ALTERNATIVE

WHITE PAPER

PHYSIOLOGICALLY BASED KINETIC MODELING IN NEW APPROACH METHODOLOGIES

MARCH 2025

HORIZON EUROPE PROJECT

ALTERNATIVE is funded by the European Commission under the EU Horizon 2020 programme

ROLE FOR TOXICOKINETICS IN NAM-BASED CHEMICAL RISK ASSESSMENT

New Approach Methodologies (NAMs) are innovative approaches designed to replace, reduce, or refine traditional animal testing paradigms. Within NAMs, in vitro toxicology assays offer significant promise due to their human relevance, ethical benefits, and cost-effectiveness. These assays can generate vast amounts of toxicological data, presenting a wealth of information that can potentially be used to make informed decisions in chemical risk assessment, without using animals. However, extrapolating in vitro findings to predict in vivo outcomes without the use of clinical experiments remains a challenge. Physiologically Based Kinetic (PBK) modeling, also known as physiologically based toxicokinetic or pharmacokinetic (PBTK/PBPK) modeling, addresses this challenge by simulating the kinetic processes of a chemical in the body based on physiological principles and chemical properties. By doing so, PBK models offer several critical applications within NAM-based chemical risk assessment frameworks, particularly aiming at bridging the gap between experimental in vitro data and real-world scenarios. Some of the more prominent applications of PBK models within NAMs are depicted in table 1, with various other applications and possibilities available. PBK models and toxicokinetics are therefore essential components of NAM-based chemical assessments, as they can cover crucial parts that would otherwise be informed by animal studies.

Table 1. Possible applications of PBK models in chemical risk assessment. Applications used in the Horizon 2020 ALTERNATIVE project are indicated in bold. Table was adopted from the OECD Guidance on PBK models for regulatory purposes[1].

1	Extrapolating across doses or exposure scenarios
2	Route to route extrapolation (of an external dose)
3	Interspecies extrapolation (reducing uncertainty factors)
4	**Intraspecies extrapolation (accounting for population variability)**
5	**(Quantitative) in vitro toxicity data to in vivo exposure extrapolation – (Q)IVIVE**
6	Setting safe levels of a chemical based on tissue dosimetry (in humans or animals)
7	**Interpreting human and wildlife biomonitoring data by retrospectively reconstructing the external dose or exposure (reverse dosimetry)**
8	Predicting biologically-relevant doses at target tissues
9	Bioaccumulation assessment

This is further highlighted by the fact that the importance of toxicokinetic data for the current system of chemical safety assessment is increasingly recognized and increasingly implemented in legislative frameworks and guidance documents[2,3].

This white paper introduces the principles of toxicokinetics within the context of NAMs, discusses the fundamentals of PBK modeling and demonstrates its possible applications, particularly focusing on NAM-based chemical risk assessment.

PRINCIPLES OF TOXICOKINETICS AND PBK MODELS

Toxicokinetics (TK) is the study of how a chemical is absorbed, distributed, metabolized, and excreted (ADME) within a living organism following exposure. It provides crucial insights into the internal exposure to a toxicant and helps predict

[1] OECD, "Guidance Document on the Characterisation, Validation and Reporting of Physiologically Based Kinetic (Pbk) Models for Regulatory Purposes, Oecd Series on Testing and Assessment, No. 331."
[2] Esther F. A. Brandon, Rivm, and Jos Bessems, "Integration of Toxicokinetics and Toxicodynamics Testing Essential for Risk Assessment."
[3] Health Canada "Science Approach Document - Bioactivity Exposure Ratio: Application in Priority Setting and Risk Assessment."

potential toxic effects. These kinetic ADME processes are essential in understanding and modeling the route that a chemical takes during its time in the body, and what the body does to the chemical. TK models characterize the ADME processes of a chemical by representing the body as one or more interconnected compartments, through which the substance can enter (absorption), move (distribution), and exit (metabolism and elimination). Mathematically, a TK model describes the time-dependent changes in the amount of a substance within each compartment using a set of mass-balance ordinary differential equations. The model parameters define the size of each compartment and the rates at which the substance enters, transfers between, and exits compartments. The overall structure of the model determines the number of compartments and their interconnections.

Physiologically based kinetic (PBK) models are structured so that their components and parameters have direct physiological significance, distinguishing them from empirical, non-physiological compartmental TK models. In PBK models, compartments typically represent individual organs, tissues, or groups of physiologically similar tissues. By integrating these physiological, biochemical, and physicochemical properties into mathematical frameworks, the kinetic behavior of chemicals in humans or animals can be predicted. The resulting simulations characterize the time-dependent behavior of a certain chemical in the body and can be used to estimate the amount that reaches the target site based on a given external dose or exposure. Alternatively, internal concentrations can be converted to external intake rates (e.g. the daily intake of a chemical that is needed to reach a certain tissue concentration), which allows a comparison with modeled or estimated daily intake for the population to ultimately assess health risk (figure 1). Therefore, toxicokinetic PBK models can be used in NAMs to bridge the gap between internal *in vitro* concentrations and external doses, ensuring relevance and applicability of NAM-derived data for human health risk assessment.

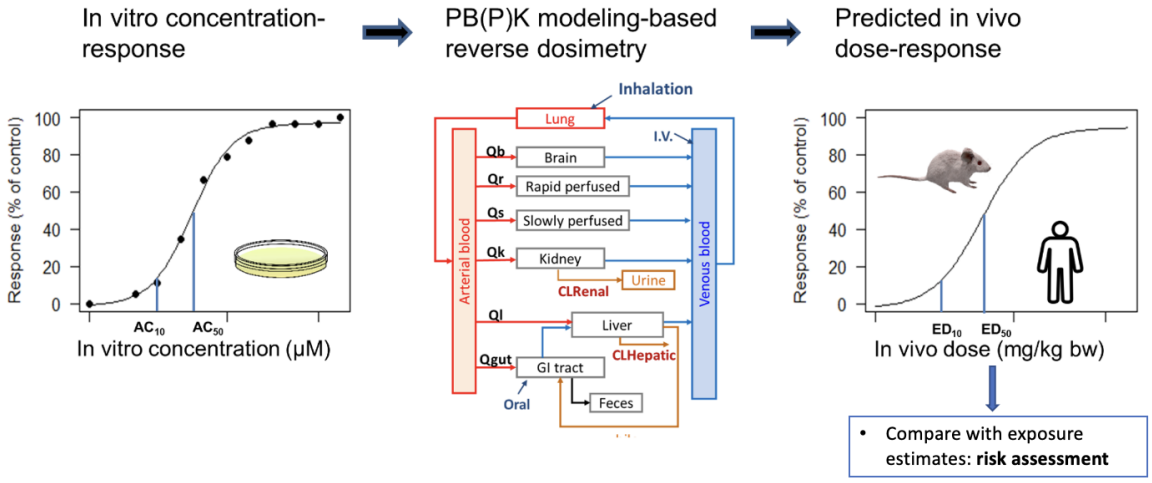

Figure 1. Principles of quantitative in vitro – in vivo extrapolation (QIVIVE), adopted from [4].

PBK models are needed in next generation risk assessment as typical results from in vitro based NAMs include bioactive concentration response data, which alone do not inform human health risk. With PBK modeling, these in vitro data can be extrapolated from internal concentrations to external exposure scenarios, a process that is referred to as quantitative in vitro – in vivo extrapolation (QIVIVE). The result is a (daily) intake rate that would lead to a bioactive concentration at the target site, which can be compared to population intake estimates to allow risk assessment and prioritization for environmentally relevant chemicals, thereby completing the risk assessment cycle.

Below, an overview is given of the most important TK processes, including absorption, distribution, metabolism and excretion, and how these processes were incorporated into the high throughput toxicokinetics (httk) R package[5], which was recently optimized specifically for QIVIVE applications in public health risk assessment[6] and is therefore highly useful for applications within NAM-based chemical risk assessment paradigms.

[4] X. Chang et al., "Ivive: Facilitating the Use of in Vitro Toxicity Data in Risk Assessment and Decision Making."
[5] Robert G. Pearce et al., "Httk: R Package for High-Throughput Toxicokinetics."
[6] Sarah Davidson-Fritz et al., "Enabling Transparent Toxicokinetic Modeling for Public Health Risk Assessment."

ABSORPTION

Absorption is the process by which chemicals enter systemic circulation from exposure sites such as the gastrointestinal tract, lungs, or skin. In PBK modeling, absorption is characterized by parameters reflecting the route-specific absorption rates and bioavailability, and rely on the specific route of exposure of each particular chemical. When modeling the exposure to chemicals, it is important to incorporate the true route(s) of exposure in the PBK model, since these can differ substantially between chemicals and chemical groups. In toxicology, commonly modeled routes of exposure are oral (via food or water), inhalation (via lungs, e.g., solvents and air pollutants) and dermal (via skin, e.g., cosmetics).

Oral absorption can be modeled via compartments representing the gastrointestinal tract, often subdivided into stomach and intestine segments, accounting for factors including the dissolution rate, solubility, intestinal permeability, and gut metabolism. Parameters such as gastric emptying time, intestinal transit, and permeability coefficients influence absorption kinetics.

In httk, absorption is modeled using a simple first-order process, with the amount absorbed depending on the absorption rate constant (k_a) and the fraction absorbed (F_abs). Conservative assumptions are made in absence of specific data (fast absoprtion, fraction absorped = 1) although these parameters can be easily modified by the user when more accurate information is available. While the httk model simplifies certain complexities (e.g., permeability limitations, gut metabolism), it provides a reasonable approximation for high-throughput toxicokinetics.

Inhalation absorption can be modeled for chemicals inhaled as vapors, gases, or aerosols through respiratory tract compartments, typically including the nasal cavity, tracheobronchial region, and alveoli. Parameters such as breathing rate, cardiac output, alveolar ventilation, and blood-air partition coefficients are critical for accurately predicting absorption via inhalation.

In httk, an inhalation compartment was recently added and evaluated[7]. Inhalation is represented in this model as a continuous uptake process where a chemical is absorbed through the respiratory system and enters systemic circulation. It describes inhalation exposure as a function of ventilation rate, blood-air partitioning and pulmonary uptake and clearance.

Dermal absorption can be modeled for chemicals that come into contact with the skin by including a skin compartment describing chemical diffusion through skin layers (e.g., stratum corneum, epidermis, dermis). Key determinants include the skin thickness, partition coefficients between the blood and skin, diffusion rates, and the surface area of exposure[8,9,10]. Currently, developments are underway to include the dermal route of exposure in the httk package[11].

DISTRIBUTION

Once absorbed, the chemical is distributed via systemic circulation to various tissues and organs. Distribution is governed by blood flow rates, tissue volumes, and tissue-specific partition coefficients. PBK models typically either assume that each tissue compartment is well-stirred and that distribution is perfusion rate-limited, or, for large, hydrophilic molecules or transporter-dependent drugs, the distribution is permeability-limited (figure 2)[12,13].

[7] M. W. Linakis et al., "Development and Evaluation of a High Throughput Inhalation Model for Organic Chemicals."
[8] Man Hu et al., "Physiologically-Based Toxicokinetic Modeling of Human Dermal Exposure to Diethyl Phthalate: Application to Health Risk Assessment."
[9] Man Hu et al., "Development of Human Dermal Pbpk Models for the Bisphenols Bpa, Bps, Bpf, and Bpaf with Parallel-Layered Skin Compartment: Basing on Dermal Administration Studies in Humans."
[10] Qiaoying Chen et al., "Contribution of Continued Dermal Exposure of Pfas-Containing Sunscreens to Internal Exposure: Extrapolation from in Vitro and in Vivo Tests to Physiologically Based Toxicokinetic Models."
[11] John Wambaugh, and Annabel Meade, "Development and Analysis of High Throughput Physiologically Based Pharmacokinetic/Toxicokinetic (Pbpk/Tk) Dermal Exposure Model."
[12] Y. Peng, Z. Cheng, and F. Xie, "Evaluation of Pharmacokinetic Drug-Drug Interactions: A Review of the Mechanisms, in Vitro and in Silico Approaches."
[13] Malcolm Rowland, Thomas N. Tozer, and Malcolm Rowland, *Clinical Pharmacokinetics and Pharmacodynamics : Concepts and Applications*.

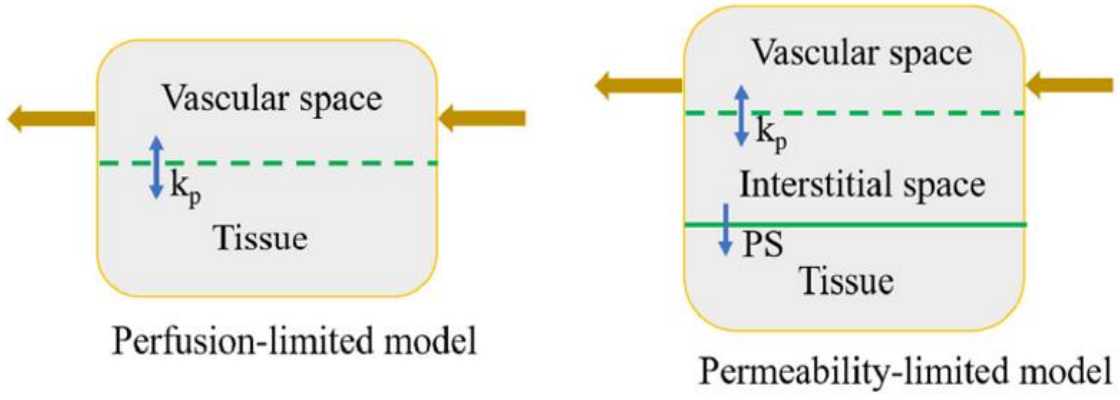

Figure 2. PBK models generally tend to be either perfusion limited or permeability limited models. kp, tissue partitioning coefficient (the concentration ratio between tissue and plasma at steady state); PS, membrane permeability coefficient. Adapted from [12].

As can be seen in figure 2, the tissue to plasma partitioning coefficients (kp, the concentration ratio between tissue and plasma at steady state) are the driving force for describing distribution (and therefore a crucial component of PBK models). Classically, these kp values have been informed by animal experimentation. Given the trend in reducing animal testing in chemical risk assessment, several in vitro and in silico approaches have been developed that can provide an estimate of kp values. By using Quantitative Structure–Activity Relationship models (QSARs), tissue to plasma partitioning coefficients can be predicted based on tissue composition and on chemical properties. The preferred QSAR method for estimating kp values is highly chemical and scenario specific, and multiple approaches exist. For a comprehensive review of some prominently used QSAR methods for estimating kp values, see[14].

For describing distribution, httk assumes perfusion-limited kinetics and bases its kp estimations on a modified version of the Schmitt method[15] for calculating tissue to plasma partition coefficients (for more details on the specific method, see[5]).

METABOLISM AND EXCRETION

Metabolism encompasses biochemical modifications of chemicals, primarily mediated by enzymatic processes occurring predominantly in the liver, but also in tissues such as intestines, lungs, and kidneys. When sufficient data are available, PBK models explicitly incorporate metabolic transformations through metabolic rate constants, enzyme kinetics, or clearance rates. When sufficient data is available, metabolites can be incorporated by using parent-metabolite linked PBK models .

Excretion involves the removal of chemicals and their metabolites from the body, primarily via kidneys (urine), liver (bile), and lungs (exhalation) . PBK models incorporate excretion processes using clearance parameters. In httk, renal excretion is assumed to be via passive glomerular filtration and is therefore directly based on the glomerular filtration rate (GFR) which is multiplied by the unbound kidney tissue concentration. Hepatic excretion is represented through scaling in vitro measured chemical specific intrinsic hepatocyte clearance (Cl_{int}) to a full liver clearance.

PBK MODEL DEVELOPMENT PIPELINE

The OECD guidance document on the Characterization, Validation and Reporting of PBK Models for Regulatory Purposes[1] serves as a vital resource for advancing the acceptance and utilization of PBK models in regulatory settings. By providing a clear framework for model characterization, validation, and reporting, it enhances the credibility of PBK models and supports their integration into chemical risk assessments, ultimately contributing to more informed and

[14] K. Utsey et al., "Quantification of the Impact of Partition Coefficient Prediction Methods on Physiologically Based Pharmacokinetic Model Output Using a Standardized Tissue Composition."
[15] W. Schmitt, "General Approach for the Calculation of Tissue to Plasma Partition Coefficients."

reliable regulatory decisions . Based on this guidance, we highlight some of the steps in the proposed framework, ranging from model conceptualization, to parameterization, implementation and evaluation, and ultimately the deployment of the PBK model within a NAM-based chemical risk assessment setting.

STEP 1: MODEL CONCEPTUALIZATION

Model conceptualization involves defining the structure and components of the model based on biological plausibility, the toxicity endpoint of interest, and available data. Clear identification of target organs, exposure routes, and relevant biological pathways is crucial at this stage. In the H2020 ALTERNATIVE project, the standard httk PBK model was modified to include additional heart and adipose compartments (figure 3) to tailor the specific needs of the project. The modification of the standard PBK model was simple due to the open-source nature of the software package[16]. Other compartments can easily be added to ensure the usability of httk for a wide variety of NAMs and applications.

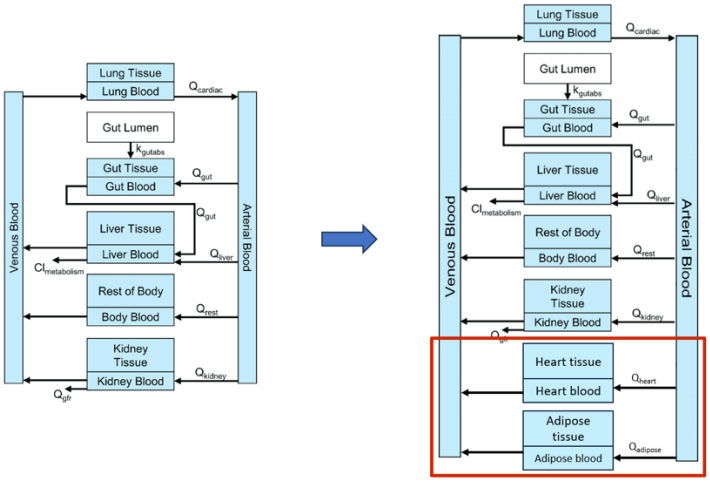

Figure 3. Adaption of the physiologically based toxicokinetic (PBTK) model structure. The original structure is shown on the left. We included additional non-metabolizing heart and adipose compartments (on the right, highlighted in red), each consisting of tissue and blood parts.

STEP 2: MODEL PARAMETERIZATION

PBK model parameters are derived from anatomical, physiological, and biochemical properties (figure 4). These parameters can represent physiological quantities, such as tissue blood flow and tissue volume, or chemical-specific properties that describe interactions between the chemical and the body, such as tissue-to-plasma equilibrium partition coefficients. The parameters can be derived from literature, experimental data, or computational predictions.

[16] Davidson-Fritz et al. Enabling Transparent Toxicokinetic Modeling for Public Health Risk Assessment

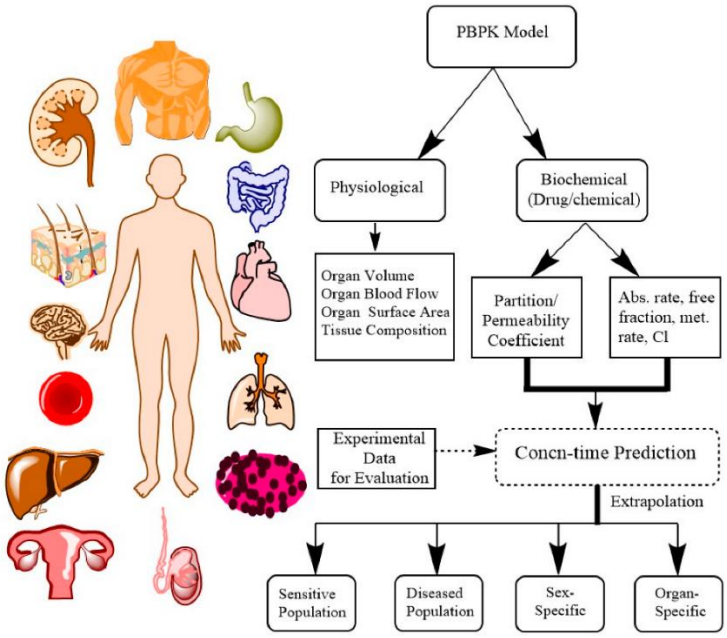

Figure 4. Parameters generally needed for a PBK model, adapted from [17]. PBK models are informed by physiological parameters (such as organ volumes, blood flows, surface areas and tissue composition) and by chemical-specific parameters (such as solubility, molecular weight etc.). Simulations can be made and are evaluated with clinical data (if available in literature). Afterwards, extrapolation studies can be performed to simulate concentration-time profiles for sensitive and diseased populations or for specific target sites in the body.

Httk uses multiple sources for its parameters. Anatomical and physiological parameters are derived from well-established literature databases[18,19,20] and are available in the software package for humans, rats, rabbits, mice, and dogs. The minimal requirements needed to run a PBK model in httk are the physicochemical properties that are used in the partitioning calculations, and in vitro measured fraction unbound in plasma (f_{ub}) and intrinsic clearance (Cl_{int}). Figure 5 gives an overview of the in vitro methods that were used in acquiring these parameters and are currently available for ~1.000 chemicals. However, the in vitro measured parameters f_{ub} and Cl_{int} can also be predicted with in silico QSAR methods, expanding the chemical library to approximately 10.000 chemicals[21,22].

[17] Deepika Deepika, and Vikas Kumar, "The Role of “Physiologically Based Pharmacokinetic Model (Pbpk)” New Approach Methodology (Nam) in Pharmaceuticals and Environmental Chemical Risk Assessment."
[18] R. P. Brown et al., "Physiological Parameter Values for Physiologically Based Pharmacokinetic Models."
[19] B. Davies, and T. Morris, "Physiological Parameters in Laboratory Animals and Humans."
[20] ICRP, "Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values. A Report of Age- and Gender-Related Differences in the Anatomical and Physiological Characteristics of Reference Individuals. Icrp Publication 89."
[21] Daniel E. Dawson et al., "Designing Qsars for Parameters of High-Throughput Toxicokinetic Models Using Open-Source Descriptors."
[22] Prachi Pradeep et al., "Using Chemical Structure Information to Develop Predictive Models for in Vitro Toxicokinetic Parameters to Inform High-Throughput Risk-Assessment."

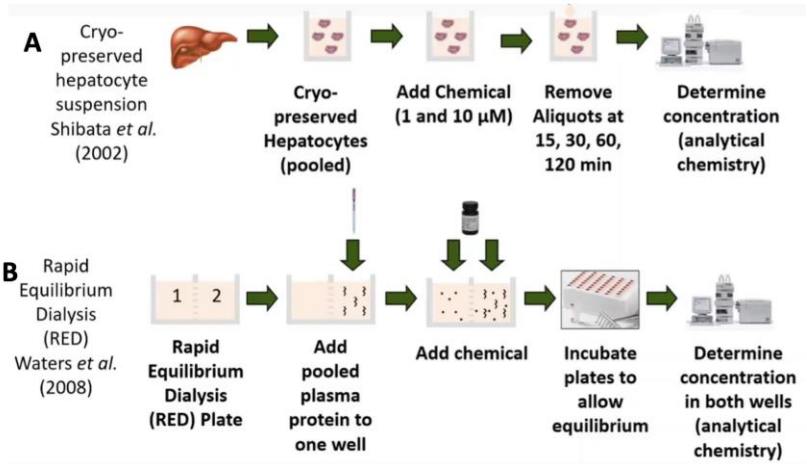

Figure 5. Overview of methods generating in vitro data used in the httk package. Adopted from httk slide deck[23]. A. Cryopreserved hepatocyte incubation method for the prediction of hepatic intrinsic clearance[24]. B. Rapid equilibrium dialysis approach for the measurement of plasma protein binding[25] .

STEP 3: MODEL IMPLEMENTATION AND EVALUATION

PBK modeling integrates absorption, distribution, metabolism, and excretion processes into a cohesive mathematical framework, often represented by sets of differential equations describing chemical transfer between compartments. PBK models consists of a series of equations, including differential equations that describe rate expressions and algebraic equations that calculate other relevant quantities. These equations are based on the mass balance principle, which states that the amount of a chemical entering a compartment equals the sum of the amount exiting or being cleared from that compartment and the amount retained within it. These changes are expressed as a function of time. An example of typical mass balance equations for compartments in a generic, simple PBK model are listed below[26]:

Rate of change of amount in tissue: $\frac{dAt}{dt} = Qt \times (Ca - Cvt) - clearance$

Where At = the amount in tissue t (that changes over time), Qt = blood flow to tissue *t*, Ca = arterial blood concentration, Cvt = chemical concentration in the venous blood leaving tissue *t*, and *clearance* is an additional rate expression describing clearance processes.

The equation describes the rate of change in the amount of a chemical within the tissue compartments, which can be solved using an ordinary differential equation (ODE) solver.

The total mass of the chemical in the compartment (area under the curve, AUC) is obtained by integrating this equation using a numerical integration method. Additional quantities are then calculated:

$Ct = At \,/\, Vt$ = concentration in each tissue compartment,

$Cvt = Ct \,/\, Pt$ = concentration in venous blood leaving the tissue,

Where At = amount of chemical in each tissue, Vt = volume of each tissue, and Pt = partition coefficient between the tissue and blood.

Supplemental rate equations can be used to include processes such as absorption from the gut lumen, elimination by the liver or kidneys, or the uptake of the chemical into lung blood through equilibrium. Various other physiological

[23] John Wambaugh, "Toxicokinetic New Approach Methodologies." https://www.epa.gov/chemical-research/new-approach-methods-nams-training

[24] Y. Shibata et al., "Prediction of Hepatic Clearance and Availability by Cryopreserved Human Hepatocytes: An Application of Serum Incubation Method."

[25] N. J. Waters et al., "Validation of a Rapid Equilibrium Dialysis Approach for the Measurement of Plasma Protein Binding."

[26] Levels National Research Council Committee on Acute Exposure Guideline, in *Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 9*.

processes may be included or excluded in the PBK model based on their relevance to the specific chemical being studied. However, these customized, chemical-specific or study-specific PBK models require extensive data on the behavior and partitioning of the chemical within the body and these data only exist for pharmaceuticals and relatively few, well-studied chemicals such as dioxin, lead, and trichloroethylene[27].

The following step in the PBK model development is to evaluate the simulations against clinical datasets not used during the development of the model. For pharmaceuticals, these data are often available in literature, either from clinical trials or pharmacokinetic analyses. In toxicology, this clinical data is generally more limited and often only available in toxicity studies in animals (if available at all).

STEP 4: DEPLOYMENT OF MODEL (USE CASE)

In order to facilitate risk assessment, the NAM-derived in vitro toxicity data has to be extrapolated to an external dose estimate. A concept that addresses this challenge is the administered equivalent dose (AED), which represents the external exposure that would result in the bioactive plasma or tissue concentration[27]. A chemical specific AED can be calculated by dividing the bioactive concentration with the steady state plasma or tissue concentration per unit of dose administered. The general equation for calculating chemical specific AEDs is listed below:

$$AED = \frac{EC_X}{C_{SS}} \times \frac{1\ mg}{kg_{BW} \times day}$$

Where EC = bioactive in vitro concentration (or target tissue concentration), Css = the steady-state plasma or tissue concentration resulting from a 1 mg/kg/day exposure. Since httk assumes linear kinetics, the steady state tissue or plasma concentration (Css) is proportional to the administered dose. Therefore, the dose (AED) is scaled accordingly to reach the bioactive concentration in a tissue or in plasma.

A recent development in the field of toxicokinetics is the assessment of the *ratio* between the AED and the modeled or estimated exposure of that chemical in the population. This ratio is referred to as the Bioactivity : Exposure Ratio (BER)[27] and allows for quick prioritization of chemicals for further toxicological evaluation by identifying those chemicals with potential risks for public health. For example, if a BER falls below the specified threshold, further action may be warranted, such as targeted testing strategies in later risk assessment tiers or a more in-depth risk evaluation.

Within a BER, the AED represent the bioactive part and is often calculated for the entire population rather than for individuals. The httk PBK model incorporates a Monte Carlo simulator, known as the Virtual Population Generator for httk (httk-pop)[28], to account for inter-individual variability within the human (US) population. The physiological parameters used by httk-pop are derived from the National Health and Nutrition Examination Survey (NHANES) data[29]. The resulting AEDs can be plotted against exposure estimates to construct BER plots.

We performed a case study to demonstrate how AEDs and BERs can utilize NAM-produced results in chemical risk assessment, specifically focusing on cardiotoxicity. To this extent, we selected the chemicals tested in an in vitro NAM for cardiotoxicity[30] for which exposure estimates could be retrieved from ExpoCast[31]. We converted the reported IC50s for this group of chemicals to an AED using httk, using the steady state heart concentration as target for the IC50s. The AEDs were calculated for populations of 1000 individuals, giving a 5th, median, and 95th percentile estimate. These AEDs were then plotted against the ExpoCast exposure estimates for the entire population (also 5th, median and 95th percentile estimates), resulting in a BER plot (figure 6). In this plot, chemicals were sorted based on a low to high BER

[27] M. Breen et al., "High-Throughput Pbtk Models for in Vitro to in Vivo Extrapolation."
[28] Caroline L. Ring et al., "Identifying Populations Sensitive to Environmental Chemicals by Simulating Toxicokinetic Variability."
[29] C. L. Johnson et al., "National Health and Nutrition Examination Survey: Sample Design, 2011-2014."
[30] O. Sirenko et al., "In Vitro Cardiotoxicity Assessment of Environmental Chemicals Using an Organotypic Human Induced Pluripotent Stem Cell-Derived Model."
[31] Caroline L. Ring et al., "Consensus Modeling of Median Chemical Intake for the U.S. Population Based on Predictions of Exposure Pathways."

from left to right. As can be seen, there are certain chemicals for which the BER is high (BER > 1.000) which would not be considered a current priority. On the other hand, chemicals on the left-hand side require careful consideration as in some cases the exposure in this scenario might be close to or higher than the bioactive concentration (BER < 10 or BER < 1). Overall, this case study demonstrates the utility of in vitro bioactivity data in quantitative risk-based prioritization and assessment.

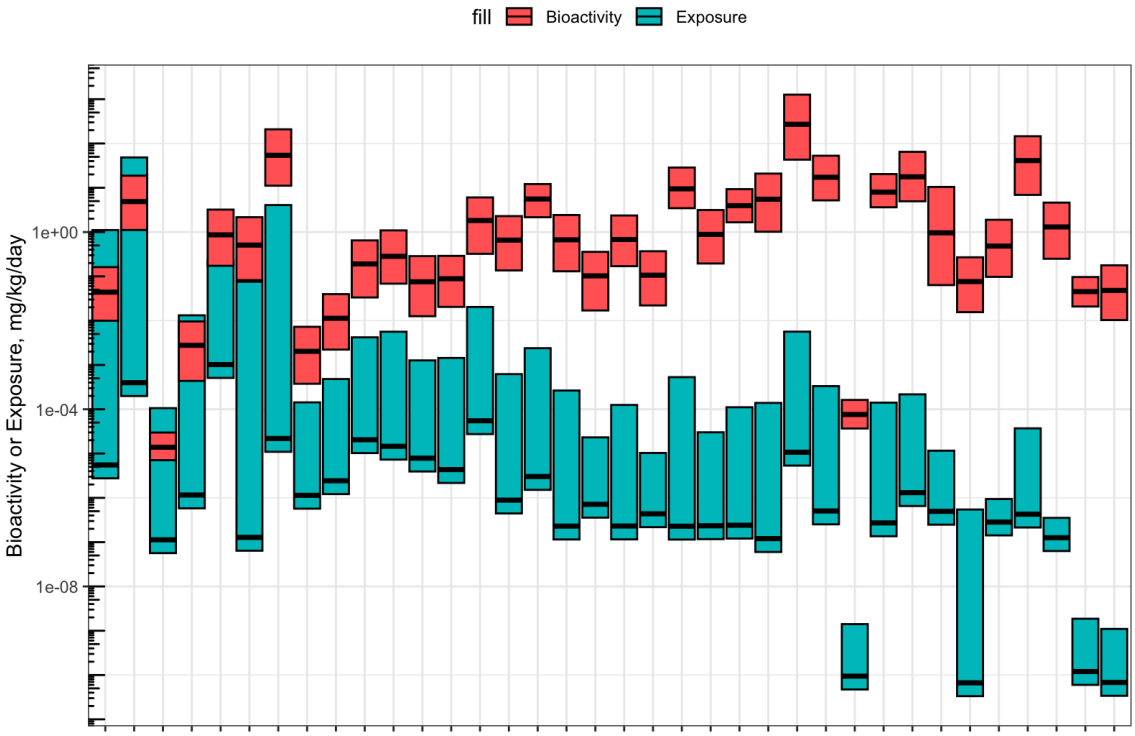

Figure 6. BER plot for a group of suspected cardiotoxicants. Each column represents a unique chemical, and chemicals are sorted based on their BER. Chemicals on the left have a BER close to or below 1, indicating that exposure is higher than bioactivity (reason for concern). In this plot, bioactivity refers to the Administered Equivalent Dose for reaching a bioactive concentration in the heart tissue, and exposure estimates were retrieved from ExpoCast [31].

CONCLUSIONS AND FUTURE PERSPECTIVES

The advancement and implementation of PBK modeling tools such as httk represents a transformative shift toward next-generation risk assessment methodologies, emphasizing non-animal methods (NAMs) and quantitative in vitro to in vivo extrapolation (QIVIVE). By integrating physiological and biochemical principles into computational frameworks, PBK models facilitate a robust prediction of chemical behavior in biological systems, thereby reducing reliance on traditional animal testing approaches. This not only aligns with evolving ethical standards but also addresses regulatory mandates for alternative methodologies in toxicological assessments.

In this regard, bioactivity exposure ratios (BERs) can evaluate the biological relevance and risk associated with chemical exposures, aiding in the interpretation of in vitro toxicity data in the context of real-world exposure scenarios. Integrating BER assessments with PBK models in NAMs can facilitate the accurate extrapolation from laboratory data to human health risk assessments.

Understanding toxicokinetics is essential for next-generation risk assessment, drug development, and regulatory toxicology because it helps predict internal exposure, optimize drug safety, refine animal testing, and support regulatory decisions. Integrating TK data into safety evaluations can improve accuracy, reduce uncertainty, and enhance public health risk assessment and protection. To further reduce or eliminate animal testing, research is underway to develop new risk assessment approaches based on cell-based tests instead of whole-animal experiments. However, these tests do not account for the kinetics of a substance within an entire organism. Therefore, they must be complemented by PBK models that simulate the substance's behavior in the body. By incorporating toxicokinetics and PBK models into in

vitro based NAM testing paradigms, we can not only improve the estimation of human health risks but also help reduce the number of test animals needed to assess the harmful effects of substances.

Authors:

Tom Roos, Utrecht University

Ronette Gehring, Utrecht University

Editors:

Anastasius Gavras, Eurescom GmbH

Pooja Mohnani, Eurescom GmbH

Date of publication:

24th March 2025

ALTERNATIVE SOCIAL MEDIA:

WEBSITE: https://alternative-project.eu/

LINKEDIN: https://www.linkedin.com/in/project-alternative-732943223/

PLATFORM X: https://x.com/EU_ALTERNATIVE

ALTERNATIVE

environmentAL Toxicity chEmical mixtuRes through aN innovative platform

The research leading to these results has received funding from the European Union Horizon 2020 Programme under grant agreement number 101037090 – ALTERNATIVE. The content of this document reflects only the author's view and the Commission is not responsible for any use that may be made of the information it contains.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°101037090