IN SILICO TOXICOKINETICS IN NEXT-GENERATION RISK ASSESSMENT: THE EVOLVING ROLE OF PBPK AND QIVIVE MODELS

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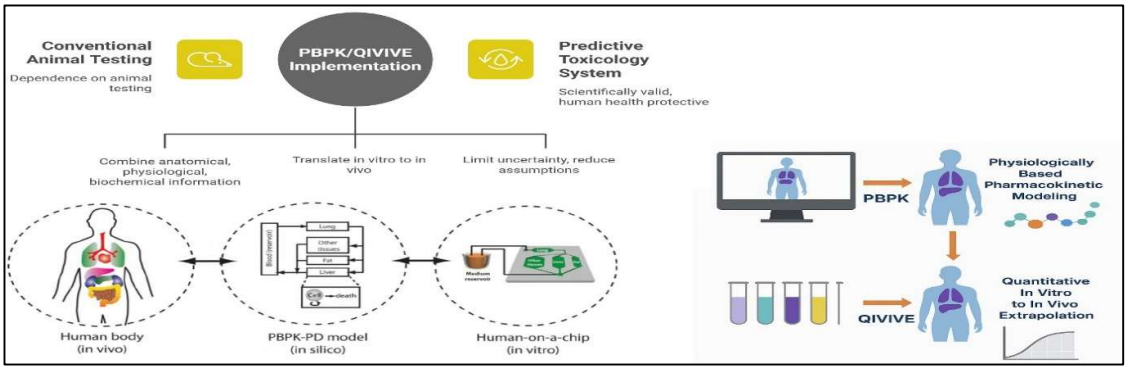
Abstract
The toxicological risk assessment paradigm is changing at breakneck speed from dependence on conventional animal testing toward predictive, human-relevant, and mechanism-based strategies. During the transition, in silico toxicokinetics has emerged as the central figure, and physiologically based pharmacokinetic (PBPK) models and quantitative in vitro–in vivo extrapolation (QIVIVE) is among the most important tools to have emerged. PBPK models combine anatomical, physiological, and biochemical information with compound-specific information to model absorption, distribution, metabolism, and excretion (ADME) processes for various species, life stages, and exposure routes. QIVIVE adds this system to the equation by allowing for in vitro toxicity value to be translated to in vivo exposure concentrations through reverse dosimetry and thus quantitatively derive human-equivalent dose estimates. Collectively, PBPK and QIVIVE enhance next-generation risk assessment (NGRA) approaches by limiting uncertainty, reducing default assumptions, and enhancing transparency of regulatory decision-making. Applications are spreading to various areas of chemical prioritization, pharmaceutical safety assessment, and defense of environmental health. Combination with high-throughput screening, omics-based biomarkers, and machine learning is also improving the predictive power, allowing for more sophisticated characterization of inter-individual variability, susceptible subpopulations, and low-dose effects. There are still, however, challenges regarding data availability, standardization, validation, and harmonization of regulatory acceptance among legislatures. This review critically analyzes the changing role of PBPK and QIVIVE in NGRA, the strengths and limitations, and the future directions. By facilitating mechanistic, computationally proficient, and ethically responsible risk assessment, the models are a milestone towards the development of a predictive toxicology system that is scientifically valid and human health protective.

Keywords
In silico toxicokinetics; Physiologically based pharmacokinetic modeling; Quantitative in vitro–in vivo extrapolation; Next-generation risk assessment; Human-relevant safety assessment.

Graphical Abstract:



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1. INTRODUCTION

The 21st century is reshaping toxicological risk assessment through an engaged integration of scientific progress, regulatory change, and ethical responsibility. Traditional historic toxicology has rested on in vivo animal testing to evaluate chemical safety. Such reliance is increasingly being questioned on ethical, low throughput, cost constraints, interspecies differences, and restricted human relevance [1,2,3]. These restrictions direct the course towards the imperative need for a general trend towards more predictive, mechanism-based, and human-relevant methods. Toward this end, the regulating authorities and the research world have progressively joined ranks behind New Approach Methodologies (NAMs) directed towards the use of non-animal data sources and in silico modeling [4,5]. Leading this revolution are physiologically based pharmacokinetic (PBPK) models and quantitative in vitro to in vivo extrapolation (QIVIVE) methods, which possess unprecedented ability to predict chemical behavior in man without the need for experiment [6,7]. PBPK models apply mathematical models of anatomical and physiological functions to model the absorption, distribution, metabolism, and excretion (ADME) of chemicals in a species-specific manner [8,9]. These models are able to forecast tissue concentrations with respect to time, gain information on internal doses, and allow extrapolation between populations with varying physiological characteristics, routes of exposure, stages of life, and species [10,11,12]. Positioned atop PBPK, QIVIVE connects in vitro toxicity data to in vivo human exposure situations via incorporation of bioactivity data and kinetic modeling [13,14]. Reverse dosimetry or forward extrapolation using QIVIVE enables the transformation of concentration from in vitro (e.g., EC50, benchmark doses) to human-equivalent dose (HED) such that risk assessors could extrapolate points of departure (PODs) from mechanistic assays [15]. Regulatory bodies have started appreciating the scientific validity and applicability of such models. The European Chemicals Agency (ECHA), the U.S. Environmental Protection Agency (EPA), and the Organisation for Economic Co-operation and Development (OECD) have promulgated guidelines favoring the application of PBPK and QIVIVE in safety assessments [16,17]. For example, OECD guidelines for PBK modeling to support regulatory decision-making (2021) establishes standards of model credibility, verification, as well as transparency [18]. Moreover, the U.S. EPA has incorporated QIVIVE into the ToxCast and Tox21 initiatives, using high-throughput screening (HTS) data to rank chemicals by estimated internal doses [19]. Figure 1 illustrates the progression of Toxicological Risk Assessment. Strategies for PBPK and QIVIVE are increasingly being established in disciplines other than environmental safety. PBPK-based projections have been utilized in drug-drug interaction studies, dosing in pediatrics, and in testing for bioequivalence in pharmaceutical development [20]. More recently, similar strategies are in use in cosmetics and food safety regulations that can substitute animal testing by modeling systemic exposure after dermal or oral exposure [21,22]. Despite these advancements, there is still much work ahead. One such major hindrance is the absence of harmonized model validation criteria among regulatory jurisdictions. Parameterization techniques, model structure, and report format differences can be a challenge for mutual recognition and replication [23]. In addition, kinetic data for most industrial and environmental chemicals are still not available, preventing the broad use of QIVIVE [24]. To rectify these deficiencies, international efforts like the OECD Good Modeling Practice framework and the European Partnership for Alternative Approaches to Animal Testing (EPAA) have focused on enhancing model transparency, documentation, and accessibility using open-source platforms [25,26]. The use of PBPK and QIVIVE tools under the auspices of NGRA is a transition from hazard-based to exposure-based evaluation. Historically, regulatory toxicology used conservatively as-tested paradigms that are not typically representative of actual exposure levels [27]. However, in silico models allow the simulation of realistic, population-derived exposure conditions, and consequently, a more advanced foundation for the development of safety margins [28]. PBPK modeling using Monte Carlo simulations is utilized to explore population variation to address age, sex, genetic polymorphism, health status, and lifestyle variations [29]. These models not only have predictive capability but are also being integrated into Adverse Outcome Pathway (AOP) frameworks. AOPs connect molecular initiating events (MIEs) to downstream key events and adverse outcomes, resulting in a mechanistic roadmap of toxicity [30]. The incorporation of AOPs into QIVIVE and PBPK

improves mechanistic understanding of bioassay results and allows quantitative inference of in vitro perturbations to in vivo hazard [31].

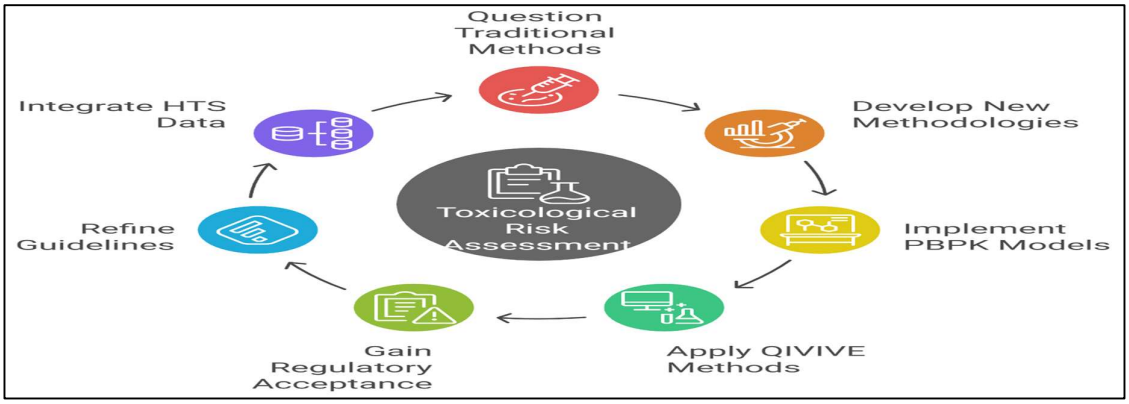

Fig. No:1: Evolution of Toxicological Risk Assessment

The use of PBPK and QIVIVE tools under the auspices of NGRA is a transition from hazard-based to exposure-based evaluation. Historically, regulatory toxicology used conservatively as-tested paradigms that are not typically representative of actual exposure levels [27]. However, in silico models allow the simulation of realistic, population-derived exposure conditions, and consequently, a more advanced foundation for the development of safety margins [28]. PBPK modeling using Monte Carlo simulations is utilized to explore population variation to address age, sex, genetic polymorphism, health status, and lifestyle variations [29]. These models not only have predictive capability but are also being integrated into Adverse Outcome Pathway (AOP) frameworks. AOPs connect molecular initiating events (MIEs) to downstream key events and adverse outcomes, resulting in a mechanistic roadmap of toxicity [30]. The incorporation of AOPs into QIVIVE and PBPK improves mechanistic understanding of bioassay results and allows quantitative inference of in vitro perturbations to in vivo hazard [31]. The combined method has already been applied for example, to endocrine disruption [32], developmental neurotoxicity [33], hepatotoxicity [34], and reproductive toxicity [35]. More recent developments still show the usefulness of such models under data-limited conditions. For instance, in vitro-to-in vivo extrapolations were able to accurately predict in vivo developmental toxicity in rodents from in vitro transcriptomic profiles and PBPK simulations only [36]. Even reverse dosimetry applied in real-world human biomonitoring studies has been used to calculate external exposure doses by reverse calculation that can help inform cumulative risk assessment of environmental pollutants [37,38]. Advancing Safety Evaluation with PBPK and QIVIVE showed in Figure 2. Software developments have also made adoption easier.

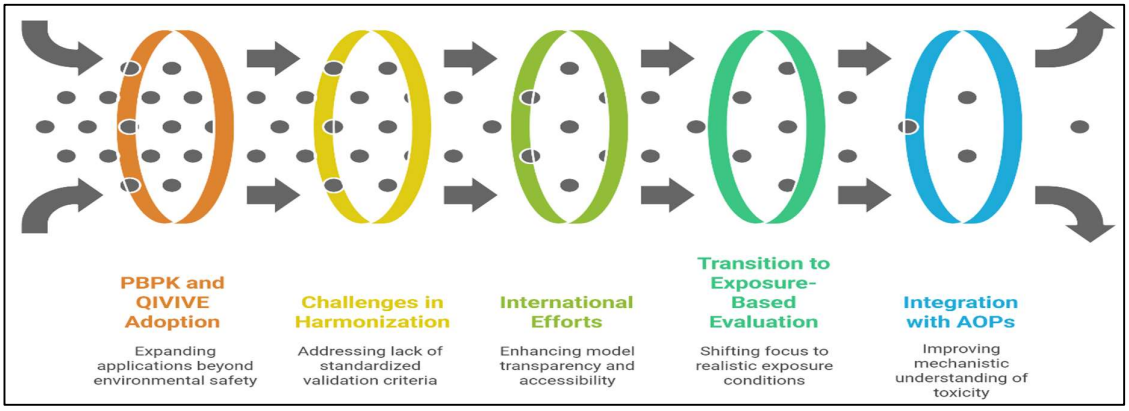

Fig. No: 2: Advancing Safety Evaluation with PBPK and QIVIVE

Simcyp, GastroPlus, and PK-Sim platforms facilitate full PBPK modeling, while toolboxes such as KNIME, R Shiny, and Virtual Cell-Based Assay (VCBA) facilitate QIVIVE workflow ease [39,40]. Parameter libraries and databases that are regulator-friendly, such as the OECD QSAR Toolbox and Open Systems Pharmacology Suite, are now widely used for facilitating data input and model validation [41,42]. Briefly, PBPK and QIVIVE models are key cornerstones of next-generation, human-translational toxicological evaluation. They not only are scientifically sound substitutes for animal experiments, but also with increasingly more potent aids for risk prediction, transparency in regulations, and regulatory decision-making. As the models continue to develop further and harmonize in the regulatory environments, full incorporation into safety evaluation methodologies holds the promise of a fairer, cost-saving, and scientifically credible future.

2. PRINCIPLES OF PBPK MODELING IN TOXICOKINETICS

2.1. Definition and Historical Context

Physiologically Based Pharmacokinetic (PBPK) modeling is a biologically mechanistic and mechanism-based modeling method that predicts the absorption, distribution, metabolism, and excretion (ADME) of chemical compounds by various organ systems of human and animal organisms. In contrast to empirical models, PBPK models are constructed to mimic real anatomy and physiology, including tissue volume data, blood flow rates, enzyme activity, and transport of biochemicals. This enables them to predict accurately internal tissue concentration with time. Its mechanistic foundation is its forte, allowing species-, age-, and condition-specific physiological and biochemical data to be combined and thereby making it extremely valuable in extrapolations over populations, life stages, or exposure scenarios. PBPK modeling emerged in the 1970s, initially being used within the framework of inhalation toxicology and occupational exposure estimation. Initial applications were to volatile organic compounds in indoor air in the work environment, but explosive growth of computational biology and availability of physiological databases soon broadened its use. With advances in in vitro assays and in silico software, PBPK modeling has become a central element in drug discovery, chemical risk assessment, and regulatory safety assessments in efforts such as Next-Generation Risk Assessment (NGRA) [43].

2.2. Basic Building Blocks of PBPK Models

A typical PBPK model considers the body to be a cascade of connected compartments where each compartment is an illustration of an organ or a tissue like the liver, kidneys, lungs, fat, brain, or gastrointestinal tract. The compartments are characterized by anatomical, physicochemical, and biochemical parameters. The anatomical parameters are organ weights and blood flow rates to different areas; physicochemical properties are solubility, lipophilicity (logP), molecular weight, and ionization constants (pKa); biochemical processes are enzyme kinetics (e.g., Vmax and Km), metabolic clearance rates, transporter activity, and protein binding. The entire collection of compartments is connected by systemic circulation, and chemical passage between them is controlled by mass-balance differential equations. This framework enables PBPK models to model the time-course of concentration of a chemical in each organ such that exposure at tissue level can be computed, not merely from plasma levels. As shown in toxicokinetic modeling studies for many environmental xenobiotics, these parameters enable accurate prediction of internal dosimetry, hence filling the gap between external exposure and internal effect levels [44]. Inputs to the PBPK model are typically grouped into three broad categories: physiological parameters (e.g., organ volume, blood perfusion, and cardiac output), biochemical parameters (e.g., intrinsic clearance, enzyme efficacy, and transport kinetics), and compound-specific parameters (e.g., partition coefficients, pKa, and lipophilicity). These are normally derived from literature, experimental data, or calculated through quantitative structure-property relationships (QSPRs). Software has also been developed to bridge data gaps for chemicals without experimental data, e.g., by prediction using QPPRs [45].

2.3. PBPK Modeling Platforms

A variety of commercial and open-source platforms is available to facilitate PBPK modeling in academia and regulation. Each platform differs in scope, flexibility, and user access. Table 1 below illustrates concise features of popular tools

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Table 1. List of PBPK Modeling Tools

Tools	Developer	Uses
Simcyp®	Certara	Drug–drug interactions, pediatrics, QIVIVE
GastroPlus®	Simulations Plus	ADME profiling, oral absorption, IVIVC
PK-Sim®	Open Systems Pharmacology	Risk assessment, whole-body PBPK
MATLAB/SimBiology®	MathWorks	Custom dynamic systems, PBPK/QSP
mrgsolve (R)	R Community	Pharmacometrics, simulation pipelines

These tool facilitate PBPK modeling under various conditions of exposure, with some platforms aimed at regulatory-grade validation and others offering research flexibility. The incorporation of population variability, Monte Carlo simulations, and graphical sensitivity analysis has further extended their application to NGRA and regulatory submissions [46].

2.4. Benefits Compared to Conventional Pharmacokinetic Models

Relative to the traditional compartmental models, PBPK modeling offers several benefits on the basis of its physiological and mechanistic basis. To begin with, PBPK models introduce biological plausibility, which enhances predictability and interpretability. PBPK models facilitate interspecies and inter-individual extrapolation, i.e., from rodent to human uses or children to the elderly. PBPK modeling further facilitates route-to-route extrapolation, i.e., transformation of oral exposure to inhalation or dermal exposure estimates [47]. A second important strength is human relevance. PBPK models allow extrapolation of in vitro test data to be used for the prediction of in vivo exposures, facilitating replacement of animal testing and adoption of New Approach Methodologies (NAMs). They are used daily to convert HTS data into human-equivalent doses, a critical part of QIVIVE pipelines. They are similarly accepted by international regulatory bodies such as the FDA, EMA, and OECD, which now promote the application of PBPK modeling in selection of dose, safety assessment, and chemical risk assessment. Their inclusion into guidance documents and frameworks, such as the OECD guidance for the validation of PBK models, is proof of increasing global consensus [48]. Moreover, PBPK models extend risk assessment by dynamic, exposure-based insight. They permit toxicologists to model exposure patterns in sensitive subpopulations (e.g., pregnant females, neonates), or those with deranged physiology. This capability supports dose–response modeling by moving away from external dose estimation towards tissue-specific internal concentration measures such as Cmax and AUC [49].

2.5. Workflow for PBPK Model Development and Evaluation

Constructing a virtual anatomical and physiological model typically forms the initial step towards the development of a physiologically based pharmacokinetic (PBPK) model, in which the body is modeled as a series of compartments that represent major organs and tissues responsible for the absorption, distribution, metabolism, and excretion (ADME) of the target chemical. The compartments are characterized by organ-specific volume and blood flow rates and linked by systemic circulation to mimic biological transport. This is subsequently followed by model parameterization in the form of a mix of physiological data, biochemical data (i.e., transport activity and enzyme kinetics), and chemical-specific properties such as partition coefficients and lipophilicity. The inputs can be

derived from the literature, in vitro data, predictive models, or curated databases. Following the initial parameterization, the model is optimized and fine-tuned by adjusting the parameters so that the resultant pharmacokinetic profiles agree with whatever experimental or clinical data may be available. Validation is then performed subsequently, employing independent sets of data to challenge whether the model handles consistently for varied cases and compounds and thereby ensure external validity. Importantly, reliability and robustness of the model are also evaluated through sensitivity analysis to determine the parameters of most sensitive output variation, and uncertainty analysis, most commonly through Monte Carlo simulation or Bayesian methods, in an effort to estimate confidence limits to enclose prediction. This intensive and iterative process guarantees the scientific validity, clarity, and regulatory acceptability of PBPK models for facilitating chemical risk assessment and decision-making in Next-Generation Risk Assessment (NGRA) systems. Figure 3 describes PBPK Model Development along with Evaluation Workflow [50]

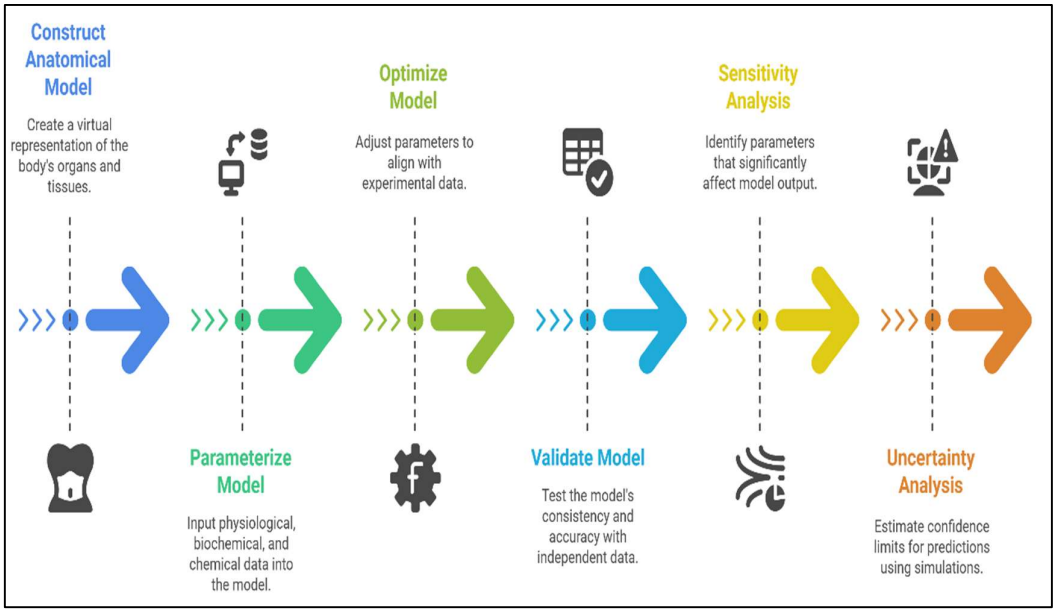

Figure 3: PBPK Model Development and Evaluation Workflow

3. QIVIVE: FROM IN VITRO TO HUMAN-RELEVANT PREDICTIONS

3.1. Definition and Rationale of QIVIVE

Quantitative In Vitro to In Vivo Extrapolation, or QIVIVE, is a mechanistic procedure through which in vitro test system concentrations or effects, e.g., with human cell cultures or tissue models, are extrapolated to predict exposure concentrations or internal doses in humans. It has a key function in transforming the non-animal assay output to regulatory-relevant parameters, mostly concerning Next-Generation Risk Assessment (NGRA) [51]. Classical toxicology assays heavily depend on animal models, which are well-documented to be beset by issues of human relevance, expense, throughput, and ethics. Conversely, QIVIVE uses physiological and pharmacokinetic modeling to extrapolate useful human exposure estimates from in vitro concentrations in order to improve efficiency and ethical merit in safety evaluation [52]. (Figure 4) QIVIVE is now pivotal for New Approach Methodologies (NAMs) mainly because it can generate Points of Departure (PODs) from in vitro data. PODs are the foundation for deriving health-based guidance values such as acceptable daily intake (ADI) or reference doses (RfDs). With such incorporation, it becomes possible to assess the risk of thousands of chemicals without much or no in vivo data and support activities in chemical prioritization, read-across, and exposure-based waiving [53].

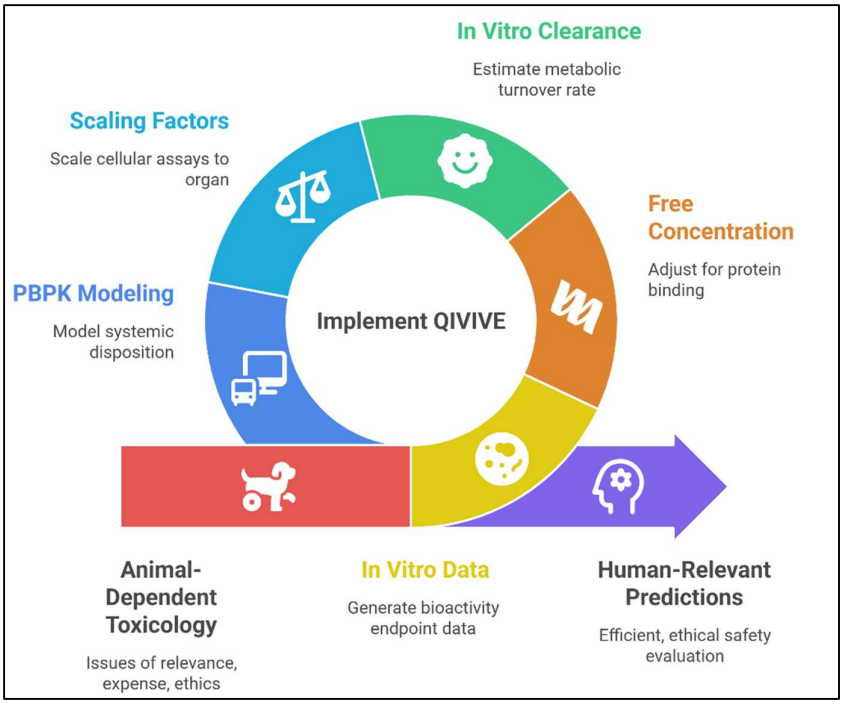

Figure 4: QIVIVE: Predicting Human Exposure from In Vitro Data

3.2. QIVIVE Method Types

There are two most prevalent types of QIVIVE methodology: forward QIVIVE and reverse dosimetry. Forward QIVIVE employs a mechanistically justifiable concentration derived in vitro, for example, EC_{50} or a benchmark concentration, which is transformed into an external dose (mg/kg/day) that would yield the same internal concentration in a human target tissue. This is the most applied technique when setting exposure limits, particularly when integrated with physiologically based pharmacokinetic (PBPK) models [54]. With dynamic ADME parameters, forward QIVIVE is able to perform route-to-route extrapolation and aid in dose selection within safety assessment paradigms. Conversely, reverse dosimetry or reverse in vitro-based QIVIVE proceeds from known or inferred human exposure levels—i.e., biomonitoring data or environmental levels—and computes the resulting internal concentrations. These are compared to in vitro bioactivity thresholds to make an estimation of potential health hazard. This is especially proficient at population biomonitoring data interpretation, evaluation of real-life exposure situations, and the ease of risk assessments where exposure monitoring is on hand but toxicity data are limited [55].

3.3. Integration with PBPK Models

PBPK modeling is the mechanistic basis on which QIVIVE was designed. It allows in vitro-to-in vivo extrapolation by dynamic simulation of tissue-specific chemical concentrations founded on human physiology and chemical-specific knowledge including lipophilicity, protein binding, metabolic clearance, and permeability [56]. PBPK models help in the determination of significant kinetic parameters like peak plasma concentration (Cmax), area under the curve (AUC), and time above threshold concentrations that are essential for the interpretation of in vivo relevance of in vitro-derived potencies. Effective QIVIVE applications are based on solid PBPK models that characterize such kinetics in realistic exposure scenarios. Simcyp, PK-Sim, GastroPlus, and mrgsolve are well-established model platforms that can accommodate QIVIVE applications. They include spaces for target tissues (e.g., liver, brain, lung) and enable enzyme and transporter kinetics and provisions for simulating population variability (e.g., age, sex, disease status), which is required for extrapolating safe exposure limits to sensitive subpopulations [57]. Routine QIVIVE steps typically involve a stepwise sequence: Step 1, quantitation in vitro of chemical effect concentration or potency; Step 2, adjustment of free (unbound) concentration by means of

protein/lipid partitioning models; Step 3, scaling up whole organ activity using empirical scale factors (e.g., hepatocytes per liver); Step 4, modeling tissue concentration with PBPK models; and Step 5, computation of an equivalent external dose with the same internal concentration as measured in vitro. Validation is achieved by comparing such extrapolated doses to available in vivo information or human exposure experiments [58].

3.4. Building Blocks of QIVIVE Implementation

QIVIVE uses a very wide variety of biological, chemical, and computational building blocks to support strong extrapolation from in vitro systems to human-relevant dose prediction. At the heart of this activity is the generation of in vitro assay data, which are usually in the form of bioactivity endpoints such as cytotoxicity, gene expression modulation, or receptor-ligand interaction. These values are meaningful only when adjusted for free concentration estimation since nominal concentrations used in in vitro experiments could be significantly affected by protein binding, lipid partitioning, or adsorption to plastic surface. This adjustment is done only for the fraction that remains biologically available in subsequent modeling. Another key piece is in vitro clearance, from hepatocyte or microsomal assays, that gives estimates of the metabolic turnover rate of the target chemical. They are used to estimate how fast a compound will be metabolized in vivo. Scaling factors are used to fill in the gap from cellular assays to whole-organ predictions—e.g., to scale enzyme activity per million hepatocytes to total liver metabolic capacity. After these parameters are set, a PBPK simulation program is used to model the systemic disposition of the compound under realistic physiological and exposure conditions. Computer programs such as Simcyp, PK-Sim, and GastroPlus are generally used to estimate tissue concentrations as a function of time from data gathered. In certain applications, e.g., in applications such as biomonitoring or population studies, reverse dosimetry is used. It makes it possible to estimate internal concentrations or exposure doses given the in vitro effects which can be taken to reflect actual exposure conditions. Considering the inherent complexity and variability of biological systems and experimental designs, uncertainty and sensitivity analysis are an absolute necessity in QIVIVE processes. The analyses enable the most impacting parameters on model predictions to be determined, evaluate the effect of parameter variability, and include confidence intervals for predicted doses. This not only renders in vitro data extrapolations mechanistically plausible but also scientifically acceptable for application in risk assessment models [59].

3.5. Advantages and Limitations of QIVIVE

The application of QIVIVE introduces a range of advantages in risk assessment. Foremost among these is its compatibility with animal-free strategies without sacrificing human-relevant dose estimates, thus enhancing the scientific and ethical attractiveness of chemical safety assessments. Its capacity to include high-throughput screening data places it well for large-scale prioritization and screening programs on chemicals. Further, QIVIVE can also be of help to read-across strategies as well as evaluation for chemicals with insufficient traditional toxicological data, especially when integrated into solid modeling platforms like PBPK and augmented with regulation-approved guidance like OECD Test Guideline 497 [60]. Nevertheless, QIVIVE is not without its challenges. One of its most significant downfalls is the availability and quality of in vitro kinetic data (e.g., metabolism, transport), which are of vital importance in valid extrapolation. Metabolite management, nonlinearity, and time-dependent toxicities are still problematic situations in the absence of total biological information. Harmonized approaches and standardized in vitro protocols and data incorporation into PBPK models also await harmonization. Reporting guidelines on models, validation needs, and acceptability criteria are also urgently needed in order to provide consistency in submissions to the regulators [61]. In spite of such challenges, ongoing technological advances in modeling, in vitro assay development, and regulatory harmonization indicate that QIVIVE will remain an anchor device for contemporary toxicology. Coupled with PBPK models, omics, and AOPs, this serves as the basis for truly integrated risk assessment systems that can produce human-relevant data without recourse to animal testing.

4. INTEGRATION OF PBPK AND QIVIVE IN PREDICTIVE TOXICOLOGY: EMERGING OPPORTUNITIES

The convergence of PBPK and QIVIVE approaches in computational toxicology has revolutionized the

predictive nature of toxicological risk assessment from empirically derived animal data towards biologically mechanistic, human-relevant approaches. Physiologically Based Pharmacokinetic (PBPK) modeling is a systematic simulation of absorption, distribution, metabolism, and excretion (ADME) processes in anatomically differentiated human body compartments, whereas Quantitative In Vitro to In Vivo Extrapolation (QIVIVE) enables in vitro concentrations to be extrapolated into context-specific external doses based on incorporation of toxicokinetic principles. Collectively, these models constitute a key building block of New Approach Methodologies (NAMs) that permit the use of in vitro-based data for informing dose-response modeling, exposure estimation, and hazard characterization within a risk assessment framework. Perhaps the most potent use of PBPK modeling is for internal chemical dosimetry modeling across various population subgroups like neonates, elderly patients, pregnant females, or hepatically or renally impaired individuals. These subgroup models transcend interindividual heterogeneity and enable more realistic human-specific safety levels from data as opposed to extrapolated animal models [61]. Moreover, this modeling enables extrapolation over exposure conditions (oral, inhalation, dermal) and species and is pivotal to connect preclinical data to human health risk assessment. Cumulative risk assessment and chemical mixture toxicology are one of the potential uses of PBPK-QIVIVE integration. Based on biomonitoring data and co-exposure data, PBPK models are able to mimic the internal levels of several compounds simultaneously. Multi-chemical modeling allows regulators to act upon environmentally representative exposures when humans are exposed to a mixture of compounds rather than one compound alone. Sophisticated co-dosing simulations also quantify pharmacokinetic interactions, particularly in cases with shared metabolic or transporter pathways [62]. Where experimental physicochemical properties for logP or permeability coefficients do not exist, Quantitative Property–Property Relationships (QPPRs) are being used more and more to estimate significant inputs required for PBPK modeling. QPPR-based PBPK models have been successful to model internal dosimetry for large categories of volatile organic compounds (VOCs), especially for inhalation exposure conditions with high-throughput screening environmental pollutants [63]. In addition, integration of PBPK-QIVIVE modeling into Adverse Outcome Pathway (AOP) schemes enables reconciliation of internal dosimetry predictions with molecular initiating events and downstream phenotypes to be made possible. These multi-scale models have been successfully used to predict chemical-induced hepatic steatosis from omics data and pathway-level perturbations. Such applications not only add mechanistic plausibility but also facilitate establishment of exposure connection with a health-relevant endpoint, thus further adding to the credibility of in silico approaches under NGRA [64]. Production of FAIR-compliant models (Findable, Accessible, Interoperable, and Reusable) marks the achievement in reproducibility and regulation accessibility of such tools. Progress is being made toward the creation of open-source PBPK platforms with transparent code, uniform input structures, and curated compound libraries. This enables data sharing and model validation by the international toxicology community to facilitate collaborative improvements and enhanced regulatory confidence [65]. Another innovation in the field is using Next-Generation PBPK (NG-PBPK) models. The models apply Bayesian statistical modeling, high-resolution omics biomarkers, and human biomonitoring exposure data to produce probabilistic dose estimates. By allowing inter-individual variability and simulating low-frequency subpopulations, NG-PBPK models generate high-resolution perspectives on sensitive populations and low-dose effects, enhancing the depth and accuracy of contemporary toxicology [66]. Even with these developments, one of the key technical challenges is the simulation of toxicokinetic saturation, a non-linear process where metabolic pathways saturate at increased doses to yield disproportionately high tissue concentrations. Such a response is especially significant in the risk estimation of endocrine disruptors or high-exposure industrial chemicals. Simulation of saturation kinetics accurately is critical to avoid underestimation of calculated safe exposure levels, especially with high-dose or chronic exposures [67]. Finally, regulatory uptake and acceptance of PBPK-QIVIVE tools persist. Guidelines are now established that have a strong emphasis on issues like biological relevance, transparency of the model, reproducibility, and predictivity. Regulators like the U.S. EPA, EMA, and OECD increasingly include these issues in dossiers and guidance documents. Inclusion of sensitivity analysis, uncertainty quantification, and plausibility checks of parameters

is now part of ascertaining the model prediction robustness, making them fit for decision-making under NAMs and NGRA paradigms [68,69,70].

5. REGULATORY ACCEPTANCE AND HARMONIZATION EFFORTS

Regulatory bodies across the globe have also appreciated the utility of PBPK and QIVIVE models in determining human health risk, but harmonization concerns and standard acceptable criteria continue to be a persisting phenomenon. The Organisation for Economic Co-operation and Development (OECD) has continued to be the forerunner agency in setting international standards. The OECD guidelines regarding PBPK model characterization, validation, and documentation have offered official recommendations regarding model reporting, transparency of parameters, and reproducibility so that in silico methods are appropriate for use in regulatory submissions [71]. Additionally, the OECD Adverse Outcome Pathway (AOP) framework in an explicit way connects mechanistic toxicology information with PBPK output to enable regulatory application of NAMs by situating in vitro results within biologically realistic mechanisms of toxicity [72].

In Europe, the European Medicines Agency (EMA) has issued comprehensive guidance on the use of PBPK modeling in drug development and pharmacokinetic assessment, especially drug–drug interaction studies and pediatric dosing [73]. The EMA requires models to be supported by clear explanation of assumptions, sensitivity analysis, and rationale for parameter values, in response to an increase in an imperative for reproducibility. In the same way, the European Chemicals Agency (ECHA) has highlighted the contribution of PBPK and QIVIVE methods towards chemical safety evaluation, especially through the inclusion of read-across and grouping techniques in the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation [74]. The models compel modelers to deploy in silico models in bridging toxicological data gaps with less dependency on animal testing.

The United States regulatory agencies, including the U.S. Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have created complementary guidelines for PBPK usage. FDA has increasingly included acceptance of PBPK-based simulations in regulatory filings to support labeling, determination of bioequivalence, and special population dosing [75]. The EPA, however, has given high priority to the use of PBPK-QIVIVE platforms in environmental risk assessment of pesticides and industrial chemicals, where high-throughput in vitro assays must be extrapolated to population-level adverse health effects [76]. Such advancements reflect a regulatory direction away from descriptive toxicology towards mechanism-based predictive toxicokinetics.

Global harmonization has also played an important role in bringing practices together. Large-scale projects such as EU-ToxRisk and RISK-HUNT3R have established working integrated computational platforms combining PBPK, QIVIVE, and omics-based strategies to provide human-relevant chemical safety predictions [77]. Such projects are leading the way for the inclusion of PBPK/QIVIVE workflows into international regulatory science, with case studies demonstrating the successful validation of computer models using human biomonitoring and epidemiology data [78]. specifically, such activities highlight the importance of standardized workflows and open-access model repositories facilitating transparency and reproducibility in regulatory decision-making.

Validation frameworks also form a critical part of regulatory acceptance. Standards such as inter-individual variability, predictive accuracy, parameter sensitivity, and biological plausibility become progressively strained across agencies. For example, recent FDA workshops and EMA consultations have indicated that independent peer review of PBPK models and transparent reporting standards such as the "Good Modeling Practices" (GMP) checklist [79] are required. Meanwhile, the OECD and WHO are making international harmonization a reality by producing harmonized guidance documents in the effort to prevent differences in regulatory interpretation between jurisdictions [80]. These harmonization steps are a step towards global agreement, and PBPK and QIVIVE models are pillars of the new risk assessment generation.

Table 2. Regulatory Adoption of PBPK and QIVIVE Models in Risk Assessment

Regulatory Body	Application	Frameworks	Contributions	References
OECD	Chemical safety, AOP-linked NAMs	OECD Guidance on PBPK model reporting; AOP framework	Standardized reporting, validation, model transparency; mechanistic link to adverse outcomes	[71,72]
EMA (European Medicines Agency)	Drug development, drug–drug interactions, pediatric dosing	EMA PBPK Guideline (2018)	Requires transparency, sensitivity analysis, parameter justification; used in drug submissions and labeling	[73]
ECHA (European Chemicals Agency)	REACH chemical safety assessment	REACH Read-across & grouping strategies	Encourages PBPK/QIVIVE to fill toxicology gaps, minimize animal testing	[74]
FDA (U.S. Food and Drug Administration)	Drug approvals, bioequivalence, special populations	FDA PBPK Modeling & Simulation Guidance	Accepts PBPK in regulatory submissions; supports clinical pharmacology and drug labeling	[75]
EPA (U.S. Environmental Protection Agency)	Environmental and pesticide risk assessment	EPA PBPK-QIVIVE guidance documents	Uses in vitro–in vivo extrapolation for human health protection; supports NAM-based risk assessment	[76]
EU-ToxRisk & RISK-HUNT3R (EU Projects)	Human-relevant chemical safety	Integrated computational toxicology platforms	Combines PBPK/QIVIVE with omics and systems biology; provides case studies validated against human biomonitoring	[77,78]
OECD–WHO Harmonization Efforts	International regulatory alignment	Joint workshops and technical reports	Promotes global consistency in PBPK/QIVIVE acceptance and reporting	[79,80]

6. ADVANCED IN SILICO TOOLS AND DATA INTEGRATION

The high-speed development of computational infrastructure and databases dramatically enhanced the scale of in silico toxicokinetics, which allows for integration of various types of biological, chemical, and pharmacokinetic data towards NGRA. Over the last decade, PBPK modeling software environments have evolved from commercial packages like Simcyp, GastroPlus, and ADMET Predictor to open-source platforms like PK-Sim, mrgsolve, and BERGMOD, which are increasingly being used both within research and for regulatory use. These software programs encompass the features of parameter estimation, virtual population simulations, and high-throughput toxicokinetic predictions, thereby allowing for thorough characterization of exposure and dose–response relationships over life stages and populations [81]. The advent of virtual cell-based assays (VCBAs) has further bridged the gap between in vitro measurement and in vivo significance. VCBAs integrate cellular processes like passive diffusion, active transport, intracellular binding, and metabolism into computational models that allow for quantitative intracellular concentration predictions that more accurately reflect exposure in human tissue [82]. These models are increasingly integrated into PBPK models, thus permitting mechanistic representations of tissue-specific toxicity such as hepatotoxicity, cardiotoxicity, and neurotoxicity.

Machine learning (ML) and artificial intelligence (AI) are also revolutionizing the in silico toxicokinetic scenario. With large databases of biology and chemicals, ML models are being used more and more to provide more precise estimations of partition coefficients, plasma protein binding, clearance rates, and transporter affinities than old-style QSARs. For example, deep learning models can be trained on chemical structure–activity data sets to make high-confidence predictions of ADME parameters, which can then be included in PBPK-QIVIVE workflows for the assessment of risks [83]. They minimize the expense of experiments and expedite the screening of thousands of chemicals

that have sparse empirical data. However another central breakthrough is the availability of web-based QIVIVE toolboxes such as workflow systems such as KNIME and R Shiny applications that are accessible for automated conversion of in vitro toxicity data into corresponding human doses [84]. Such systems make model tools accessible, so that non-experts in toxicology and regulation can carry out QIVIVE with little coding ability. In addition, expanding availability of well-validated physiologically based biokinetic (PBBK) databases has made it easier to calibrate models, benchmark, and share parameters. These databases, since they are being established under EU-funded consortia such as EU-ToxRisk and RISK-HUNT3R, compile species-specific biochemical and physiological constants with in vitro–in vivo extrapolation factors validated, and thereby harmonize between laboratories and agencies [85]. Upcoming advancements will propose that omics-based mechanistic data integration, AI-assisted parameterization, and open-source PBPK-QIVIVE models will be able to bridge the current gap in toxicokinetics. These developments will not only enhance predictability but also support FAIR (Findable, Accessible, Interoperable, Reusable) principles of model and data sharing, thereby ensuring transparency, reproducibility, and regulatory trust in NGRA applications [86].

Table 3. Advances in In Silico Tools and Data Integration for PBPK-QIVIVE in NGRA

Tool	Features	Merits	Demerits	References
Simcyp, GastroPlus (commercial PBPK platforms)	Virtual population simulations, parameter estimation, drug–drug interaction modeling	High accuracy, regulatory acceptance, broad library of physiological parameters	Expensive licenses, limited transparency of proprietary algorithms	[81]
PK-Sim, mrgsolve (open-source PBPK platforms)	Mechanistic PBPK modeling, customizable workflows, open access	Cost-free, transparent, widely used in academia and risk assessment	Steeper learning curve, fewer pre-loaded compound libraries	[81]
Virtual Cell-Based Assays (VCBAs)	Cellular-level modeling of diffusion, transport, metabolism	Mechanistic insight into intracellular concentrations and tissue-specific toxicity	Requires extensive in vitro input data; limited regulatory familiarity	[82]
AI/ML-based ADME Prediction Models	Deep learning and QSAR-enhanced models for clearance, binding, partitioning	Rapid high-throughput predictions, handles large datasets	Data quality dependent, often "black box" in interpretation	[83]
Web-based QIVIVE Toolboxes (e.g., KNIME, R Shiny apps)	Automated workflows for IVIVE, user-friendly interfaces	Democratizes modeling, accessible to non-specialists	May lack advanced customization, still under regulatory evaluation	[84]
Curated Biokinetic Databases (EU-ToxRisk, RISK-HUNT3R)	Shared physiological/biochemical constants, harmonized data for PBBK	Supports model calibration, fosters standardization and transparency	Still expanding coverage, requires community-wide contributions	[85]
Integrated Omics + PBPK-QIVIVE	Incorporation of transcriptomics, proteomics, metabolomics into PBPK frameworks	Mechanistic depth, supports systems toxicology approaches	Data complexity, computationally intensive	[86]

7. APPLICATIONS ACROSS RISK ASSESSMENT DOMAIN

PBPK and QIVIVE modeling applications have expanded significantly across the wide spectrum of risk assessment fields due to their capacity for delivering human-relevant, mechanistic information. In the field of environmental chemical safety assessment, PBPK-QIVIVE strategies have

extensively been applied to extrapolate in vitro bioactivity data to external exposure estimates for prioritizing thousands of chemicals screened within high-throughput platforms like ToxCast and Tox21 [87]. This has been especially valuable for industrial chemicals with sparse data, where read-across and structural analog approaches are facilitated by incorporating biokinetic modeling that minimizes uncertainty in extrapolating across species [88]. For pharmaceuticals, PBPK models have been used for decades to predict ADME properties of novel drug candidates. Their utility in early safety assessment has been boosted by integration with QIVIVE via enabling reverse dosimetry, with toxic thresholds elicited in vitro converted into initiation doses that are safe for first-in-human clinical trials [89]. Such approaches are now being fully acknowledged by the regulatory agencies for use in drug development, e.g., drug–drug interaction assessment and organ-specific toxicity forecasting [90]. PBPK-QIVIVE also comes into play in the safety assessment of food additives and cosmetics ingredients, where animal testing is significantly limited by regulatory and ethical requirements (e.g., EU Cosmetics Directive). In this context, in vitro assays that detect endpoints like endocrine activity or dermal absorption can be quantitatively extrapolated to predict human margins of exposure through the use of PBPK models, which offer a practical pathway for safety demonstration without animal experimentation [91].

These applications have emerged in the biocides and pesticide industry, for example, where predictive toxicokinetics is employed to support both acute and chronic exposure modeling and interspecies scaling to support ecological risk assessments [92]. The other critical application area is to cumulative risk assessment and mixture toxicity. Traditional toxicological strategies find it challenging to handle concurrent exposures to more than one chemical, whereas the PBPK-based mixture models are able to handle typical metabolic routes, competitive inhibition, or additive dose contribution. Mixture PBPK models, for instance, have been used to predict joint hepatotoxicity of volatile organic compounds and endocrine activity of phthalates and illustrated their potential in real-world exposure settings [93]. Additionally, PBPK-QIVIVE has shown potential for extrapolation across species to environmental toxicology, particularly to risk assessment of wildlife. For instance, fish, avian, and amphibians' scaling models have been established for the extrapolation of internal dosimetry of contaminants and to give mechanistic understanding to ecological risk assessments as a supplement to conventional field and laboratory data [94]. These uses are greatest examples of the utility of PBPK-QIVIVE in human and ecological health assessment integration in a One Health approach. Finally, PBPK and QIVIVE are increasingly applied in human biomonitoring research. Observed blood, urine, or tissue chemical or metabolite concentrations can be combined with reverse dosimetry models to predict external exposures, which can be applied in epidemiological interpretation as well as risk assessment. This has been applied successfully with per- and polyfluoroalkyl substances (PFAS), bisphenols, and phthalates, so biomonitoring data can be normalized to health-based guidance values [95]. Together, these examples reveal that PBPK and QIVIVE methodologies are no longer the exclusive domain of research research studies but are reaching into the wider regulatory, industry, pharmaceutical, and environmental tools. Their mechanistic bridging of exposure to internal dose and biological response makes them indispensable in taking NGRA methodologies forward to animal-free, human-relevant risk assessments [96].

8. CHALLENGES, LIMITATIONS, AND FUTURE DIRECTIONS

The outstanding improvements in PBPK and QIVIVE modeling have been hindered by a number of challenges restricting their widespread application as routine columns of next-generation risk assessment. One of the most stubborn challenges is the absence of kinetic parameterization, especially for enzyme kinetics, transport activities, and tissue partition coefficients. Although databases like Simcyp, PK-Sim, and Open Systems Pharmacology are useful starting points, for the majority of industrial chemicals and novel contaminants, there still remains a general data gap [97]. The gap is even more significant for compounds with poorly characterised metabolism, where assumptions automatically made about clearance or bioavailability can generate high uncertainty for extrapolations [98]. Another problem is dealing with sophisticated ADME scenarios. Substances that are susceptible to active metabolite formation, saturable transport, or enterohepatic recycling are likely to require highly sophisticated models that may be computationally intensive and difficult to validate. Enterohepatic

recycling of drugs such as ethinyl estradiol or mycophenolic acid, for example, is a formidable challenge to the accuracy of dose prediction since typical compartmental models can lead to underestimation of systemic exposure [99]. Furthermore, parent compound-metabolite interactions and mixture exposures are still a very uninvestigated area requiring further methodological advancements [100]. The lack of in vitro protocols for the generation of standardized input data also limits model reliability. Heterogeneity in cell line choice, culture medium, readout assay, and scaling factors hinders harmonization of kinetic parameters between labs. Worldwide initiatives like OECD's In vitro–in vivo extrapolation guideline and the PBK template are trying to harmonize these differences, but application across regulatory platforms remains heterogeneous [101]. Most directly related to this problem is that there needs to be strong reporting guidelines and data transparency. While efforts such as the FAIR (Findable, Accessible, Interoperable, Reusable) principles and the OECD PBK template are steps toward standardization, heterogeneity between research groups does occur in model description, validation, and exchange [102]. This is a problem to peer review, regulatory approval, and reproducibility across sectors. Uncertainty analysis is another key frontier. Sensitivity analyses are now the standard in PBPK papers, but thorough uncertainty frameworks like Bayesian methods and probabilistic modelling are not yet standard. This deficit undermines confidence in QIVIVE-based decision-making when model output prescribes regulatory limits or human health guidance values directly [103].

Lastly, the future of PBPK-QIVIVE modeling relies strongly on community-sourced resources and open science. Shared repositories like GitHub-based PBPK libraries, OpenPBPK initiatives, and crowd-sourced model repositories are pivotal in accelerating reproducibility and transparency. It will take more incentives for model sharing, peer review, and connection to high-throughput in vitro and -omics data before unwidespread uptake can occur, although [104]. Integration of artificial intelligence (AI) and machine learning (ML) in the future will help with kinetic behavior extrapolation and parameter gap-filling based on limited experimental data. Multi-scale modeling at molecular, cellular, organ-level, and body response scales will be critical to address real-world exposure complexity. In summary, these advancements demonstrate that while PBPK and QIVIVE have long revolutionized toxicology, more innovation is predicated on greater data standardization, transparency, uncertainty management, and cooperative innovation [105].

9. CONCLUSION

The dynamic nature of toxicology and risk assessment increasingly requires mechanistic, predictive, and directly human biology translatable approaches. Toward this purpose, physiologically based pharmacokinetic (PBPK) models and quantitative in vitro–in vivo extrapolation (QIVIVE) platforms have been revolutionary tools. They promise to link in vitro experimental results with human-pertinent dose–response predictions, take into account interindividual variation, and handle real-world chemical exposure complexities. Differing from conventional recourse to default uncertainty factors or large-scale animal testing, PBPK and QIVIVE provide a rational, quantitative, and mechanistic basis for next-generation risk assessment (NGRA). Characteristic among these methods is that they are able to synthesize various streams of chemical and biological data into cohesive, predictive systems models. By coupling experimental observation with physiologically meaningful parameters like tissue volumes, blood flows, enzyme activities, and clearance rates, PBPK-QIVIVE models not only calculate systemic exposure but also enable reverse dosimetry—relativization of external exposures to quantified internal concentrations. This has been a mainstay of reassessments of safety margins, of the establishment of exposure limits, and of human health risk assessments for drugs, pesticides, industrial chemicals, and environmental toxins. Both PBPK and QIVIVE also mirror each other's use of the 3Rs principle (Replacement, Reduction, Refinement). Both aim to reduce dependence on animal testing, providing an ethical, environmentally sustainable, and cost-saving alternative which is scientifically valid. Both also allow extrapolation across species, for test species-man difference, and can be modified for susceptible populations like infants, pregnant women, or individuals with existing health problems. This ability renders them particularly useful in regulatory environments where the safety of vulnerable populations is the highest priority. Regulatory agencies around the globe are recognizing the

potential of PBPK-QIVIVE approaches more and more. Regulatory agencies in North America, Europe, and Asia have already commenced the inclusion of PBPK models in guidelines and case-by-case assessment. These models are increasingly utilized to guide drug discovery, chemical safety assessment, and food safety risk analysis, their development from research tools to regulatory pillars. Global deployment will be delayed pending standardization of report formats, open validation processes, and quality data infrastructures to ensure reproducibility and global acceptability. In the future, PBPK and QIVIVE will advance in parallel with technological convergence and cross-disciplinary integration. Convergence of artificial intelligence, machine learning, and high-dimensional biological data in genomics, transcriptomics, proteomics, and metabolomics is expected to enhance model precision, reduce uncertainty, and enhance predictability. Further, construction of multi-scale models that link cell-level events to body-scale dynamics will provide more precise predictions for intricate exposure scenarios like chemical mixtures, chronic low-dose exposures, and environment persistent pollutants. Finally, success of PBPK and QIVIVE in NGRA will depend on a cooperation system that combines academia, industry, and regulation. Open-source model platforms, community-maintained parameter databases, and open-validation approaches will facilitate world-wide application. Most importantly, these approaches constitute a revolution in toxicology: from descriptive, animal-reliant observations to mechanistic, human-focused predictions that harmonize with scientific advancement and citizens' demands for sustainable, animal-free risk assessments. In totality, PBPK and QIVIVE are not technical devices but pillars of the future generation of risk assessment. Through the convergence of mechanistic understanding, computation, and ethics, they can possibly redefine regulatory toxicology as a predictive, preventive, and coherent science that keeps pace with the changing challenges to global environmental health and public welfare.

Conflict of interests

Authors declare that there is no conflict of interests.

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REFERENCES

1. Leist, M., Ghallab, A., Graepel, R., Marchan, R., Hassan, R., Bennekou, S.H., et al., 2017. Adverse outcome pathways: opportunities, limitations and open questions. *Arch. Toxicol.* 91, 3477–3505. https://doi.org/10.1007/s00204-017-2045-3
2. Hartung, T., 2009. Toxicology for the twenty-first century. *Nature* 460(7252), 208–212. https://doi.org/10.1038/460208a
3. Rovida, C., Alépée, N., Api, A.M., Basketter, D.A., Bois, F.Y., Caloni, F., et al., 2020. Integrated Testing Strategies (ITS) for safety assessment. *ALTEX* 37(3), 305–317. https://doi.org/10.14573/altex.2001281
4. Bal-Price, A., Leist, M., Schildknecht, S., et al., 2018. New approach methodologies for neurotoxicity testing. *NeuroToxicology* 67, 1–2. https://doi.org/10.1016/j.neuro.2018.04.004
5. OECD, 2021. Guidance Document on the Characterisation, Validation and Reporting of Physiologically Based Kinetic (PBK) Models for Regulatory Purposes. *Series on Testing and Assessment No. 331*, ENV/JM/MONO(2021)30. Organisation for Economic Co-operation and Development, Paris. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2021)30&doclanguage=en
6. Dent, M., Trosko, J.E., Carmichael, P.L., 2021. NGRA: The principles and application of new approach methodologies. *Regul. Toxicol. Pharmacol.* 125, 105024. https://doi.org/10.1016/j.yrtph.2021.105024
7. Jones, H.M., Chen, Y., Gibson, C., Heimbach, T., Parrott, N., Peters, S.A., et al., 2015. Physiologically based pharmacokinetic modeling in drug discovery and development: a pharmaceutical industry perspective. *Clin. Pharmacol. Ther.* 97(3), 247–262. https://doi.org/10.1002/cpt.37
8. Wetmore, B.A., 2015. Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. *Toxicol. In Vitro* 29(1), 231–238. https://doi.org/10.1016/j.tiv.2014.10.004

9. Loizou, G.D., McNally, K., Hogg, A., 2020. Integrating QIVIVE and PBPK modeling in risk assessment. *Comput. Toxicol.* 14, 100126. https://doi.org/10.1016/j.comtox.2020.100126
10. Ellison, C.M., Madden, J.C., Cronin, M.T.D., 2019. Integrating in silico tools for NGRA: An expert review. *ALTEX* 36(4), 643–653. https://doi.org/10.14573/altex.1907031
11. Kuepfer, L., Niederalt, C., Wendl, T., Schlender, J.F., Willmann, S., Lippert, J., et al., 2016. Applied concepts in PBPK modeling: How to build a PBPK/PD model. *CPT Pharmacometrics Syst. Pharmacol.* **5**(10), 516–531. https://doi.org/10.1002/psp4.12134
12. Jones, H.M., Chen, Y., Gibson, C., Heimbach, T., Parrott, N., Peters, S.A., et al., 2015. Physiologically based pharmacokinetic modeling in drug discovery and development. *Clin. Pharmacol. Ther.* **97**(3), 247–262. https://doi.org/10.1002/cpt.37
13. Ramsey, J.C., Andersen, M.E., 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* **73**(1), 159–175. https://doi.org/10.1016/0041-008X(84)90014-3
14. Kuepfer, L., Niederalt, C., Wendl, T., Schlender, J.F., Willmann, S., Lippert, J., et al., 2016. Applied concepts in PBPK modeling: How to build a PBPK/PD model. *CPT Pharmacometrics Syst. Pharmacol.* **5**(10), 516–531. https://doi.org/10.1002/psp4.12134
15. Rietjens, I.M.C.M., Louisse, J., Punt, A., 2021. Physiologically based kinetic modeling as a tool to support the application of non-animal methods in chemical safety assessment. *Toxicol. In Vitro* **73**, 105133. https://doi.org/10.1016/j.tiv.2021.105133
16. Rostami-Hodjegan, A., 2012. PBPK joined with IVIVE: A marriage under the arch of systems pharmacology. *Clin. Pharmacol. Ther.* **92**(1), 50–61. https://doi.org/10.1038/clpt.2012.65
17. Edginton, A.N., Schmitt, W., Voith, B., Willmann, S., 2006. Physiologically based pharmacokinetics in pediatric drug development. *Clin. Pharmacokinet.* **45**(11), 1053–1074. https://doi.org/10.2165/00003088-200645110-00004
18. Wetmore, B.A., Wambaugh, J.F., Ferguson, S.S., Sochaski, M.A., Rotroff, D.M., Freeman, K., et al., 2012. Integration of dosimetry, exposure, and high-throughput screening data for prioritization of environmental chemicals. *Toxicol. Sci.* **125**(1), 157–174. https://doi.org/10.1093/toxsci/kfr254
19. McLanahan, E.D., El-Masri, H.A., Sweeney, L.M., Ring, C.L., Krishnan, K., Phillips, R.D., et al., 2012. Physiologically based pharmacokinetic model use in risk assessment—why being published is not enough. *Toxicol. Sci.* **126**(1), 5–15. https://doi.org/10.1093/toxsci/kfr295
20. Jamei, M., 2016. Recent advances in development and application of physiologically-based pharmacokinetic (PBPK) models: A transition from academic curiosity to regulatory acceptance. *Curr. Pharmacol. Rep.* **2**, 161–169. https://doi.org/10.1007/s40495-016-0059-9
21. Jamei, M., Marciniak, S., Feng, K., Barnett, A., Tucker, G., Rostami-Hodjegan, A., 2009. The Simcyp population-based ADME simulator. *Expert Opin. Drug Metab. Toxicol.* **5**(2), 211–223. https://doi.org/10.1517/17425250802691074
22. Adam, A.H.B., Zhang, M., de Haan, L.H.J., van Ravenzwaay, B., Louisse, J., Rietjens, I.M.C.M., 2019. The in vivo developmental toxicity of diethylstilbestrol (DES) in rats evaluated by an alternative testing strategy. *Arch. Toxicol.* **93**(7), 2021–2033. https://doi.org/10.1007/s00204-019-02487-6
23. Armitage, J.M., Wania, F., Arnot, J.A., 2014. Application of mass balance models and the chemical activity concept to facilitate the use of in vitro toxicity data for risk assessment. *Environ. Sci. Technol.* **48**(16), 9770–9779. https://doi.org/10.1021/es501955g
24. Bale, A.S., Kenyon, E.M., Flynn, T.J., Lipscomb, J.C., Mendrick, D.L., Hartung, T., et al., 2014. Correlating in vitro data to in vivo findings for risk assessment. *ALTEX* **31**(1), 79–90. https://doi.org/10.14573/altex.1310011
25. Bell, S.M., Chang, X., Wambaugh, J.F., Allen, D.G., Bartels, M., Brouwer, K.L.R., et al., 2018. In vitro to in vivo extrapolation for high-throughput prioritization and decision making. *Toxicol. In Vitro* **47**, 213–227. https://doi.org/10.1016/j.tiv.2017.11.016
26. Berggren, E., White, A., Ouedraogo, G., Paini, A., Richarz, A. N., Bois, F. Y., et al. (2017). Ab initio chemical safety assessment: A workflow based on exposure

considerations and non-animal methods. *Comput. Toxicol.*, 4, 31–44. https://doi.org/10.1016/j.comtox.2017.10.001

27. Boonpawa, R., Spenkelink, A., Punt, A., & Rietjens, I. M. C. M. (2017). In vitro–in silico-based analysis of the dose-dependent in vivo oestrogenicity of the soy phytoestrogen genistein in humans. *Br. J. Pharmacol.*, 174(16), 2739–2757. https://doi.org/10.1111/bph.13900
28. McNally, K., & Loizou, G. D. (2015). A probabilistic model of human variability in physiology for future application to dose reconstruction and QIVIVE. *Front. Pharmacol.*, 6, 213. https://doi.org/10.3389/fphar.2015.00213
29. 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. *Front. Pharmacol.*, 12, 692442. https://doi.org/10.3389/fphar.2021.692442
30. Mesnage, R., Phedonos, A., Arno, M., Balu, S., Corton, J. C., & Antoniou, M. N. (2017). Editor's highlight: Transcriptome profiling reveals bisphenol A alternatives activate estrogen receptor alpha in human breast cancer cells. *Toxicol. Sci.*, 158(2), 431–443. https://doi.org/10.1093/toxsci/kfx101
31. Offman, E., Phipps, C., & Edginton, A. N. (2016). Population physiologically based pharmacokinetic model incorporating lymphatic uptake for a subcutaneously administered pegylated peptide. *Silico Pharmacol.*, 4(3), 3–14. https://doi.org/10.1186/s40203-016-0018-5
32. Pacifici, G. M., Franchi, M., Bencini, C., Repetti, F., Di Lascio, N., & Muraro, G. B. (1988). Tissue distribution of drug-metabolizing enzymes in humans. *Xenobiotica*, 18(7), 849–856. https://doi.org/10.3109/00498258809041723
33. Paini, A., Sala Benito, J. V., Bessems, J., & Worth, A. P. (2017). From in vitro to in vivo: Integration of the virtual cell based assay with physiologically based kinetic modelling. *Toxicol. In Vitro*, 45(2), 241–248. https://doi.org/10.1016/j.tiv.2017.06.015
34. Paini, A., Tan, Y. M., Sachana, M., & Worth, A. (2021). Gaining acceptance in next generation PBK modelling approaches for regulatory assessments—An OECD international effort. *Comput. Toxicol.*, 18, 100163. https://doi.org/10.1016/j.comtox.2021.100163
35. European Medicines Agency (EMA). (2018). *Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation*. EMA/CHMP/458101/2016.
36. Zhao, P., et al. (2011). Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clin. Pharmacol. Ther.*, 89(2), 259–267. https://doi.org/10.1038/clpt.2010.298
37. Wetmore, B. A., et al. (2015). Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to evaluate thousands of chemicals. *Environ. Sci. Technol.*, 49(11), 6760–6771. https://doi.org/10.1021/acs.est.5b00401
38. European Chemicals Agency (ECHA). (2021). *Read-Across Assessment Framework (RAAF) and Guidance on Information Requirements*. ECHA, Helsinki. https://echa.europa.eu/documents/10162/13628/raaf_en.pdf
39. Punt, A., Pinckaers, N., Peijnenburg, A., & Louisse, J. (2021). Development of a web-based toolbox to support quantitative in vitro-to-in vivo extrapolations (QIVIVE) within non-animal testing strategies. *Chem. Res. Toxicol.*, 34(2), 460–472. https://doi.org/10.1021/acs.chemrestox.0c00307
40. Ankley, G. T., et al. (2010). Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ. Toxicol. Chem.*, 29(3), 730–741. https://doi.org/10.1002/etc.34
41. Strikwold, M., Spenkelink, B., Woutersen, R. A., Rietjens, I. M. C. M., & Punt, A. (2017). Development of a combined in vitro physiologically based kinetic (PBK) and Monte Carlo modelling approach to predict interindividual human variation in phenol-induced developmental toxicity. *Toxicol. Sci.*, 157(2), 365–376. https://doi.org/10.1093/toxsci/kfx054
42. Wetmore, B. A., et al. (2012). Incorporating dosimetry into in vitro toxicity screening: Application of in vitro data to in vivo dose-response modeling. *Toxicol. Sci.*, 125(1), 157–174. https://doi.org/10.1093/toxsci/kfr280
43. Rietjens, I. M. C. M., Boersma, M. G., Zaleska, M., & Punt, A. (2008). Differences in simulated liver

concentrations of toxic coumarin metabolites in rats and different human populations evaluated through physiologically based biokinetic (PBBK) modeling. *Toxicol. In Vitro*, 22(8), 1890–1901. https://doi.org/10.1016/j.tiv.2008.09.004

44. Pletz, J., Blakeman, S., Paini, A., et al. (2020). Physiologically based kinetic (PBK) modelling and human biomonitoring data for mixture risk assessment. *Environ. Int.*, 143, 105978. https://doi.org/10.1016/j.envint.2020.105978
45. Chebekoue, S. F., & Krishnan, K. (2019). A framework for application of quantitative property–property relationships (QPPRs) in physiologically based pharmacokinetic (PBPK) models for high-throughput prediction of internal dose of inhaled organic chemicals. *Chemosphere*, 215, 634–646. https://doi.org/10.1016/j.chemosphere.2018.10.041
46. Ortega-Vallbona, R., Palomino-Schätzlein, M., Tolosa, L., Benfenati, E., Ecker, G. F., Gozalbes, R., & Serrano-Candelas, E. (2024). Computational strategies for assessing adverse outcome pathways: Hepatic steatosis as a case study. *Int. J. Mol. Sci.*, 25(20), 11154. https://doi.org/10.3390/ijms252011154
47. Cronin, M. T., Belfield, S. J., Briggs, K. A., Enoch, S. J., Firman, J. W., Frericks, M., et al. (2023). Making in silico predictive models for toxicology FAIR. *Regul. Toxicol. Pharmacol.*, 140, 105385. https://doi.org/10.1016/j.yrtph.2023.105385
48. Paini, A., Leonard, J. A., Joossens, E., Bessems, J. G., Desalegn, A., Dorne, J. L., et al. (2019). Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. *Comput. Toxicol.*, 9, 61–72. https://doi.org/10.1016/j.comtox.2018.11.002
49. Tan, Y. M., Barton, H. A., Boobis, A., Brunner, R., Clewell, H., Cope, R., et al. (2021). Opportunities and challenges related to saturation of toxicokinetic processes: Implications for risk assessment. *Regul. Toxicol. Pharmacol.*, 127, 105070. https://doi.org/10.1016/j.yrtph.2021.105070
50. Clark, L. H., Setzer, R. W., & 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/10.1111/j.0272-4332.2004.00561.x
51. OECD. Case study on the use of integrated approaches for testing and assessment for systemic toxicity arising from cosmetic exposure to caffeine. OECD; 2020a.
52. Pletz J, Blakeman S, Paini A, et al. Physiologically based kinetic (PBK) modelling and human biomonitoring data for mixture risk assessment. Environ Int. 2020;143:105978. https://doi.org/10.1016/j.envint.2020.105978
53. Peters SA. Evaluation of a generic physiologically based pharmacokinetic model for lineshape analysis. Clin Pharmacokinet. 2008;47(4):261–275. https://doi.org/10.2165/00003088-200847040-00004
54. Najjar A, Schepky A, Krueger C-T, et al. Use of physiologically based kinetics modelling to reliably predict internal concentrations of the UV filter, homosalate, after repeated oral and topical application. Front Pharmacol. 2021;12:802514.
55. Punt A, Pinckaers N, Peijnenburg A, Louisse J. Development of a web-based toolbox to support quantitative in-vitro-to-in-vivo extrapolations (QIVIVE) within nonanimal testing strategies. Chem Res Toxicol. 2021;34(2):460–472. https://doi.org/10.1021/acs.chemrestox.0c00307
56. Rotroff DM, Wetmore BA, Dix DJ, et al. Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. Toxicol Sci. 2010;117(2):348–358. https://doi.org/10.1093/toxsci/kfq220
57. OECD. Case study on use of an integrated approach to testing and assessment (IATA) and New approach methods to inform a theoretical read-across for dermal exposure to propylparaben from cosmetics. OECD; 2020b.
58. Pradeep P, Patlewicz G, Pearce R, Wambaugh J, Wetmore B, Judson R. Using chemical structure information to develop predictive models for in vitro toxicokinetic parameters to inform high-throughput risk-assessment. Comput Toxicol. 2020;14:100136. https://doi.org/10.1016/j.comtox.2020.100136
59. Paini A, Tan Y-M, Sachana M, Worth A. Gaining acceptance in next generation PBK modelling approaches for regulatory assessments—an OECD international

effort. Comput Toxicol. 2021;18:100163. https://doi.org/10.1016/j.comtox.2021.100163
60. Rowland M, Balant L, Peck C. Physiologically based pharmacokinetics in drug development and regulatory science: a workshop report (Georgetown University, Washington, DC, May 29–30, 2002). AAPS PharmSci. 2004;6(1):E6. https://doi.org/10.1208/ps060106
61. Moxon TE, Li H, Lee MY, et al. Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicol in Vitro. 2020;63:104746. https://doi.org/10.1016/j.tiv.2019.104746
62. Rietjens IMCM, Boersma MG, Zaleska M, Punt A. Differences in simulated liver concentrations of toxic coumarin metabolites in rats and different human populations evaluated through physiologically based biokinetic (PBBK) modeling. Toxicol in Vitro. 2008;22(8):1890–1901. https://doi.org/10.1016/j.tiv.2008.09.004
63. Parish ST, Aschner M, Casey W, et al. An evaluation framework for new approach methodologies (NAMs) for human health safety assessment. Regul Toxicol Pharmacol. 2020;112:104592. https://doi.org/10.1016/j.yrtph.2020.104592
64. OECD. Guidance document on good in vitro method practices (GIVIMP). OECD; 2018.
65. Richardson SJ, Bai A, Kulkarni AA, Moghaddam MF. Efficiency in drug discovery: liver S9 fraction assay as a screen for metabolic stability. Drug Metab Lett. 2016;10(2):83–90. https://doi.org/10.2174/1872312810666160223121836
66. Paini A, Leonard JA, Kliment T, Tan Y-M, Worth A. Investigating the state of physiologically based kinetic modelling practices and challenges associated with gaining regulatory acceptance of model applications. Regul Toxicol Pharmacol. 2017;90:104–115. https://doi.org/10.1016/j.yrtph.2017.08.019
67. OECD. Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion. OECD; 2021.
68. Rodgers T, Rowland M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J Pharm Sci. 2006;95(6):1238–1257. https://doi.org/10.1002/jps.20502
69. OECD Environment, Health and Safety Publications. Guidance document on the characterisation, validation and reporting of PBK models for regulatory purposes. OECD; 2021.
70. Punt A, Louisse J, Pinckaers N, Fabian E, van Ravenzwaay B. Predictive performance of next generation physiologically based kinetic (PBK)-model predictions in rats based on in vitro and in silico input data. Toxicol Sci. 2021;180(1):150–162. https://doi.org/10.1093/toxsci/kfab150
71. Pearce RG, Setzer RW, Strope CL, Wambaugh JF, Sipes NS. httk: R package for high-throughput toxicokinetics. J Stat Softw. 2017;79(4):1–26. https://doi.org/10.18637/jss.v079.i04
72. Ruiz P, Ray M, Fisher J, Mumtaz M. Development of a human physiologically based pharmacokinetic (PBPK) toolkit for environmental pollutants. Int J Mol Sci. 2011;12(11):7469–7480. https://doi.org/10.3390/ijms12117469
73. Pearce RG, Setzer RW, Davis JL, Wambaugh JF. Evaluation and calibration of high-throughput predictions of chemical distribution to tissues. J Pharmacokinet Pharmacodyn. 2017;44(6):549–565. https://doi.org/10.1007/s10928-017-9548-7
74. Punt A, Louisse J, Beekmann K, et al. Predictive performance of next generation human physiologically based kinetic (PBK) model predictions based on in vitro and in silico input data. ALTEX. 2022;39(2):187–202. https://doi.org/10.14573/altex.2108301
75. Nrc NRC. Toxicity testing in the 21st century: a vision and a strategy. The National Academies Press, Washington; 2007.
76. Tan Y-M, Chan M, Chukwudebe A, et al. PBPK model reporting template for chemical risk assessment applications. Regul Toxicol Pharmacol. 2020;115:104691. https://doi.org/10.1016/j.yrtph.2020.104691
77. Wambaugh JF, Wetmore BA, Ferguson SS, et al. Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. Toxicol Sci. 2012;125(1):157–174. https://doi.org/10.1093/toxsci/kfr254
78. Shebley M, Sandhu P, Emami Riedmaier A, et al. Physiologically based

pharmacokinetic model qualification and reporting procedures for regulatory submissions: a consortium perspective. Clin Pharmacol Ther. 2018;104(1):88–110. https://doi.org/10.1002/cpt.1013

79. Thompson CV, Firman JW, Goldsmith MR, et al. A systematic review of published physiologically-based kinetic models and an assessment of their chemical space coverage. Altern Lab Anim. 2021;49(5):197–208. https://doi.org/10.1177/02611929211060264
80. Wetmore BA. Quantitative in vitro-to-in vivo extrapolation in a high-throughput environment. Toxicology. 2015;332:94–101. https://doi.org/10.1016/j.tox.2014.05.012
81. Wang YH. Confidence assessment of the Simcyp time-based approach and a static mathematical model in predicting clinical drug-drug interactions for mechanism-based CYP3A inhibitors. Drug Metab Dispos. 2010;38(7):1094–1104. https://doi.org/10.1124/dmd.110.032177
82. Zhang M, van Ravenzwaay B, Rietjens IMCM. Development of a generic physiologically based kinetic model to predict in vivo uterotrophic responses induced by estrogenic chemicals in rats based on in vitro bioassays. Toxicol Sci. 2019;173(1):19–31. https://doi.org/10.1093/toxsci/kfz216
83. Stillhart C, Pepin X, Tistaert C, et al. PBPK absorption modeling: establishing the in vitro–in vivo link—industry perspective. AAPS J. 2019;21(2):19. https://doi.org/10.1208/s12248-019-0292-3
84. Wetmore BA, Wambaugh JF, Ferguson SS, et al. Relative impact of incorporating pharmacokinetics on predicting in vivo hazard and mode of action from high-throughput in vitro toxicity assays. Toxicol Sci. 2013;132(2):327–346. https://doi.org/10.1093/toxsci/kft01
85. Shibata Y, Takahashi H, Ishii Y. A convenient in vitro screening method for predicting in vivo drug metabolic clearance using isolated hepatocytes suspended in serum. Drug Metab Dispos. 2000;28(12):1518–1523.
86. Wambaugh JF, Wetmore BA, Ring CL, et al. Assessing toxicokinetic uncertainty and variability in risk prioritization. Toxicol Sci. 2019;172(2):235–251. https://doi.org/10.1093/toxsci/kfz205
87. Yoon M, Campbell JL, Andersen ME, Clewell HJ. Quantitative in vitro to in vivo extrapolation of cell-based toxicity assay results. Crit Rev Toxicol. 2012;42(8):633–652. https://doi.org/10.3109/10408444.2012.692115
88. Zhang Q, Li J, Middleton A, Bhattacharya S, Conolly RB. Bridging the data gap from in vitro toxicity testing to chemical safety assessment through computational modeling. Front Public Health. 2018;6:261. https://doi.org/10.3389/fpubh.2018.00261
89. Statelova M, Holm R, Fotaki N, Reppas C, Vertzoni M. Successful extrapolation of paracetamol exposure from adults to infants after oral administration of a pediatric aqueous suspension is highly dependent on the study dosing conditions. AAPS J. 2020;22(6):126. https://doi.org/10.1208/s12248-020-00504-6
90. Watford S, Ly Pham L, Wignall J, Shin R, Martin MT, Friedman KP. ToxRefDB version 2.0: improved utility for predictive and retrospective toxicology analyses. Reprod Toxicol. 2019;89:145–158. https://doi.org/10.1016/j.reprotox.2019.07.012
91. Ahmad A, Pepin X, Aarons L, et al. IMI—oral biopharmaceutics tools project—evaluation of bottom-up PBPK prediction success part 4: prediction accuracy and software comparisons with improved data and modelling strategies. Eur J Pharm Biopharm. 2020;156:50-63. doi:10.1016/j.ejpb.2020.08.006
92. Andersen ME. Development of physiologically based pharmacokinetic and physiologically based pharmacodynamic models for applications in toxicology and risk assessment. Toxicol Lett. 1995;79(1–3):35-44. doi:10.1016/0378-4274(95)03355-o
93. Basketter DA, Clewell H, Kimber I, et al. A roadmap for the development of alternative (non-animal) methods for systemic toxicity testing. Altex. 2012;29(1):3-91. doi:10.14573/altex.2012.1.003
94. Bell SM, Chang X, Wambaugh JF, et al. In vitro to in vivo extrapolation for high throughput prioritization and decision making. Toxicol In Vitro. 2018;47:213-27. doi:10.1016/j.tiv.2017.11.016
95. Berezhkovskiy LM. Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. J Pharm Sci. 2004;93(6):1628-40. doi:10.1002/jps.20073
96. Berggren E, White A, Ouedraogo G, et al. Ab initio chemical safety assessment: a workflow based on exposure

considerations and non-animal methods. Comput Toxicol. 2017;4:31-44. doi:10.1016/j.comtox.2017.10.001
97. Bessems JG, Loizou G, Krishnan K, et al. PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment: recommendations from a joint EPAA-EURL ECVAM ADME workshop. Regul Toxicol Pharmacol. 2014;68(1):119-39. doi:10.1016/j.yrtph.2013.11.008
98. Blaauboer BJ. Biokinetic modeling and in vitro–in vivo extrapolations. J Toxicol Environ Health B. 2010;13(2–4):242-52. doi:10.1080/10937404.2010.483940
99. Bopp SK, Kienzler A, Richarz A-N, et al. Regulatory assessment and risk management of chemical mixtures: challenges and ways forward. Crit Rev Toxicol. 2019;49(2):174-89. doi:10.1080/10408444.2019.1579169
100. Breen M, Ring CL, Kreutz A, Goldsmith MR, Wambaugh JF. High-throughput PBTK models for in vitro to in vivo extrapolation. Expert Opin Drug Metab Toxicol. 2021;17(8):903-21. doi:10.1080/17425255.2021.1935867
101. Campbell JL Jr, Clewell RA, Gentry PR, Andersen ME, Clewell HJ 3rd. Physiologically based pharmacokinetic/toxicokinetic modeling. Methods Mol Biol. 2012;929:439-99. doi:10.1007/978-1-62703-050-2_18
102. Chebekoue SF, Krishnan K. A framework for application of quantitative property-property relationships (QPPRs) in physiologically based pharmacokinetic (PBPK) models for high-throughput prediction of internal dose of inhaled organic chemicals. Chemosphere. 2019;215:634-46. doi:10.1016/j.chemosphere.2018.10.041
103. Chiu WA, Barton HA, DeWoskin RS, et al. Evaluation of physiologically based pharmacokinetic models for use in risk assessment. J Appl Toxicol. 2007;27(3):218-37. doi:10.1002/jat.1225
104. Choi G-W, Lee Y-B, Cho H-Y. Interpretation of non-clinical data for prediction of human pharmacokinetic parameters: in vitro-in vivo extrapolation and allometric scaling. Pharmaceutics. 2019;11(4):168. doi:10.3390/pharmaceutics11040168
105. Clark LH, Setzer RW, Barton HA. Framework for evaluation of physiologically-based pharmacokinetic models for use in safety or risk assessment. Risk Anal. 2004;24(6):1697-717. doi:10.1111/j.0272-4332.2004.00561.x

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