to cells or tissues, i.e., the concentration that is available to interact with the cells/cellular targets. Also known as the dose (target concentration) at the target site (e.g. DNA, cytoplasm or membrane receptors) in cells or tissues that causes a (toxicological) effect (e.g. µmol/µmol receptor), Groothuis et al. (2015).

Forward dosimetry
Forward dosimetry refers to the process of predicting or estimating the internal concentration of a substance, e.g., in blood/plasma or target tissues or organs, based on given external exposure information (e.g., oral, inhalation, or dermal exposure) applying PBK models**.**

In vitro point of departure (POD)
In the context of this document, the in vitro point of departure refers to the concentration or dose of a substance obtained from an in vitro study used for the QIVIVE.

Nominal concentration
The nominal concentration refers to the intended or specified concentration used in an experimental setting of a substance in a solution, i.e., the total amount of substance divided by the volume of exposure medium to which the substance is added (e.g. mol/L medium).

Reverse dosimetry
Reverse dosimetry refers to the process of predicting or estimating an external dose level of a substance based on given internal exposure information (concentration, e.g., in blood/plasma or target tissues or organs) applying PBK models**.** In the context of the quantitative interpretation of in vitro toxicity data, a link between the in vitro exposure and the internal concentration is made, and related reverse dosimetry provides insight into external dose levels related to bioactivity measured in vitro. In the context of human biomonitoring data, reverse dosimetry refers to the process of determining the amount of a substance a person is expected to have been exposed to, based on concentrations determined in human samples, such as blood, urine, or tissue.

Toxicophore
A toxicophore is a chemical structure or a portion of a structure (e.g., a functional group) that is related to the toxic properties of a chemical.

Unbound concentration

The unbound concentration of a test chemical in exposure medium or in a biological matrix (e.g., plasma or tissue), also called free concentration or freely available concentration. The free concentration can be lower than the nominal concentration, due to substance binding to medium constituents or plastics, non-specific cell binding, and/or evaporation.

9 Abbreviations

AC50 = Concentration at which 50% of maximum activity is observed
ACC = Activity concentration at cut off
ADME = Absorption, Distribution, Metabolism, Excretion
AED = Administered equivalent dose
AUC = Area under the curve
BMC = Benchmark concentration
BMD = Benchmark dose
BMR = Benchmark response
CDC = Centers for Disease Control and Prevention
DNT = Developmental neurotoxicity
EC50 = Half maximal effective concentration
EFSA = European Food Safety Authority
HC = Health Canada
HED = Human equivalent dose
IVB = In vitro battery
OED = Oral equivalent dose
PBBK = Physiologically based biokinetic
PBK = Physiologically based kinetic
PBPK = Physiologically based pharmacokinetic
PBTK = Physiologically based toxicokinetic
POD = Point of departure
QIVIVE = Quantitative in vitro to in vivo extrapolation
RP = Reference point
US EPA = United States Environmental Protection Agency

Annex A.

Assays Currently in the DNT IVB mapped with information relevant for the QIVIVE approach.								
Test Method (Assay)	**Test System (Cell culture)**	**Assay Duration/ Chemical exposure**	**DNT Endpoint**	**Viability/ Cytotoxicity Endpoint**	**Single/repeated exposure**	**Experimental set up** (Medium composition, e.g., % FBS, lipid %, protein concentration) for in vitro distribution models	**Experimental set up** (Medium volume/plate format (12, 24, 48, 96)/plate material. For in vitro distribution models	**Is the plate coated?**
Proliferation								
NPC1	Human NPC grown as proliferating 3D neurospheres	72 h / 72 h	Neurosphere area, BrdU incorporation in dividing cells	Resazurin reduction /LDH release	Single exposure over 3 days	DMEM (#31966-021, Thermo Fisher, United States) and Hams F12 (#31765-027, Thermo Fisher, United States) in a 2:1 ratio (v:v) supplemented with 2% B27 (#17504044, Thermo Fisher, United States), 20 ng/ml EGF (#PHG0313, Thermo Fisher, United States), 20 ng/ml FGF basic (#233-FB, R&D Systems, United States), and 100 U/ml penicillin and 100 µg/ml streptomycin (#P06-07100, Pan-Biotech, Germany)	100 µL/ 96-well plate / PS (Polystyrene), #351177 (Falcon)	Yes – poly-(2-hydroxyethyl methacrylate)

hNP1 Prolif	Human NPC	24 h / 24 h	BrdU incorporation in dividing cells	ATP level	Single	No FBS, protein or lipid StemPro® Neural Supplement in Knockout DMEM/F12(Gibco, Cat. No. A10508-01)	96 well polystyrene	Yes – poly-L-ornithine and laminin
Apoptosis								
hNP1 Apop	Human NPC	24 h / 24 h	Apoptosis pathway (Caspase) activation	ATP level	Single	No FBS, protein or lipid StemPro® Neural Supplement in Knockout DMEM/F12(Gibco, Cat. No. A10508-01)	96 well polystyrene	Yes – poly-L-ornithine and laminin
Migration								
UKN2	Human NSC-derived neural crest cells	72 h / 24 h	Number of cells moving into defined area	Calcein-AM vital dye	Single	2.8 ng/ml Lipid content in Medium, 5.6 µM Protein content in Medium, no FBS (Krebs 2020 et al.)	100 µL, 96 well plates, from Corning	Yes - poly-L-ornithine /Laminin/ Fibronectin
NPC2a	Human NPC grown as differentiated 3D neurospheres	72 h / 72 h 120 h / 120 h	Mean distance of radial glia (nuclei negative for neuronal and oligodendrocyte markers) from edge of sphere	Resazurin reduction/ LDH release	Repeated exposure on d0 and d3, assay runs over 5 days	DMEM (#31966-021, Thermo Fisher, United States) and Ham's F12 (#31765-027, Thermo Fisher, United States) in a 2:1 ratio (v:v) supplemented with 1% N2 (#17502-048, Thermo Fisher, United States) and 100U/mL penicillin and 100 µg/ml streptomycin (#P06-07100, Pan-Biotech, Germany)	100 µL/ 96-well plate / PS (Polystyrene), free of heavy metal, #655180 (Greiner)	Yes – Poly D-lysine and Laminin
NPC2b	Human NPC grown as differentiated	120 h / 120 h	Mean distance of tubulin-positive	Resazurin reduction/	Repeated exposure on d0 and d3,	DMEM (#31966-021, Thermo Fisher, United States) and Ham's F12 (#31765-027, Thermo Fisher, United States)	100 µL/ 96-well plate /	Yes – Poly D-lysine and Laminin

	3D neurospheres		neurons from edge of sphere	LDH release	assay runs over 5 days	in a 2:1 ratio (v:v) supplemented with 1% N2 (#17502-048, Thermo Fisher, United States) and 100U/mL penicillin and 100 µg/ml streptomycin (#P06-07100, Pan-Biotech, Germany)	PS (Polystyrene), free of heavy metal, #655180 (Greiner)	
NPC2c	Human NPC grown as differentiated 3D neurospheres	120 h / 120 h	Mean distance of O4-positive oligodendrocytes from edge of sphere	Resazurin reduction/ LDH release	Repeated exposure on d0 and d3, assay runs over 5 days	DMEM (#31966-021, Thermo Fisher, United States) and Ham's F12 (#31765-027, Thermo Fisher, United States) in a 2:1 ratio (v:v) supplemented with 1% N2 (#17502-048, Thermo Fisher, United States) and 100U/mL penicillin and 100 µg/ml streptomycin (#P06-07100, Pan-Biotech, Germany)	100 µL/ 96-well plate / PS (Polystyrene), free of heavy metal, #655180 (Greiner)	Yes – Poly D-lysine and Laminin
Neuronal Differentiation								
NPC3	Human NPC grown as differentiated 3D neurospheres	120 h / 120 h	Number of tubulin-positive neurons	Resazurin reduction/ LDH release	Repeated exposure on d0 and d3, assay runs over 5 days	DMEM (#31966-021, Thermo Fisher, United States) and Ham's F12 (#31765-027, Thermo Fisher, United States) in a 2:1 ratio (v:v) supplemented with 1% N2 (#17502-048, Thermo Fisher, United States) and 100U/mL penicillin and 100 µg/ml streptomycin (#P06-07100, Pan-Biotech, Germany)	100 µL/ 96-well plate / PS (Polystyrene), free of heavy metal, #655180 (Greiner)	Yes – Poly D-lysine and Laminin
Neurite outgrowth								

NPC4	Human NPC grown as differentiated 3D neurospheres	120 h / 120 h	Neurite length & area	Resazurin reduction /LDH release	Repeated exposure on d0 and d3, assay runs over 5 days	DMEM (#31966-021, Thermo Fisher, United States) and Ham's F12 (#31765-027, Thermo Fisher, United States) in a 2:1 ratio (v:v) supplemented with 1% N2 (#17502-048, Thermo Fisher, United States) and 100U/mL penicillin and 100 µg/ml streptomycin (#P06-07100, Pan-Biotech, Germany)	100 µL/ 96-well plate / PS (Polystyrene), free of heavy metal, #655180 (Greiner)	Yes – Poly D-lysine and Laminin
UKN4	Human NSC-line (v-myc transformed)	72 h / 24 h	Neurite area	Calcein-AM vital dye	Single	2.9 ng/ml Lipid content in Medium, 5.8 µM Protein content in Medium, no FBS (Krebs 2020 et al.)	100 µL, 96 well plates, from Sarstedt	Yes – Poly-L-orthinine and fibronectin
UKN5	Human iPSC-derived peripheral (sensory) neurons	24 h / 24 h	Neurite area	Calcein-AM vital dye	Single	25 ng/ml Lipid content in Medium, 50 µM Protein content in Medium, no FBS (Krebs 2020 et al.)	100 µL, 96 well plates, from Falcon	Yes – Matrigel
Icell-gluta	Human iPSC-derived neurons	48 h / 48 h	Neurite length	Cell morphology	Single	BrainPhys Neuronal Medium (STEMCELL Technologies Cat. No. 05790), 1% iCell Nervous System Supplement (FCDI, Cat. No. M1031), 2% iCell Neural Supplement B (FCDI, Cat. No. C1029), 1% N-2 Supplement, 100X (Thermo Fisher Scientific Cat. No. 17502048), 1 µg/mL Laminin (Millipore-Sigma, Cat. No. L2020-1MG), 1% Penicillin-streptomycin, 100X	96 well polystyrene	Yes – Poly-L-ornithine and laminin

						(Thermo Fisher Scientific Cat. No. 15140122)		
Cortical initiation	Rat primary neocortex	48 h / 48 h	Neurite length	Cell morphology	Single	Neurobasal A media with B27 supplement. No FBS, protein or lipid	96 well polystyrene	Poly-L-Lysine coated
Neurite Maturation and Synaptogenesis								
Cortical maturation	Rat primary neocortex	288 h / 120 h	Dendrite length	Cell morphology	Repeated exposure on DIV7 and DIV10, assay runs over 5 days	Neurobasal A media (Thermo Fisher Scientific Cat. No. 10888), 2 B-27 Supplement (Thermo Fisher Scientific Cat. No. 17504), 1% GlutaMax (Thermo Fisher Scientific Cat. No. 35050), 1% Penicillin-streptomycin, 100X (Thermo Fisher Scientific Cat. No. 15140122). No FBS, protein or lipid	96 well polystyrene	Yes - Poly-L-Lysine coated
Cortical synaptogenesis	rRat primary neocortex	288 h / 120 h	Synapse number	Cell morphology	Repeated exposure on DIV7 and DIV10, assay runs over 5 days	Neurobasal A media (Thermo Fisher Scientific Cat. No. 10888), 2 B-27 Supplement (Thermo Fisher Scientific Cat. No. 17504), 1% GlutaMax (Thermo Fisher Scientific Cat. No. 35050), 1% Penicillin-streptomycin, 100X (Thermo Fisher Scientific Cat. No. 15140122). No FBS, protein or lipid	96 well polystyrene	Yes - Poly-L-Lysine coated
Glial Differentiation								

NPC5	Human NPC grown as differentiated 3D neurospheres	120 h / 120 h	Number of O4-positive oligodendrocytes	Resazurin reduction /LDH release	Repeated exposure on d0 and d3, assay runs over 5 days	DMEM (#31966-021, Thermo Fisher, United States) and Ham's F12 (#31765-027, Thermo Fisher, United States) in a 2:1 ratio (v:v) supplemented with 1% N2 (#17502-048, Thermo Fisher, United States) and 100U/mL penicillin and 100 µg/ml streptomycin (#P06-07100, Pan-Biotech, Germany)	100 µL/ 96-well plate / PS (Polystyrene), free of heavy metal, #655180 (Greiner)	Yes – Poly D-lysine and Laminin
Neural Network Formation								
Cortical MEA	Rat primary neocortex	288 h / 288 h	Action potential spike, burst, and network connectivity related parameters	Resazurin reduction/total LDH	Repeated (DIV 0, 5 & 9)	Neurobasal A media with B27 supplement. No FBS, protein or lipid	48 well kapton	Yes – Polyethyleneimine and Laminin

Annex B.

The Excel file that describes the available gestation and lactational PBK models is available at the following link: https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/assessment-of-chemicals/annex-b-overview-of-gestational-and-lactational-physiologically-based-kinetic-models.xlsx