Review

The Role of "Physiologically Based Pharmacokinetic Model (PBPK)" New Approach Methodology (NAMs) in Pharmaceuticals and Environmental Chemical Risk Assessment

Deepika Deepika[a,b], Vikas Kumar[a,b,*]

[a]Environmental Engineering Laboratory, Departament d' Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007, Tarragona, Catalonia, Spain
[b]IISPV, Hospital Universitari Sant Joan de Reus, Universitat Rovira I Virgili, Reus, Spain
* Correspondence: vikas.kumar@urv.cat

Abstract: Physiologically Based Pharmacokinetic Models (PBPK) are mechanistical tools generally employed in the pharmaceutical industry and environmental health risk assessment. These models are recognised by regulatory authorities for predicting organ concentration-time profile, pharmacokinetic and daily intake dose of xenobiotics. Extension of PBPK models to capture sensitive populations like pediatric, geriatric, pregnant females, fetus etc. and diseased population like renal impairment, liver cirrhosis etc. is a must. However, the current modelling practice and existing models are not mature enough to confidently predict the risk in these populations. A multidisciplinary collaboration between clinicians, experimental and modeler scientist is vital to improve the physiology, and calculation of biochemical parameters for integrating the knowledge and refining existing PBPK models. Specific PBPK covering compartments like cerebrospinal fluid, and hippocampus are required to gain mechanistic understanding about xenobiotic disposition in these sub-parts. The PBPK model assists in building quantitative adverse outcome pathways (qAOPs) for several endpoints like developmental neurotoxicity (DNT), hepatotoxicity and cardiotoxicity. Machine learning algorithms can predict physicochemical parameters required to develop in-silico models where experimental data is unavailable. Integrating machine learning with PBPK carries the potential to revolutionize the field of drug discovery and development and environmental risk. Overall, this review tried to summarize the recent developments in the in-silico models, building qAOPs, use of machine learning for improving existing models along with a regulatory perspective. This review can act as a guide for toxicologists who wish to build their careers in kinetic modeling.

Keywords: Physiologically Based Pharmacokinetic Model (PBPK); Drugs; environmental chemicals; Adverse outcome pathway (AOP); machine learning

1. Introduction

PBPK models are mathematical models encompassing of multiple compartments with physiology, anatomy, biochemical and physicochemical parameters for describing ADME (absorption, distribution, metabolism and excretion) of xenobiotics and its metabolites (Fig 1) (Deepika et al., 2020, 2021; Deepika, Sharma, Schuhmacher, et al., 2022; Sharma et al., 2017a). These models vary from empirical, semi-mechanistic to compartmental models based on the complexity of the problem (Espié et al., 2009). The major challenge with empirical or semi-mechanistic model is difficulty to interpret questions like "How to predict concentration-time profile of compound in target organ" or "How to accurately predict the exact dosing while extrapolating from animal to human". This led to development of compartmental models which are close to human anatomy and physiology. Currently these models are widely acknowledged in the field of pharmaceutical and

 doi:10.20944/preprints202212.0564.v1

environmental for the prediction of PK behavior of xenobiotic (drug/chemical) with respect to dose, route, and species (Jones & Rowland-Yeo, 2013).

In past, allometric scaling was used to predict some of PK parameters like clearance, volume of distribution for human from PK profile in animal or other species and also for sensitive population like pediatric from adult but this scaling fail to predict the parameters in diseased or compromised states. Also, it is bit challenging to incorporate the drug-drug interaction or mixture assessment during allometric scaling which led to the reduction in popularity of such approaches (Deepika, Sharma, Schuhmacher, et al., 2022; Espié et al., 2009; Malik & Edginton, 2019). PBPK models can overcome such challenges as they consist of systems data (like physiology) and biochemical parameters like metabolism, excretion including mechanisms like saturation of enzymes or presence of specific receptors (Stader et al., 2019).

PBPK models have multiple applications and recently the toxicokinetic is being integrated with adverse outcome pathways (AOPs) for improving the overall risk assessment in the context of New Approach Methodology (NAM) (V. Kumar et al., 2021). In-vitro systems are currently accepted in drug discovery and toxicological assessments especially for predicting molecular initiating event and key events (Halappanavar et al., 2020). But the question arises that if the chemical is reaching in enough concentration in target tissue to cause a perturbation which can affect cellular biology and cause adverse effect. This points towards quantification of kinetics for evaluating the internal concentration at molecular initiating event site (MIE) considering all the possible scenarios during in-vitro testing like binding of chemical to lipids and proteins, partitioning between medium and chemical, and binding to the plastic in cell culture system which can reduce the free chemical. IVIVE-based mechanistic PBPK model (integral component of NAM) can help in taking all the factors into consideration for predicting the kinetic and also the in-vitro exposure equivalent to human target organ for successful human health risk assessment (Leist et al., 2017; Sharma et al., 2017b). The development of PBPK is sometime considered time- and resource- intensive and complicated due to multiple parameters with many having unknown values which are often fitted. Therefore, utilizing machine learning and artificial intelligence approach can help in predicting some of the PK parameters of new substances which can speed up the process of developing robust NAM based PBPK with limited experimental data (Chou & Lin, 2022; S. Kumar et al., 2022).

In this review article, we summarize the method to develop a PBPK model, feasibility to extend them for different human population and diseased patients. The focus is also on existing challenges in PBPK and overcoming then by building organ-specific models and their application for AOP development. The comprehensive idea about the usage of machine learning and artificial intelligence for improving and advancing the existing PBPK framework is also discussed. Additionally, the ways by which regulatory acceptance of these models can be improved is mentioned providing a complete picture about current PBPK models.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0564.v1

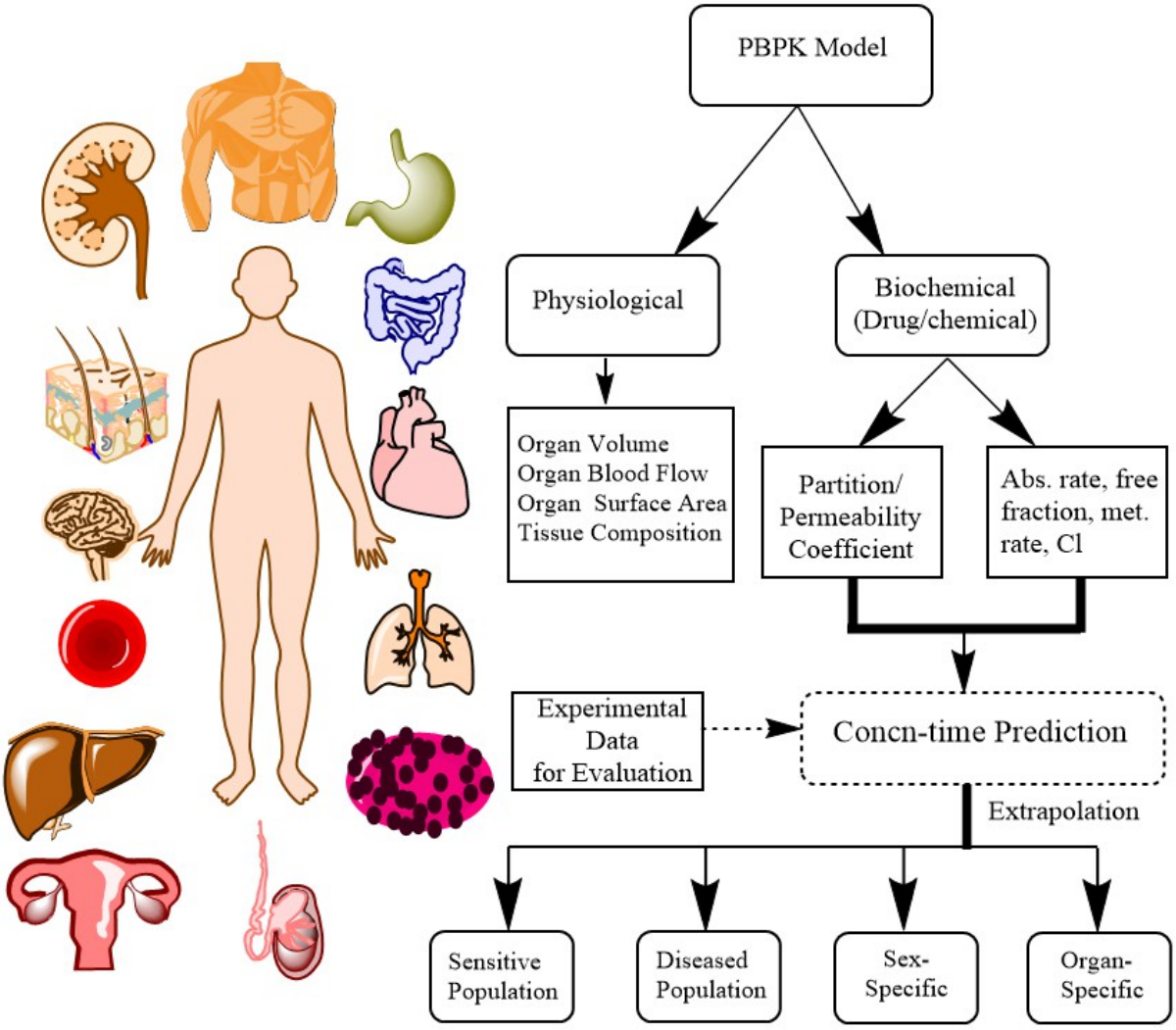

Figure 1: Conceptual Diagram for PBPK Model representing physiological, biochemical parameters and concentration extrapolation for chemical risk assessment.

2. Unravelling the art of developing PBPK

The PBPK model explicitly considers different organs which are called compartments but the choice of the number of compartments solely depends on the creator and required outcome. Typically, the compartments utilized are the liver, gut, kidney, heart, stomach, spleen, brain, muscle, bone, and skin. But some researchers prefer two compartmental models which are easy to build (Jones & Rowland-Yeo, 2013). They depend on the assumption that the compound is getting absorbed to the gut and then moving to primary compartment with excretion in urine (Eq. 1-3). The challenge being these models fail to capture the complex human physiology and the events like metabolism, reabsorption etc.

Simplistic equation for two-Compartment model

$$\frac{d(A_{gut})}{dt} = -k_{abs} * A_{gut} + oral\ dose \quad (1)$$

$$\frac{d(A_{pri})}{dt} = k_{abs} * A_{gut} - k_{el} * A_{pri} \quad (2)$$

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0564.v1

$$\frac{d(A_{urine})}{dt} = k_{el} * A_{pri} \quad (3)$$

The differential equation (dA/dt) defines the amount with time (gut, primary compartment, or urine), k_{abs} refers to the absorption rate constant, and k_{el} is elimination rate constant. All the equations can be converted to the concentration by dividing amount with volume of a particular tissue.

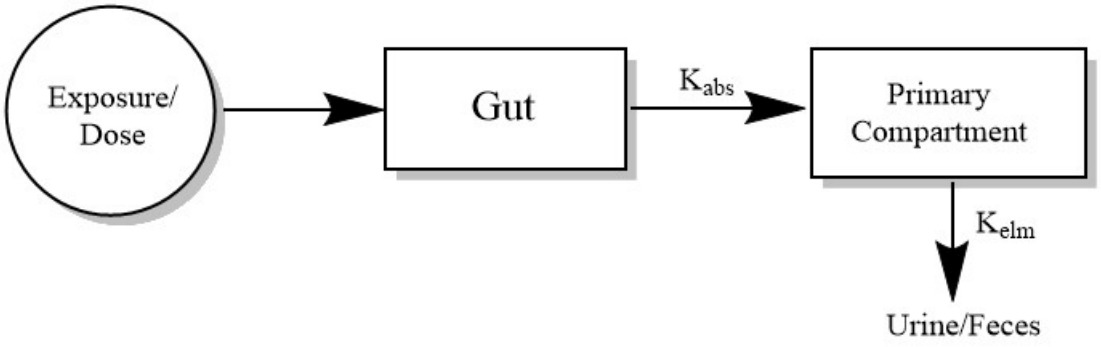

Figure 2: Overall structure for building two compartment model considering absorption and elimination rate constant.

Multi-compartment models often provide detailed information about various tissues and most importantly they include intrinsic physiological processes like liver metabolism, gut metabolism, kidney transporters and elimination, reabsorption, and enterohepatic recirculation (EHR) to capture the linear and non-linear kinetics along with anatomy (Fig 3) (Campbell et al., 2018; Deepika et al., 2021, 2022; Fàbrega et al., 2014; Sharma et al., 2018). But, the point worth mentioning is adding a lot of compartments often makes the model complex and impractical since values are required for multiple parameters. Often the choice of compartment is governed by target chemical, species, and the route of administration.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0564.v1

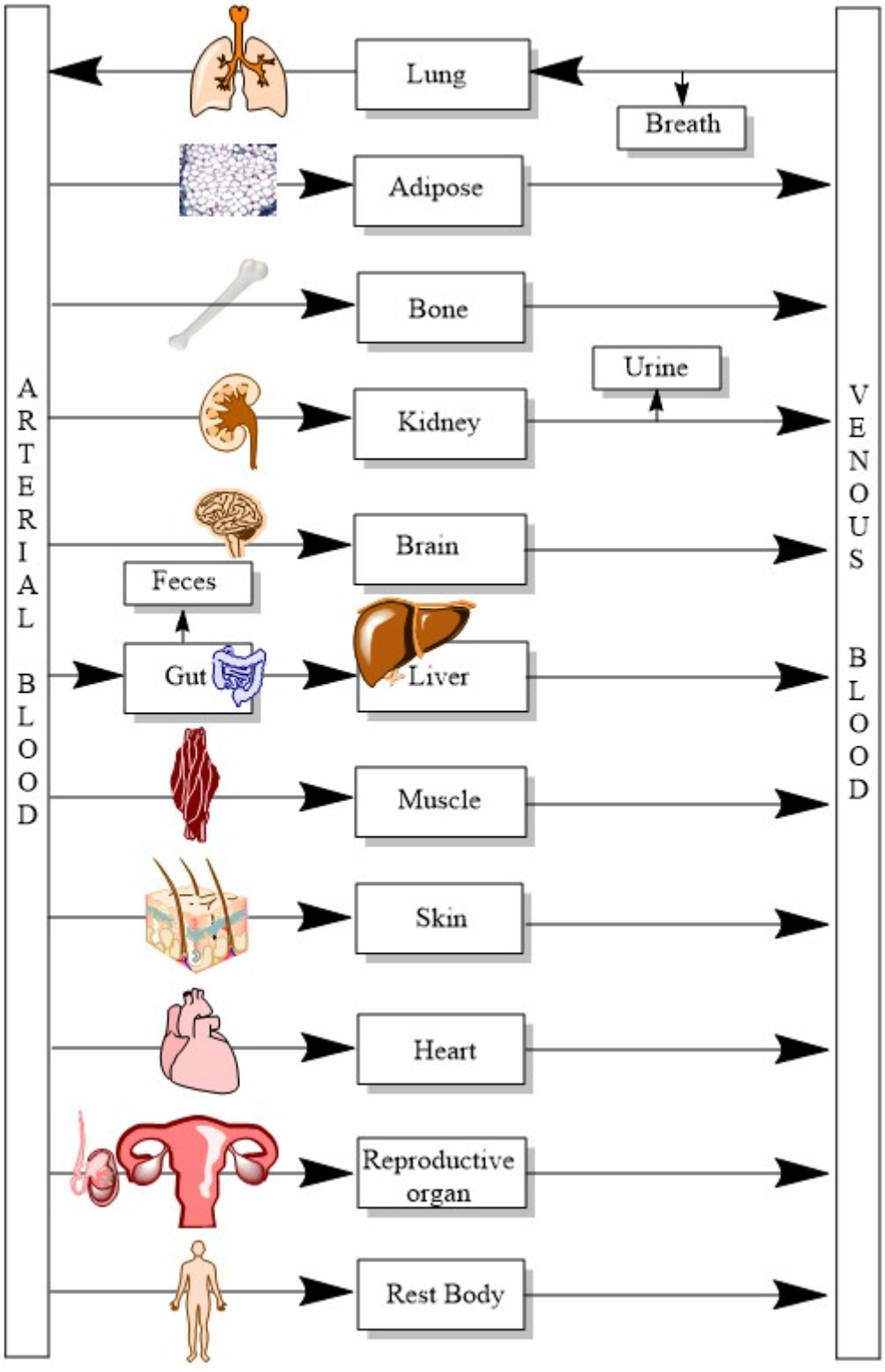

Figure 3: Multi-compartment PBPK model with 12 compartments. Excretion of the compound is from feces via gut, urine via kidney and breath via lung respectively.

2.1. Model Parameterization

2.1.1. Physiological Information

For building any PBPK model, we need anatomical and physiological parameters of the respective species like rat, dog, human etc. (Espié et al., 2009; V. Kumar et al., 2021). Parameters include body weight, height, organ volume, cardiac output and organ blood

 doi:10.20944/preprints202212.0564.v1

flow. There are some literatures available where authors have collected the physiological data or developed equations for human lifetime (Brown et al., 1997; Deepika et al., 2021). Mass balance equations are often used to explain blood flows and other parameters connecting all tissues. All PBPK contains a compartment called "rest of the body" which can be calculated for volume by subtracting the body weight by volume of other organ and for blood flow by subtracting the cardiac output by blood flow for other organs respectively. This data can be fed in PBPK model to account for variation in ADME profile based on the physiology.

2.1.2. Biochemical information

There are multiple biochemical parameters depending on the type of PBPK model but the most basic being a) absorption and EHR, b) distribution and fu, c) metabolism with IVIVE and d) excretion. These parameters can be either calculated based on PK principles or fitted using multiple methods like Bayesian with Markov Chain Monte Carlo (MCMC) algorithm, other being Metropolis-Hasting algorithm, reversible jump MCMC, the Gibbs sampler and Hamiltonian Monte Carlo (HMC) (Tsiros et al., 2019). The choice of algorithm depends on the ease of use and type of clinical data available.

Absorption and Enterohepatic Recirculation

Absorption is defined as the movement of a xenobiotic from its site of delivery to systemic circulation or another compartment (Subhani et al., 2022). In the PBPK model, absorption is defined by the absorption rate constant (k_{abs}) which is the rate of absorption of the xenobiotic with time. It is often a first-order rate constant. This parameter becomes tricky when instead of a simple formulation, there is a delayed absorption which can be due to a modified formulation or a meal effect (Rebeka et al., 2019). Parameters like intestinal permeability or dissolution rate can be measured to calculate the absorption rate constant. Absorption-related processes like enterohepatic recirculation (EHR) need to be introduced sometimes to predict the terminal phase of the plasma-concentration time profile (Fig 4) (Deepika et al., 2022; Roberts et al., 2002). Compounds which have high biliary excretions, faster absorption rates and limited clearance are often the substrates for EHR (Li et al., 2014). The equations for EHR often includes liver, bile and gut as the main compartments (eq. 4-5).

$$\frac{d(A_{liver_1})}{dt} = k_{abs} * A_{gut} + Q_{liver} * C_x - k_{liver-bile} * A_{liver1} * C_{liver1} \quad (4)$$

$$\frac{d(A_{bile})}{dt} = k_{liver-bile} * A_{liver1} - k_{bile-gut} * A_{bile} \quad (5)$$

The bile-gut is first order rate constant, and it could change with time based on bile flow rate which can be described by a turnover model. More details about this model can be found in this article (Kim et al., 2015). This phenomenon become important in case of non-linear kinetics.

 doi:10.20944/preprints202212.0564.v1

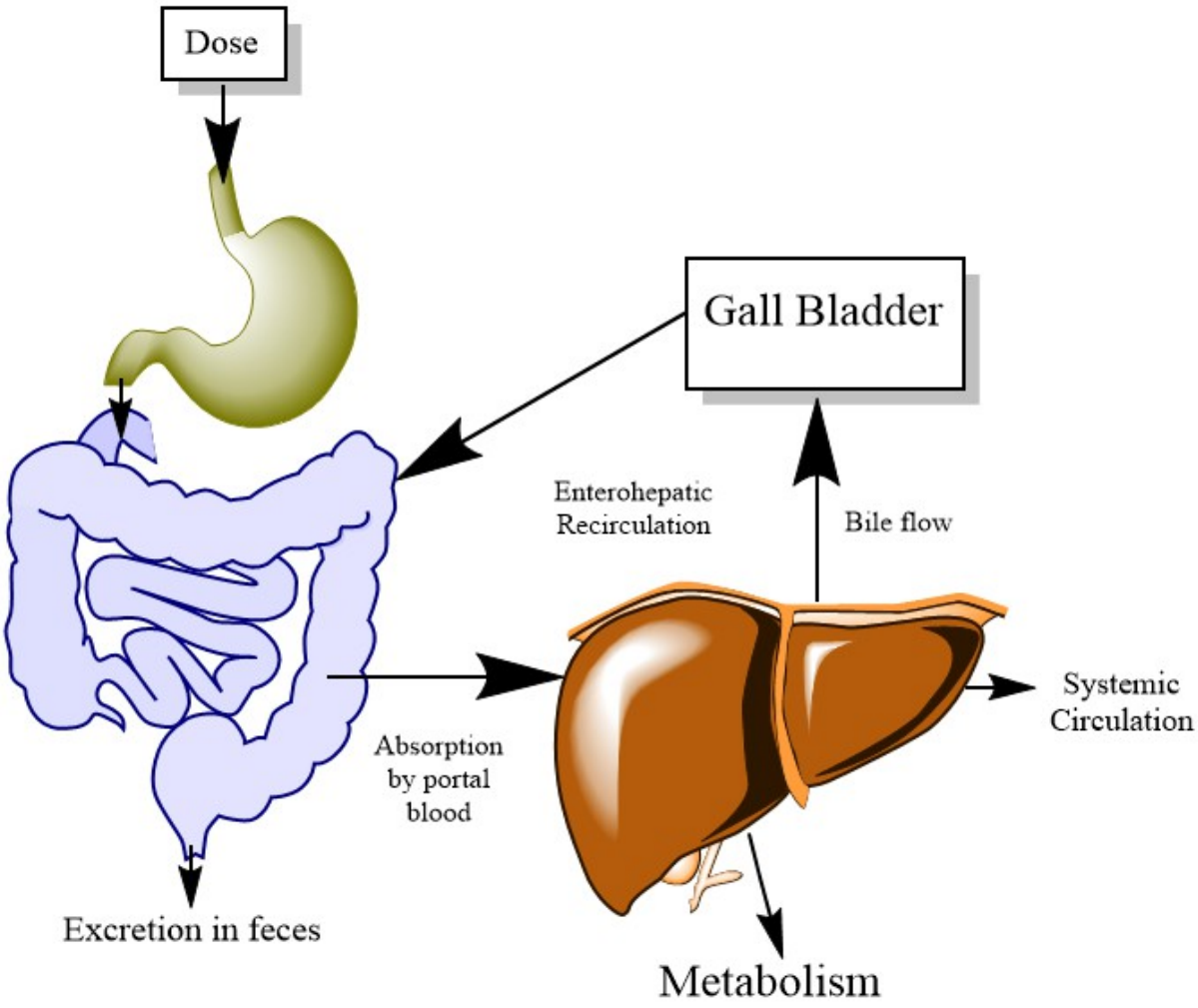

Figure 3: Oral absorption of xenobiotic followed by enterohepatic recirculation (EHR). EHR occurs by biliary excretion followed by intestinal reabsorption of xenobiotic along with hepatic conjugation and intestinal deconjugation.

Sometime the experimental data shows multiple peaks and the generic PBPK framework fails to capture it. For instance, PBPK model was developed for DPHP using lymphatic uptake and EHR to predict the plasma and urine concentration including the delayed peak. The author explained that inclusion of three processes (systemic circulation to lymph movement, high protein binding, and effluxing absorbed chemical from liver via hepatic route) were able to explain the data (McNally et al., 2021). The important point here is that for kinetic modeling, it is imperative to understand the experimental data and gain mechanistic knowledge before diving in the calibration and optimisation of the model.

Distribution and fraction unbound

Fraction unbound is of the utmost importance and an input parameter which can affect the output from the PBPK model. Albumin, alpha acid glycoproteins (AAG) and lipoproteins (LPs) are among 60 plasma proteins which bind to drugs/chemicals (Yun & Edginton, 2021). Basic compounds bind to AAG and LPs while acidic and neutral compounds bind to albumin. Generally, for environmental chemicals, the experimental fup for adults as well as children is not available (Yun & Edginton, 2021). For adults, in-silico models like QSPR based on the learned relationship between structure and protein binding can help. For fup in infants, equations are available in literature (McNamara & Alcorn,

 doi:10.20944/preprints202212.0564.v1

2002)for extrapolating fu from adult (Eq. 6). Ye et al. demonstrated that a wrong calculation of fup could result in Vss prediction error for neutral drugs and error in clearance for low-cleared compounds (Ye et al. 2016) pointing towards importance of fu in PK.

$$fu_{infant} = \frac{1}{1 + \frac{P_{infant}}{P_{adult}} \frac{(1 - fu_{adult})}{fu_{adult}}} \quad (6)$$

Here P refers to molar protein concentration.

Another important parameter in PBPK model is partition coefficient for perfusion limited model required to distribute the xenobiotic in tissues. The partition coefficient for PBPK can be computed either by calculating AUC based on experimental data (eq. 7) or by the equation derived by multiple authors (Haddad et al., 2000; Poulin & Krishnan, 1995; Rodgers et al., 2005; Rodgers & Rowland, 2006; Schmitt, 2008). Among this Rodger approach is widely accepted for most drugs due to consideration of binding with lipids and proteins. Nonetheless, all these methods need tissue composition data and physiochemical properties as inputs to quantify the organ: plasma/blood partition. In a recent study, standardized tissue composition was proposed for human which can be used as a common input for five partition coefficient prediction methods (Berezhkovskiy, 2004; Lippert et al., 2019; Poulin & Krishnan, 1995; Rodgers & Rowland, 2006; Schmitt, 2008). The author compared the results utilizing PBPK model and found that all partition coefficient methods should be considered in the process of drug development (Utsey et al., 2020).

$$Partition\ coefficient = \frac{AUC_{organ}}{AUC_{plasma/blood}} \quad (7)$$

Metabolism with IVIVE

Metabolism through hepatic and extrahepatic enzymes is an important aspect from PK perspective and account for toxicity or detoxification. Most parent drugs and chemicals are metabolized by phase I and phase II reactions into several metabolites or inactive excretion products in liver. Phase I reactions are majorly catalysed by cytochrome P-450 (CYP) enzymes and phase II by UDP-glucuronosyltransferase (UGT-s), sulfotransferase (SULT), glutathione S-transferase (GSTs), N-acetyl transferases (NAT) etc. (Abouir et al., 2021). Apart from this, some drug transporters like ATP binding cassettes (ABC) and solute carrier transporter (SLC) are responsible for influx and efflux of compounds and metabolites contributing to phase 0 (absorption) and III (elimination) (Döring & Petzinger, 2014). All these metabolic kinetics can be incorporated in PBPK model with specific parameters like enzymatic expression in organs, maximum rate of metabolism (Vmax) and Michaelis constant (Km) especially for non-linear metabolism (eq. 8) (Reddy et al., 2021).

$$\frac{dp}{dt} = \frac{V_{max} \cdot [S]}{K_m + [S]} \quad (8)$$

Where P is the product and S is the substrate.

Vmax can be calculated in the model using in vitro-to-in vivo extrapolation (IVIVE) technique utilizing in-vitro data with the following equation 9. Vmax is in pmol/min/pmol of enz, enz refers to enzyme abundance in a particular organ like liver (pmol/mg of microsomal protein), MPPGL is microsomal protein per gram of human liver (mg/g), V_{org} refers to organ volume (L) and MW is molecular weight of chemical (g/mol) (Sharma et al., 2018; Tylutki & Polak, 2017). This equation can be incorporated in the PBPK model to account for metabolism in the gut, intestine or any other organ.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0564.v1

$$V_{max}\left(\frac{mg}{h}\right) = V_{\text{max\_pmol (in−vitro)}} * enz * MPPGL * V_{org} * MW * 60/10^9 \quad (9)$$

Excretion

Excretion can mainly happen through urine, feces and via exhalation depending on the compound. Other route of excretion may include lactational, saliva, mucosal, sweat etc. but generally they account for minor fraction and hence not considered. However based on the toxicological risk, they can be included in the PBPK model.

In one compartment model, excretion via urine is through plasma explained by equation 3 (mentioned earlier). Even in muti-compartment model, many authors modeled urinary excretion of compound directly from plasma or blood respectively (Campbell et al., 2018; Campbell Jr et al., 2016). The excretion from kidney is also modeled mostly by first order kinetic which is quite simplest expression in first order excretion (eq. 10). Here k_{el} refers to elimination rate constant and Akidney is amount of compound present in kidney.

$$Urine = k_{el} * Akidney \quad (10)$$

For long acting compounds like PFOA, multiple mechanisms like glomerular filtration, basolateral active transport, and apical transport in proximal tubular cells along with simple diffusion is often modeled to account for slow renal excretion (Cheng & Ng, 2017). In one specific study, a generic PBPK model was developed including mechanistic kidney (proximal, loop of henle, distal and collecting segment, bowman´s capsule, GFR and bladder) incorporating active secretion, and bidirectional passive diffusion. The model was able to predict very well the renal clearance for 46 drugs (87% values within two-fold) utilizing in-vitro permeability data (Huang & Isoherranen, 2018). This shows the application of PBPK in utilizing in-vitro data for parameterization of the model.

Excretion by feces is possible if the compound excretes in bile or excretes in intestine or not absorbed in GI tract (Deepika, Sharma, García-Cortés, et al., 2022). In PBPK, simply excretion can be modeled using first order kinetic with eq. 11.

$$Feces = K_{el} \cdot Afeces \quad (11)$$

Very few compounds are being excreted via exhalation mostly being volatile organic compounds. Mostly PBPK model incorporates a tissue and air partition coefficient (P_b) to account for respiratory equilibrium (eq. 12-14). The arterial blood is dependent on cardiac output (Qc), concentration in venous blood (C_v), alveolar blood flow (Q_{alv}), and C_{inh} (inhaled concentration). Exhaled concentration can be provided by eq. 14 assuming 70% alveolar respiration (Haddad & Nong, 2020). But the inclusion of this route is quite limited for PBPK in pharmaceutical drugs and chemicals due to most compounds being non-volatile in nature.

$$C_a = \frac{(Q_c \cdot C_v + Q_{alv} \cdot C_{inh})}{\left(Q_c + \frac{Q_{alv}}{P_b}\right)} \quad (12)$$

$$C_{alv} = \frac{C_a}{P_b} \quad (13)$$

$$C_{exh} = 0.7 \cdot C_{alv} + 0.3 \cdot C_{inh} \quad (14)$$

 doi:10.20944/preprints202212.0564.v1

2.2. Route of administration

PBPK model holds the flexibility of adding one route of administration or multiple routes based on the exposure. Oral route is the most common both in drugs and environmental chemical exposure which often limits the bioavailability due to first pass metabolism. Nonetheless, oral administration is most preferred due to ease of administration and considered as the alternative to intravenous (IV) infusion depending on the specific case. For instance, gemcitabine PBPK model was developed using Gastroplus software with IV and oral route of administration to evaluate plasma-concentration time profile. Author showed that the drug Cmax was lower, but AUC was higher for the tablet dosage regimens (1000 mg, three*/day and 1500 mg 2-3*/day) compared to the IV infusion (Ferreira et al., 2021) Often the availability of the compound in systemic circulation is dependent on multiple factors. For instance, absorption is often considered the critical parameter for oral route along with influence from fasting or fed state.

Compounds like bisphenols which can be absorbed through skin and have higher exposure for certain people (e.g., cashiers) requires specific dermal PBPK models (Mielke et al., 2011). It is observed that dermal exposure of this compound results in higher half-life (approx. 8 h) due to bypassing first pass metabolism (Biedermann et al., 2010; Gundert-Remy et al., 2013). Pregnancy PBPK model was developed for BPA including both oral and dermal exposure (Sharma et al., 2018). Another PBPK model was developed for BPA and its analogues including the dermal exposure. Dermal exposure was found to be predominant route along with peroral and BPS exposure led to the highest internal concentration of unconjugated compound (Cecile et al., 2022).

Inhalation route of exposure is quite successful in respiratory diseases and also for rapid systemic drug delivery. PBPK model was built for inhaled nemirasalib consisting of extra-thoracic, thoracic, bronchiolar and alveolar tissues for evaluating pulmonary drug absorption (Ferreira et al., 2021). For environmental chemical like xylene, PBPK with lungs as a route for inhalation and exhalation was modeled for reconstructing the human exposure (McNally et al., 2012). Another model was for ethanol inhalation through first-generation and second-generation electronic cigarette (More et al., 2020). The study found that estimated BAC results were below the toxicological threshold showing the application in screening exposure assessment for safety of the product.

For some compound like chloroform where ingestion and inhalation is most significant route, multi-route PBPK model are required (Yang et al., 2010). The author showed utilization of Bayesian approach in PBPK considering ingestion, inhalation and dermal route for typical household exposure scenarios. Realistically, for environmental chemicals multi-exposure PBPK are required but since the exposure from some route is minor, so it is often ignored. However, in case of drugs, mostly the exposure is defined, so multi-route modeling is not required.

2.3. Model Simulation

The last step in building PBPK model is to introduce all the parametric values in the software or write your own code for simulating the output. Some of the software for developing PBPK are GastroPlus, PK-Sim, BioDMET, Maxsim2, PKQuest, Phoenix WinNonlin, and Berkeley Madonna which are very specific and easy to use. But most of them are commercial and hence not freely available. Free available and generalized softwares include GNU MCSIM, COPASI, PKSim etc. which also have easy interface. Additionally, user can write their own code in softwares like R and python which utilize solvers like desolve for ODE solving. In Rstudio, there are specific packages like httk (high throughput toxicokinetic) dedicated to make the simulation easy by containing chemical-specific data and physiological information (Pearce et al., 2017).

After building the model, next ideal step is to validate it with experimental data before moving towards prospective predictions. Generally, the simulated and predicted result within two-fold is considered as the good validation for building robust PBPK models

 doi:10.20944/preprints202212.0564.v1

but, it is mostly applicable for wide therapeutic indexed drugs and chemicals. Sometimes, it is not possible to validate the output from all compartments of the model. In these scenarios, the robustness of the model is ensured based on well-supported assumptions with high confidence in underlying mechanisms (Peters & Dolgos, 2019).

3. Applications of PBPK for Pharmaceuticals and Environmental Risk Assessment

3.1. PBPK model capturing sensitive and diseased population

Originally the PBPK models were focused on exposure prediction and toxicokinetic of xenobiotics for adult population (Johnson et al., 2022a). Overtime, PBPK models have matured and currently they are being utilized for sensitive population like infants and patient suffering from renal or kidney diseases (Fig 5). The study by Michelet et al. utilized propofol for developing PBPK in children and neonates (term and preterm) using top-down and bottom-up approach (Michelet et al., 2018). Propofol is extensively metabolized in liver and kidney with glucuronidation being the major pathway. Adult model was extrapolated to pediatric population incorporating changes in tissue volume, blood flow, and variation in ontogeny function with age. Model was able to predict the concentration-time profile similar to experimental data for preterm neonatal population. Similarly for BPA, pediatric PBPK model was developed after extrapolating from adult considering ontogeny changes to account for age and gender-specific risk (Deepika, Sharma, Schuhmacher, et al., 2022).

Different software has different physiological information, ontogeny functions for enzymes, and principle for utilizing unbound drug concentration in hepatocytes for pediatric population. For instance, Simcyp model use unbound drug concentration with unionized fraction as the major driving force for metabolism whereas Gastroplus utilizes unbound with both ionized and unionized concentration for metabolism (T'jollyn et al., 2018). In PK-Sim, drug-tissue distribution is being handled by permeability*surface area product and tissue-plasma partition coefficient. In this software, based on step speed, perfusion or permeability limited model runs for the output. Such differences lead to significant variation in PK parameters in pediatric population. Additionally, the physiological parameters used to build these models were taken from different datasets and literature sources and hence variation in the values. There is a need to harmonize the pediatric dataset and extrapolation principle for improving the existing PBPK models. Also, besides refining these models for small molecules, they should be also extended to therapeutic proteins and large molecular entity to evaluate age-related changes that are impossible to predict in-vitro or in-vivo (Smits et al., 2019).

Another application of PBPK is predicting compound concentration in geriatric population which is the largest population for pharmaceutical market. Unfortunately, elderly population aged over 65 is least studies in clinical trials but they are the biggest consumers taking two to five types of medication per day (Cui et al., 2021). Geriatric PBPK model was modified for Chinese population incorporating physiological parameters and drug dependent parameter for six drugs. Refinement of the model based on age, weight, height, BSA, creatinine levels etc. improved the prediction performance of PBPK, assessing age-specific risk. Human lifetime PBPK model was developed for forever chemical like PFOS to evaluate long term risk and disposition in organs for improving risk assessment (Deepika et al., 2021)

By incorporating pathophysiological changes occurring in a disease, PBPK model are being extended for drug-disease model. They can be used for predicting the ADME of a given drug or chemical in case of diseased population like liver or renal failure. For instance, Rasool et al. developed PBPK drug-disease model for rifampicin in tuberculosis and Cirrhosis patients (Rasool et al., 2019). Variation in albumin concentration in TB patients improved the clearance prediction. Simulated results showed increase in AUC in cirrhosis patients after oral dosing. Such model can be helpful in dose selection for diseased patients. PBPK model can provide clinical valuable insight about dosage design in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0564.v1

diseased patients like renal failure but still they are not being widely accepted by regulatory bodies like U.S. Food and Drug Administration (FDA). However, PBPK model application for renal incidences has been highlighted in FDA guidance of 2020 for supporting inclusion of patients with RI in clinical trials for improving the dosage regimen (Rowland Yeo & Gil Berglund, 2021). Kidney disease affect the PK profile of both renally and non-renally cleared drugs impacting the likelihood of drug or chemical induced toxicity. In such cases specialized PBPK models like organ-specific can unravel the true kinetic or the mechanism happening inside specific sub-compartments.

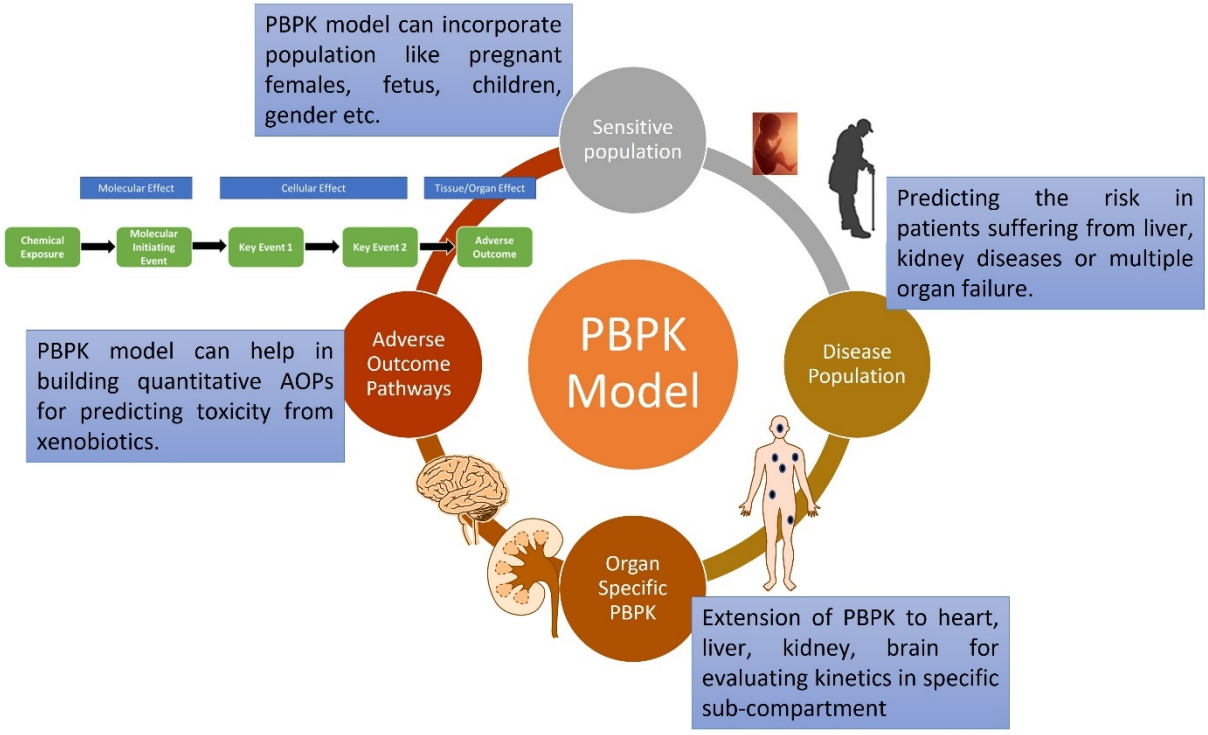

Figure 4: Applications of PBPK model in different fields (sensitive population, diseased population, organ-specific and adverse outcomes) for therapeutics and toxicological risk assessment.

3.2. Current trend towards organ specific PBPK Model

Organ-specific PBPK models are next generation kinetic models which are one-step ahead in evaluating the kinetic inside a specific organ (Fig 5). Multiple organ-specific models with focus on liver, heart, brain, kidney etc. have been developed by researchers. Inclusion of permeability limited model with perfusion limited PBPK improves prediction of transporter mediated interaction for specific organs. Often the integration of these models is being used by researchers to define the complex PK of some drugs or environmental chemicals (Ramoju et al., 2017).

Translational model for antibody disposition in brain consist of blood-brain barrier and blood-cerebrospinal fluid barrier with two separate sub-compartments: CSF circulation system and brain parenchyma (Chang et al., 2019). This model predicts quantitatively monoclonal antibodies PK at the targeted site in rats, mice, monkeys and human and hence can be used for preclinical to clinical translation for CNS disorders. Another population-PBPK model was developed based on brain micro dialysis data and Simcyp was used for bottom-up prediction to model CNS with brain ECF and brain tissue (Ball et al., 2014). Interestingly both the models provided similar prediction in brain, but ECF concentration was accurately predicted by population-PBPK. In short, the type of PBPK model to be utilized depend on the output required and mechanistic knowledge regarding the ADME profile of drug along with in-vitro (Deepika, Kumar, et al., 2022) and in-vivo data.

 doi:10.20944/preprints202212.0564.v1

PBPK with sub-compartments like globus pallidus, pituitary gland, olfactory bulb, cerebellum and blood brain has been developed for compounds like Manganese to evaluate the central nervous system toxicity (Ramoju et al., 2017). These are multiple examples for successful PBPK model developed either to capture the kinetic in hippocampus, cortex or in ECF section of brain (Ball et al., 2014; Zakaria & Badhan, 2018)to target neurological disorders or neurotoxic risk assessment.

For cardiac toxicity and safety assessment, PBPK model consisting of four heart compartments: epicardium, midmyocardium, endocardium and pericardial fluid was developed in R software. Whole body PBPK model of seventeen compartment was built and integrated with heart model along with cardiac metabolism using CYP450. Heart model was used to predict amitriptyline concentration in plasma and heart and was a first attempt to develop organ-specific model for this case scenario (Tylutki & Polak, 2017).

Forever chemicals like PFOA which are not metabolized and renally reabsorbed needs specialized PBPK model accounting for transporter mediated renal elimination. PBPK model involving kidney transporters was developed for both sexes in rat incorporating proximal tubule lumen, proximal tubule cells with diffusion and active transport to defined PFOA kinetics. Activity in proximal tubule cells and kidney transporters were found to be important component for PFOA serum clearance and also the half-life for both sexes (Worley & Fisher, 2015). Recently the mechanistic kidney model incorporating ontogeny and physiology of renal processes was able to predict the PK of five renally excreted drugs for pediatric population (Salem et al., 2022). Permeability limited model was incorporated with conventional model for evaluating effect of cimetidine on the kinetics of metformin (Burt et al., 2016). PBPK incorporated active uptake and efflux with electrochemical driving force for OCT1 and OCT2 in kidney and permeability limited uptake in liver with mechanistically modelling to define PK. The model was able to predict OCT and MATE mediated drug-drug interaction highlighting importance of such models in drug development. Mechanistic kidney model was integrated with full-body PBPK to evaluate the urine pH effect on drug ADME profile for methamphetamine and amphetamine (Huang et al., 2020). The model was successfully able to simulate the plasma-concn. time profile and urinary profile for both compounds. Such kind of models can evaluate effect of urine pH on drug renal excretion avoiding the toxicity in the case of patients who are taking multiple medicines. In a nutshell, such organ-specific models have the potential for predicting toxicity and improving risk assessment for sensitive population.

3.3. PBPK role in IATA and AOP

Integrated approaches for testing and assessment (IATA) focus on chemical safety minimising environmental and human health risk utilizing advanced *in silico*, *in vitro* and *in chemico* approaches. In IATA strategy, adverse outcome pathway (AOP) is one of the frameworks helpful in developing it through evaluating relationship between key events and adverse effects and identifying data gaps (Fig 5 and 6) (El-Masri et al., 2016; *Integrated Approaches to Testing and Assessment (IATA) - OECD*, n.d.; Meek, 2017). Mostly the developed AOPs are qualitative for evaluating the toxicity but only few are quantitative with dose response relationship or mechanistic modeling to evaluate risk. Integrating PBPK with AOP can help in predicting the kinetics of chemical and hence endpoint toxicity utilizing IATA approach (V. Kumar et al., 2021). Fusion of PBPK and pharmacodynamic model for developing quantitative AOPs can be one of the strategies for next generation risk assessment (Bois). AOP development along with ADME prediction can help in using AOPs for chemical-specific exposure and PK considerations for both data rich and data poor chemicals. For instance, PBPK was used to evaluate pollutant concentration in an organ with time for long term exposure. It is important for risk assessment as it may be possible that at low exposure the internal dose never reach the threshold activation and hence may not initiate the particular event or in this case the chronic inflammation as suggested by author (Cox et al., 2020). The other point worth mentioning is that some

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 December 2022 doi:10.20944/preprints202212.0564.v1

pollutants may not behave linearly and there can be increased accumulation due to saturated clearance. All these scenarios can be captured by detailed PBPK models providing improved estimate of cell concentration and hence probability of developing a particular adverse outcome.

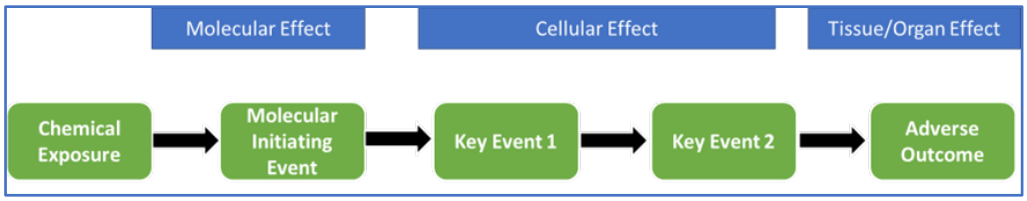

Figure 6: AOP Scheme.

Another example is a study where life stage PBPK model was integrated with AOPs to describe the fetal blood levels equivalent to concentration at which in-vitro activity related to angiogenesis/vasculogenesis was observed (El-Masri et al., 2016). In-silico models like systems biology can be integrated with PBPK to provide broad understanding about AOPs. For instance, developing qAOP for oxidative stress induced chronic kidney diseases, three approaches were used: 1) dose-response, 2) Bayesian network (BN) and 3) systems biology (SBs) model. Author found that dose-response model provides good fit, but they should be accompanied by SBs to provide mechanistic quantitative understanding (Zgheib et al., 2019). BN was more precise than dose-response but simpler than SB, but their usage needs more experience. In case of quantifying the AO for human of different ages, PBPK model can serve as gold standard tool to evaluate the risk.

4. Machine Learning with PBPK

Currently PBPK models consist of mathematical equations which are basically ordinary differential equation (ODE) being solved by ODE solver to compute the output. Recent advancement in machine learning and artificial intelligence can help in advancing this field. Neural PK/PD model was developed through deep learning for patient response time course to analyse drug concentration and platelet response from 600 patients (Lu, Bender, et al., 2021). Interestingly, neural model performed better than pop PK/PD model making it a promising methodology for forecasting precisely patient´s future response (Lu, Deng, et al., 2021). Another neural ODE model developed for trastuzumab emtansine showed good performance while predicting PK for a new treatment regimen. Variety of machine learning models like LSTM, NLME, LightGBM and neural ODE were used but are only limited to neural-ODE generated continuous PK profile with correct concentration-time pattern in artificially simulated experiment.

Apart from training for ODE, machine learning can also be used for calculating the biochemical parameters required to build PBPK models. Machine learning algorithm was used for calculating absorption rate constant, hepatic intrinsic clearance and volume of systemic circulation using 14-26 physicochemical properties generated by cheminformatics software. PBPK model with in-silico derived parameters was able to accurately predict the concentration-time for plasma after oral dosing (Kamiya et al., 2021). In another work, read across and QSAR model was developed for 1487 environmental chemicals of TK parameters to estimate steady state concentration and derive bioactivity exposure ratio (BER) for helping in risk based chemical prioritization (Pradeep et al., 2020). Such machine learning techniques carry the potential to improve existing PBPK models and made them more robust for regulatory by reducing their dependency for experimental data.

5. Challenges and improving existing PBPK for regulatory acceptance

Advancement in PBPK and enough data for validation and calibration of the model has led to acceptance by regulatory authorities. PBPK models have improved model-informed drug development by assessing drug-drug interaction, dosing prediction, dose

 doi:10.20944/preprints202212.0564.v1

validation in pediatric patients, pregnancy dose calibration, lactational exposure and much more in pharmaceutical field. Percentage of new drug approvals with PBPK analysis has increased over years with more acceptance rate by US FDA, and EMA (European Medicinal Agency). A lot of workshops are being organized to inform companies and scientific personnel about advantages of using such mechanistic models and building robust model with good practices (Lin et al., 2022).

Environmental organizations like EFSA (European Food Safety Authority), US EPA (Environmental Protection Agency), ATSDR (agency for toxic substances and disease registry) ATSDR considers the PBPK model for risk assessment based on the strength and robustness of the model. PBPK for perchlorate was assessed by EPA based on six-step framework and was found to be suitable for calculating human health risk for different life stages (McLanahan et al., 2014). Framework can be referred through this reference (Clark et al., 2004; Hoogenboom, n.d.). Recent total weekly intake (TWI) set by EFSA for four perfluoroalkyl substance (PFAS) is based on the evaluation using PBPK modeling. Lowest $BMDL_{10}$ found in children of 1 year was used to estimate maternal exposure of 0.63 ng/Kg BW/day. Based on this value and considering accumulation TWI of 4.4 ng/Kg BW/week was established (in the Food Chain (EFSA CONTAM Panel) et al., 2020).

PBPK models offers multiple advantages from dose selection/daily exposure prediction, drug-drug interactions, concentration-time profile in multiple organs etc. But still their use in assessment of trial design, pediatric formulation and toxicology are limited (Johnson et al., 2022b). There exists a scope of improving existing PBPK model for integrating them in the regulatory framework. One of the biggest challenges in PBPK model building is the estimation of unknown parameter values. Sometime the model parameter is assumed equivalent to the other species for the same chemical which is acceptable. In other scenarios, the parameter value needs to be estimated based on MCMC optimization algorithms like Metropolis-Hastings, the Gibbs sampler, and Hamiltonian Monte Carlo. The user should set a setting or initial values for estimation and also need to check for convergence after the run. This is the most important step in PBPK and is not discussed in detail while developing PBPK often reducing the confidence in the model.

In addition, PBPK model support NAM based research by focusing on in-silico toxicology combining in-vitro testing to assess health risk. NAM is being considered by regulatory bodies for almost fifteen years but still assessing biokinetic/toxicokinetic through this methodology is a bottleneck in chemical risk assessment (Punt, 2020). For increasing the acceptance of toxicokinetic models like PBPK by regulatory bodies, proper guidance protocols are a critical step along with capturing transporter mediated processes of a particular organ. Another important point being the PBPK model development and validation lack consistency in quality assessment practices making it difficult to reproduce the model (Sager et al., 2015). Stringent criteria for accepting the PBPK model based on the toxic potency of the chemical or therapeutic index of the drug needs to be defined. Recommendations for accepting the PBPK models need to be clearer and criterion like Akaike information criterion, correlation analysis should be used to predict the best fit rather than visual approach (Sager et al., 2015).

6. Conclusion

In this paper, we showed how to develop a single or multi-compartment PBPK model based on the requirement and provided data. These models have multiple applications in clinical, pharmacological, and toxicological scenarios for both drugs and environmental chemicals. PBPK models have wide application for diseased population and being used for building qAOPs. Machine learning can help in easing the process of building PBPK through providing biochemical parameters. Currently, PBPK models are being accepted by regulatory authorities especially for sensitive populations. Further, they can be combined with pharmacodynamic models to evaluate efficacy and potency of xenobiotics.

Collaboration between companies, academia and health authorities are required to improve the existing models and enhance their use in predicting PK, dose/daily exposure and much more.

7. Acknowledgement

This study was financially supported in part by the European Union co-funded project European Partnership for the Assessment of Risks from Chemicals (PARC) under Grant Agreement No 101057014. This publication reflects only the authors' views, and they have no potential competing financial interests. The Community and other funding organizations are not liable for any use made of the information contained therein. The authors confirm that the present review and conclusion drawn reflect the professional work of the authors, and, over the last five years, none of them have engaged in any legal, regulatory or advocacy activities related to this work.

Reference

1. Abouir, K., Samer, C. F., Gloor, Y., Desmeules, J. A., & Daali, Y. (2021). Reviewing Data Integrated for PBPK Model Development to Predict Metabolic Drug-Drug Interactions: Shifting Perspectives and Emerging Trends. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.708299
2. Ball, K., Bouzom, F., Scherrmann, J. M., Walther, B., & Decleves, X. (2014). Comparing translational population-PBPK modelling of brain microdialysis with bottom-up prediction of brain-to-plasma distribution in rat and human. *Biopharmaceutics and Drug Disposition*, *35*(8), 485–499. https://doi.org/10.1002/bdd.1908
3. Berezhkovskiy, L. M. (2004). Volume of Distribution at Steady State for a Linear Pharmacokinetic System with Peripheral Elimination. *Journal of Pharmaceutical Sciences*, *93*(6), 1628–1640. https://doi.org/10.1002/jps.20073
4. Biedermann, S., Tschudin, P., & Grob, K. (2010). Transfer of bisphenol A from thermal printer paper to the skin. *Analytical and Bioanalytical Chemistry*, *398*(1), 571–576. https://doi.org/10.1007/s00216-010-3936-9
5. Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., & Beliles, R. P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. *Toxicology and Industrial Health*, *13*(4), 407–484. https://doi.org/10.1177/074823379701300401
6. Burt, H. J., Neuhoff, S., Almond, L., Gaohua, L., Harwood, M. D., Jamei, M., Rostami-Hodjegan, A., Tucker, G. T., & Rowland-Yeo, K. (2016). Metformin and cimetidine: Physiologically based pharmacokinetic modelling to investigate transporter mediated drug–drug interactions. *European Journal of Pharmaceutical Sciences*, *88*, 70–82. https://doi.org/https://doi.org/10.1016/j.ejps.2016.03.020
7. Campbell, J. L., Yoon, M., Ward, P. L., Fromme, H., Kessler, W., Phillips, M. B., Anderson, W. A., Clewell, H. J., & Longnecker, M. P. (2018). Excretion of Di-2-ethylhexyl phthalate (DEHP) metabolites in urine is related to body mass index because of higher energy intake in the overweight and obese. *Environment International*, *113*, 91–99. https://doi.org/https://doi.org/10.1016/j.envint.2018.01.023
8. Campbell Jr, J. L., Andersen, M. E., Hinderliter, P. M., Yi, K. D., Pastoor, T. P., Breckenridge, C. B., & Clewell III, H. J. (2016). PBPK Model for Atrazine and Its Chlorotriazine Metabolites in Rat and Human. *Toxicological Sciences*, *150*(2), 441–453. https://doi.org/10.1093/toxsci/kfw014
9. Cecile, K., Thomas, R., Natalie, von G., Darja, G. S., Lucija, P. M., & Konrad, H. (2022). Physiologically Based Pharmacokinetic (PBPK) Modeling of the Bisphenols BPA, BPS, BPF, and BPAF with New Experimental Metabolic Parameters: Comparing the Pharmacokinetic Behavior of BPA with Its Substitutes. *Environmental Health Perspectives*, *126*(7), 077002. https://doi.org/10.1289/EHP2739
10. Chang, H.-Y., Wu, S., Meno-Tetang, G., & Shah, D. K. (2019). A translational platform PBPK model for antibody disposition in the brain. *Journal of Pharmacokinetics and Pharmacodynamics*, *46*(4), 319–338. https://doi.org/10.1007/s10928-019-09641-8
11. Cheng, W., & Ng, C. A. (2017). A Permeability-Limited Physiologically Based Pharmacokinetic (PBPK) Model for Perfluorooctanoic acid (PFOA) in Male Rats. *Environmental Science & Technology*, *51*(17), 9930–9939. https://doi.org/10.1021/acs.est.7b02602
12. Chou, W.-C., & Lin, Z. (2022). Machine Learning and Artificial Intelligence in Physiologically Based Pharmacokinetic Modeling. *Toxicological Sciences*, kfac101. https://doi.org/10.1093/toxsci/kfac101
13. Clark, L. H., Woodrow Setzer, R., & Barton, H. A. (2004). Framework for Evaluation of Physiologically-Based Pharmacokinetic Models for Use in Safety or Risk Assessment. *Risk Analysis*, *24*(6), 1697–1717. https://doi.org/https://doi.org/10.1111/j.0272-4332.2004.00561.x
14. Cox, L. A., Goodman, J. E., & Engel, A. M. (2020). Chronic inflammation, Adverse Outcome Pathways, and risk assessment: A diagrammatic exposition. *Regulatory Toxicology and Pharmacology*, *114*, 104663. https://doi.org/https://doi.org/10.1016/j.yrtph.2020.104663

15. Cui, C., Valerie Sia, J. E., Tu, S., Li, X., Dong, Z., Yu, Z., Yao, X., Hatley, O., Li, H., & Liu, D. (2021). Development of a physiologically based pharmacokinetic (PBPK) population model for Chinese elderly subjects. *British Journal of Clinical Pharmacology*, *87*(7), 2711–2722. https://doi.org/https://doi.org/10.1111/bcp.14609
16. Deepika, D., Kumar, S., Bravo, N., Esplugas, R., Capodiferro, M., Sharma, R. P., Schuhmacher, M., Grimalt, J. O., Blanco, J., & Kumar, V. (2022). Chlorpyrifos, permethrin and cyfluthrin effect on cell survival, permeability, and tight junction in an in-vitro model of the human blood-brain barrier (BBB). *NeuroToxicology*, *93*, 152–162. https://doi.org/https://doi.org/10.1016/j.neuro.2022.09.010
17. Deepika, D., Sharma, R. P., García-Cortés, H., González-Alzaga, B., Hérnandez, A. F., Lacasaña, M., Schuhmacher, M., & Kumar, V. (2022). P04-03 Evaluation of the generic physiologically based pharmacokinetic model (PBPK) for organophosphate flame retardants (OPFRs) and neurotoxic risk assessment in children. *Toxicology Letters*, *368*, S99. https://doi.org/10.1016/j.toxlet.2022.07.287
18. Deepika, D., Sharma, R. P., Schuhmacher, M., & Kumar, V. (2020). An integrative translational framework for chemical induced neurotoxicity – a systematic review. *Critical Reviews in Toxicology*, *50*(5), 424–438. https://doi.org/10.1080/10408444.2020.1763253
19. Deepika, D., Sharma, R. P., Schuhmacher, M., & Kumar, V. (2021). Risk Assessment of Perfluorooctane Sulfonate (PFOS) using Dynamic Age Dependent Physiologically based Pharmacokinetic Model (PBPK) across Human Lifetime. *Environmental Research*, *199*, 111287. https://doi.org/https://doi.org/10.1016/j.envres.2021.111287
20. Deepika, D., Sharma, R. P., Schuhmacher, M., Sakhi, A. K., Thomsen, C., Chatzi, L., Vafeiadi, M., Quentin, J., Slama, R., Grazuleviciene, R., Andrušaitytė, S., Waiblinger, D., Wright, J., Yang, T. C., Urquiza, J., Vrijheid, M., Casas, M., Domingo, J. L., & Kumar, V. (2022). Unravelling sex-specific BPA toxicokinetics in children using a pediatric PBPK model. *Environmental Research*, *215*, 114074. https://doi.org/https://doi.org/10.1016/j.envres.2022.114074
21. Döring, B., & Petzinger, E. (2014). Phase 0 and phase III transport in various organs: Combined concept of phases in xenobiotic transport and metabolism. In *Drug Metabolism Reviews* (Vol. 46, Issue 3, pp. 261–282). Informa Healthcare. https://doi.org/10.3109/03602532.2014.882353
22. El-Masri, H., Kleinstreuer, N., Hines, R. N., Adams, L., Tal, T., Isaacs, K., Wetmore, B. A., & Tan, Y.-M. (2016). Integration of Life-Stage Physiologically Based Pharmacokinetic Models with Adverse Outcome Pathways and Environmental Exposure Models to Screen for Environmental Hazards. *Toxicological Sciences*, *152*(1), 230–243. https://doi.org/10.1093/toxsci/kfw082
23. Espié, P., Tytgat, D., Sargentini-Maier, M.-L., Poggesi, I., & Watelet, J.-B. (2009). Physiologically based pharmacokinetics (PBPK). *Drug Metabolism Reviews*, *41*(3), 391–407. https://doi.org/10.1080/10837450902891360
24. Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J. L., & Nadal, M. (2014). PBPK modeling for PFOS and PFOA: Validation with human experimental data. *Toxicology Letters*, *230*(2), 244–251. https://doi.org/https://doi.org/10.1016/j.toxlet.2014.01.007
25. Ferreira, A., Lapa, R., & Vale, N. (2021). Permeability of gemcitabine and PBPK modeling to assess oral administration. *Current Issues in Molecular Biology*, *43*(3), 2189–2198. https://doi.org/10.3390/cimb43030153
26. Gundert-Remy, U., Mielke, H., & Bernauer, U. (2013). Commentary: Dermal penetration of bisphenol A—Consequences for risk assessment. *Toxicology Letters*, *217*(2), 159–161. https://doi.org/https://doi.org/10.1016/j.toxlet.2012.12.009
27. Haddad, S., & Nong, A. (2020). Physiologically based pharmacokinetic model: excretion via urine, feces, and breath. In *Physiologically Based Pharmacokinetic (PBPK) Modeling* (pp. 175–209). Elsevier. https://doi.org/10.1016/b978-0-12-818596-4.00008-4
28. Haddad, S., Poulin, P., & Krishnan, K. (2000). Relative lipid content as the sole mechanistic determinant of the adipose tissue:blood partition coefficients of highly lipophilic organic chemicals. *Chemosphere*, *40*(8), 839–843. https://doi.org/https://doi.org/10.1016/S0045-6535(99)00279-9
29. Halappanavar, S., van den Brule, S., Nymark, P., Gaté, L., Seidel, C., Valentino, S., Zhernovkov, V., Høgh Danielsen, P., de Vizcaya, A., Wolff, H., Stöger, T., Boyadziev, A., Poulsen, S. S., Sørli, J. B., & Vogel, U. (2020). Adverse outcome pathways as a tool for the design of testing strategies to support the safety assessment of emerging advanced materials at the nanoscale. *Particle and Fibre Toxicology*, *17*(1), 16. https://doi.org/10.1186/s12989-020-00344-4
30. Hoogenboom, R. (n.d.). Dose response assessment, PBPK modelling, HBGV derivation, Risk Characterisation, Uncertainties and Recommendations.
31. Huang, W., Czuba, L. C., & Isoherranen, N. (2020). Mechanistic PBPK Modeling of Urine pH Effect on Renal and Systemic Disposition of Methamphetamine and Amphetamine. *Journal of Pharmacology and Experimental Therapeutics*. https://doi.org/10.1124/jpet.120.264994
32. Huang, W., & Isoherranen, N. (2018). Development of a Dynamic Physiologically Based Mechanistic Kidney Model to Predict Renal Clearance. *CPT: Pharmacometrics & Systems Pharmacology*, *7*(9), 593–602. https://doi.org/https://doi.org/10.1002/psp4.12321
33. in the Food Chain (EFSA CONTAM Panel), E. P. on C., Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. (Ron), Leblanc, J.-C., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregård, L., Ceccatelli, S., … Schwerdtle, T. (2020). Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA Journal*, *18*(9), e06223. https://doi.org/https://doi.org/10.2903/j.efsa.2020.6223
34. Integrated Approaches to Testing and Assessment (IATA) - OECD. (n.d.).
35. Johnson, T. N., Small, B. G., & Rowland Yeo, K. (2022a). Increasing application of pediatric physiologically based pharmacokinetic models across academic and industry organizations. *CPT: Pharmacometrics and Systems Pharmacology*, *11*(3), 373–383. https://doi.org/10.1002/psp4.12764

36. Johnson, T. N., Small, B. G., & Rowland Yeo, K. (2022b). Increasing application of pediatric physiologically based pharmacokinetic models across academic and industry organizations. *CPT: Pharmacometrics and Systems Pharmacology*, *11*(3), 373–383. https://doi.org/10.1002/psp4.12764
37. Jones, H. M., & Rowland-Yeo, K. (2013). Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT: Pharmacometrics and Systems Pharmacology*, *2*(8). https://doi.org/10.1038/psp.2013.41
38. Kamiya, Y., Handa, K., Miura, T., Yanagi, M., Shigeta, K., Hina, S., Shimizu, M., Kitajima, M., Shono, F., Funatsu, K., & Yamazaki, H. (2021). In Silico Prediction of Input Parameters for Simplified Physiologically Based Pharmacokinetic Models for Estimating Plasma, Liver, and Kidney Exposures in Rats after Oral Doses of 246 Disparate Chemicals. *Chemical Research in Toxicology*, *34*(2), 507–513. https://doi.org/10.1021/acs.chemrestox.0c00336
39. Kim, T. H., Shin, S., Landersdorfer, C. B., Chi, Y. H., Paik, S. H., Myung, J., Yadav, R., Horkovics-Kovats, S., Bulitta, J. B., & Shin, B. S. (2015). Population Pharmacokinetic Modeling of the Enterohepatic Recirculation of Fimasartan in Rats, Dogs, and Humans. *AAPS Journal*, *17*(5), 1210–1223. https://doi.org/10.1208/s12248-015-9764-2
40. Kumar, S., Deepika, D., & Kumar, V. (2022). Pharmacophore Modeling Using Machine Learning for Screening the Blood–Brain Barrier Permeation of Xenobiotics. *International Journal of Environmental Research and Public Health*, *19*(20). https://doi.org/10.3390/ijerph192013471
41. Kumar, V., Deepika, D., & Sharma, R. P. (2021). Chapter 4 Integrated Translation Framework for Endocrine Disruptors in the area of Computational Toxicology. In *Challenges in Endocrine Disruptor Toxicology and Risk Assessment* (pp. 80–120). The Royal Society of Chemistry. https://doi.org/10.1039/9781839160738-00080
42. Leist, M., Ghallab, A., Graepel, R., Marchan, R., Hassan, R., Bennekou, S. H., Limonciel, A., Vinken, M., Schildknecht, S., Waldmann, T., Danen, E., van Ravenzwaay, B., Kamp, H., Gardner, I., Godoy, P., Bois, F. Y., Braeuning, A., Reif, R., Oesch, F., … Hengstler, J. G. (2017). Adverse outcome pathways: opportunities, limitations and open questions. *Archives of Toxicology*, *91*(11), 3477–3505. https://doi.org/10.1007/s00204-017-2045-3
43. Li, R., Barton, H. A., & Varma, M. v. (2014). Prediction of Pharmacokinetics and Drug–Drug Interactions When Hepatic Transporters are Involved. *Clinical Pharmacokinetics*, *53*(8), 659–678. https://doi.org/10.1007/s40262-014-0156-z
44. Lin, W., Chen, Y., Unadkat, J. D., Zhang, X., Wu, D., & Heimbach, T. (2022). Applications, Challenges, and Outlook for PBPK Modeling and Simulation: A Regulatory, Industrial and Academic Perspective. *Pharmaceutical Research*, *39*(8), 1701–1731. https://doi.org/10.1007/s11095-022-03274-2
45. Lippert, J., Burghaus, R., Edginton, A., Frechen, S., Karlsson, M., Kovar, A., Lehr, T., Milligan, P., Nock, V., Ramusovic, S., Riggs, M., Schaller, S., Schlender, J., Schmidt, S., Sevestre, M., Sjögren, E., Solodenko, J., Staab, A., & Teutonico, D. (2019). Open Systems Pharmacology Community—An Open Access, Open Source, Open Science Approach to Modeling and Simulation in Pharmaceutical Sciences. *CPT: Pharmacometrics & Systems Pharmacology*, *8*(12), 878–882. https://doi.org/https://doi.org/10.1002/psp4.12473
46. Lu, J., Bender, B., Jin, J. Y., & Guan, Y. (2021). Deep learning prediction of patient response time course from early data via neural-pharmacokinetic/pharmacodynamic modelling. *Nature Machine Intelligence*, *3*(8), 696–704. https://doi.org/10.1038/s42256-021-00357-4
47. Lu, J., Deng, K., Zhang, X., Liu, G., & Guan, Y. (2021). Neural-ODE for pharmacokinetics modeling and its advantage to alternative machine learning models in predicting new dosing regimens. *IScience*, *24*(7), 102804. https://doi.org/https://doi.org/10.1016/j.isci.2021.102804
48. Malik, P. R. V., & Edginton, A. N. (2019). Physiologically-Based Pharmacokinetic Modeling vs. Allometric Scaling for the Prediction of Infliximab Pharmacokinetics in Pediatric Patients. *CPT: Pharmacometrics and Systems Pharmacology*, *8*(11), 835–844. https://doi.org/10.1002/psp4.12456
49. McLanahan, E. D., White, P., Flowers, L., & Schlosser, P. M. (2014). The use of PBPK models to inform human health risk assessment: Case study on perchlorate and radioiodide human lifestage models. *Risk Analysis*, *34*(2), 356–366. https://doi.org/10.1111/risa.12101
50. McNally, K., Cotton, R., Cocker, J., Jones, K., Bartels, M., Rick, D., Price, P., & Loizou, G. (2012). Reconstruction of exposure to m-xylene from human biomonitoring data using PBPK modelling, bayesian inference, and Markov chain Monte Carlo simulation. *Journal of Toxicology*, *2012*. https://doi.org/10.1155/2012/760281
51. McNally, K., Sams, C., Hogg, A., Lumen, A., & Loizou, G. (2021). Development, Testing, Parameterisation and Calibration of a Human PBPK Model for the Plasticiser, Di-(2-propylheptyl) Phthalate (DPHP) Using in Silico, in vitro and Human Biomonitoring Data. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.692442
52. McNamara, P. J., & Alcorn, J. (2002). Protein binding predictions in infants. *AAPS PharmSci*, *4*(1), 19–26. https://doi.org/10.1208/ps040104
53. Meek, M. E. (Bette). (2017). AOPs in hazard characterization for human health. *Current Opinion in Toxicology*, *3*, 80–86. https://doi.org/https://doi.org/10.1016/j.cotox.2017.06.002
54. Michelet, R., van Bocxlaer, J., Allegaert, K., & Vermeulen, A. (2018). The use of PBPK modeling across the pediatric age range using propofol as a case. *Journal of Pharmacokinetics and Pharmacodynamics*, *45*(6), 765–785. https://doi.org/10.1007/s10928-018-9607-8

55. Mielke, H., Partosch, F., & Gundert-Remy, U. (2011). The contribution of dermal exposure to the internal exposure of bisphenol A in man. *Toxicology Letters*, *204*(2), 190–198. https://doi.org/https://doi.org/10.1016/j.toxlet.2011.04.032
56. More, S. L., Thornton, S. A., Maskrey, J. R., Sharma, A., de Gandiaga, E., Cheng, T. J., Fung, E. S., Bernal, A. J., & Madl, A. K. (2020). PBPK modeling characterization of potential acute impairment effects from inhalation of ethanol during e-cigarette use. *Inhalation Toxicology*, *32*(1), 14–23. https://doi.org/10.1080/08958378.2020.1720867
57. Pearce, R. G., Setzer, R. W., Strope, C. L., Sipes, N. S., & Wambaugh, J. F. (2017). Httk: R package for high-throughput toxicokinetics. *Journal of Statistical Software*, *79*, 1–26. https://doi.org/10.18637/jss.v079.i04
58. Peters, S. A., & Dolgos, H. (2019). Requirements to Establishing Confidence in Physiologically Based Pharmacokinetic (PBPK) Models and Overcoming Some of the Challenges to Meeting Them. *Clinical Pharmacokinetics*, *58*(11), 1355–1371. https://doi.org/10.1007/s40262-019-00790-0
59. Poulin, P., & Krishnan, K. (1995). An algorithm for predicting tissue : Blood partition coefficients of organic chemicals from n-octanol: Water partition coefficient data. *Journal of Toxicology and Environmental Health*, *46*(1), 117–129. https://doi.org/10.1080/15287399509532021
60. Pradeep, P., Patlewicz, G., Pearce, R., Wambaugh, J., Wetmore, B., & Judson, R. (2020). Using chemical structure information to develop predictive models for in vitro toxicokinetic parameters to inform high-throughput risk-assessment. *Computational Toxicology*, *16*, 100136. https://doi.org/https://doi.org/10.1016/j.comtox.2020.100136
61. Punt, A. (2020). New approach methodologies (NAMs) for human-relevant biokinetics predictions. *ALTEX*. https://doi.org/10.14573/altex.2003242
62. Ramoju, S. P., Mattison, D. R., Milton, B., McGough, D., Shilnikova, N., Clewell, H. J., Yoon, M., Taylor, M. D., Krewski, D., & Andersen, M. E. (2017). The application of PBPK models in estimating human brain tissue manganese concentrations. *NeuroToxicology*, *58*, 226–237. https://doi.org/https://doi.org/10.1016/j.neuro.2016.12.001
63. Rasool, M. F., Khalid, S., Majeed, A., Saeed, H., Imran, I., Mohany, M., Al-Rejaie, S. S., & Alqahtani, F. (2019). Development and evaluation of physiologically based pharmacokinetic drug–disease models for predicting rifampicin exposure in tuberculosis and cirrhosis populations. *Pharmaceutics*, *11*(11). https://doi.org/10.3390/pharmaceutics11110578
64. Rebeka, J., Jerneja, O., Igor, L., Boštjan, P., Aleksander, B., Simon, Ž., & Albin, K. (2019). PBPK Absorption Modeling of Food Effect and Bioequivalence in Fed State for Two Formulations with Crystalline and Amorphous Forms of BCS 2 Class Drug in Generic Drug Development. *AAPS PharmSciTech*, *20*(2), 59. https://doi.org/10.1208/s12249-018-1285-8
65. Reddy, M. B., Bolger, M. B., Fraczkiewicz, G., Frari, L. del, Luo, L., Lukacova, V., Mitra, A., Macwan, J. S., Mullin, J. M., Parrott, N., & Heikkinen, A. T. (2021). Pbpk modeling as a tool for predicting and understanding intestinal metabolism of uridine 5′-diphospho-glucuronosyltransferase substrates. In *Pharmaceutics* (Vol. 13, Issue 9). MDPI. https://doi.org/10.3390/pharmaceutics13091325
66. Roberts, M. S., Magnusson, B. M., Burczynski, F. J., & Weiss, M. (2002). Enterohepatic Circulation. *Clinical Pharmacokinetics*, *41*(10), 751–790. https://doi.org/10.2165/00003088-200241100-00005
67. Rodgers, T., Leahy, D., & Rowland, M. (2005). Physiologically based pharmacokinetic modeling 1: Predicting the tissue distribution of moderate-to-strong bases. *Journal of Pharmaceutical Sciences*, *94*(6), 1259–1276. https://doi.org/10.1002/jps.20322
68. Rodgers, T., & Rowland, M. (2006). Physiologically based pharmacokinetic modelling 2: Predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *Journal of Pharmaceutical Sciences*, *95*(6), 1238–1257. https://doi.org/10.1002/jps.20502
69. Rowland Yeo, K., & Gil Berglund, E. (2021). An Integrated Approach for Assessing the Impact of Renal Impairment on Pharmacokinetics of Drugs in Development: Pivotal Role of PBPK Modelling. *Clinical Pharmacology & Therapeutics*, *110*(5), 1168–1171. https://doi.org/https://doi.org/10.1002/cpt.2243
70. Sager, J. E., Yu, J., Ragueneau-Majlessi, I., & Isoherranen, N. (2015). Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: A systematic review of published models, applications, and model verification. In *Drug Metabolism and Disposition* (Vol. 43, Issue 11, pp. 1823–1837). American Society for Pharmacology and Experimental Therapy. https://doi.org/10.1124/dmd.115.065920
71. Salem, F., Small, B. G., & Johnson, T. N. (2022). Development and application of a pediatric mechanistic kidney model. *CPT: Pharmacometrics & Systems Pharmacology*, *11*(7), 854–866. https://doi.org/https://doi.org/10.1002/psp4.12798
72. Schmitt, W. (2008). General approach for the calculation of tissue to plasma partition coefficients. *Toxicology in Vitro*, *22*(2), 457–467. https://doi.org/https://doi.org/10.1016/j.tiv.2007.09.010
73. Sharma, R. P., Schuhmacher, M., & Kumar, V. (2017a). Developing integrated PBPK/PD coupled mechanistic pathway model (miRNA-BDNF): An approach towards system toxicology. *Toxicology Letters*, *280*, 79–91. https://doi.org/https://doi.org/10.1016/j.toxlet.2017.08.003
74. Sharma, R. P., Schuhmacher, M., & Kumar, V. (2017b). Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. *Environment International*, *99*, 1–14. https://doi.org/https://doi.org/10.1016/j.envint.2016.09.016
75. Sharma, R. P., Schuhmacher, M., & Kumar, V. (2018). The development of a pregnancy PBPK Model for Bisphenol A and its evaluation with the available biomonitoring data. *Science of The Total Environment*, *624*, 55–68. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.12.023

76. Smits, A., de Cock, P., Vermeulen, A., & Allegaert, K. (2019). Physiologically based pharmacokinetic (PBPK) modeling and simulation in neonatal drug development: how clinicians can contribute. *Expert Opinion on Drug Metabolism & Toxicology*, *15*(1), 25–34. https://doi.org/10.1080/17425255.2019.1558205
77. Stader, F., Penny, M. A., Siccardi, M., & Marzolini, C. (2019). A Comprehensive Framework for Physiologically-Based Pharmacokinetic Modeling in Matlab. *CPT: Pharmacometrics & Systems Pharmacology*, *8*(7), 444–459. https://doi.org/https://doi.org/10.1002/psp4.12399
78. Subhani, S., Kim, C., Muniz, P., Rodriguez, M., van Os, S., Suarez, E., Cristofoletti, R., Schmidt, S., & Vozmediano, V. (2022). Application of physiologically based absorption and pharmacokinetic modeling in the development process of oral modified release generic products. *European Journal of Pharmaceutics and Biopharmaceutics*, *176*, 87–94. https://doi.org/https://doi.org/10.1016/j.ejpb.2022.05.007
79. T'jollyn, H., Vermeulen, A., & van Bocxlaer, J. (2018). PBPK and its Virtual Populations: the Impact of Physiology on Pediatric Pharmacokinetic Predictions of Tramadol. *The AAPS Journal*, *21*(1), 8. https://doi.org/10.1208/s12248-018-0277-7
80. Tsiros, P., Bois, F. Y., Dokoumetzidis, A., Tsiliki, G., & Sarimveis, H. (2019). Population pharmacokinetic reanalysis of a Diazepam PBPK model: a comparison of Stan and GNU MCSim. *Journal of Pharmacokinetics and Pharmacodynamics*, *46*(2), 173–192. https://doi.org/10.1007/s10928-019-09630-x
81. Tylutki, Z., & Polak, S. (2017). A four-compartment PBPK heart model accounting for cardiac metabolism - model development and application. *Scientific Reports*, *7*(1), 39494. https://doi.org/10.1038/srep39494
82. Utsey, K., Gastonguay, M. S., Russell, S., Freling, R., Riggs, M. M., & Elmokadem, A. (2020). Quantification of the impact of partition coefficient prediction methods on physiologically based pharmacokinetic model output using a standardized tissue composition. *Drug Metabolism and Disposition*, *48*(10), 903–916. https://doi.org/10.1124/DMD.120.090498
83. Worley, R. R., & Fisher, J. (2015). Application of physiologically-based pharmacokinetic modeling to explore the role of kidney transporters in renal reabsorption of perfluorooctanoic acid in the rat. *Toxicology and Applied Pharmacology*, *289*(3), 428–441. https://doi.org/https://doi.org/10.1016/j.taap.2015.10.017
84. Yang, Y., Xu, X., & Georgopoulos, P. G. (2010). A Bayesian population PBPK model for multiroute chloroform exposure. *Journal of Exposure Science and Environmental Epidemiology*, *20*(4), 326–341. https://doi.org/10.1038/jes.2009.29
85. Yun, Y. E., & Edginton, A. N. (2021). Evaluation of models for predicting pediatric fraction unbound in plasma for human health risk assessment. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, *84*(2), 67–83. https://doi.org/10.1080/15287394.2020.1835761
86. Zakaria, Z., & Badhan, R. (2018). Development of a Region-Specific Physiologically Based Pharmacokinetic Brain Model to Assess Hippocampus and Frontal Cortex Pharmacokinetics. *Pharmaceutics*, *10*(1), E14. https://doi.org/10.3390/pharmaceutics10010014
87. Zgheib, E., Gao, W., Limonciel, A., Aladjov, H., Yang, H., Tebby, C., Gayraud, G., Jennings, P., Sachana, M., Beltman, J. B., & Bois, F. Y. (2019). Application of three approaches for quantitative AOP development to renal toxicity. *Computational Toxicology*, *11*, 1–13. https://doi.org/https://doi.org/10.1016/j.comtox.2019.02.001