Physiologically-Based Pharmacokinetic Modeling to Support Modernized Chemical Safety Assessment

Continuing Education Course AM07

Sunday, March 11
8:15 AM to 12:00 Noon

Chairpersons:

Miyoung Yoon
Alicia Paini

Presenters:

Alicia Paini
Hugh A. Barton
Lisa M. Sweeney
Miyoung Yoon
Cecilia Tan
Jos Bessems

SOT 2018 San Antonio
★ March 11–15 ★

SOT

2018 San Antonio

Chairpersons

Miyoung Yoon

Alicia Paini

Primary Endorser

Biological Modeling Specialty Section

Other Endorsers

In Vitro and Alternative Methods Specialty Section

Risk Assessment Specialty Section

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Physiologically-Based Pharmacokinetic Modeling to Support Modernized Chemical Safety Assessment

8:15 AM–8:30 AM	**Physiologically-Based Pharmacokinetic Models as a Critical Component in Moving Forward with the New Toxicity Testing Strategies Based on *In Vitro* and Computational Approaches** Alicia Paini, European Commission Joint Research Centre, Ispra, Italy	5
9:05 AM–9:40 AM	**Physiologically-Based Pharmacokinetic Models for Risk and Safety Assessment: Basic Principles and Examples of the Applications in Traditional Risk Assessment** Hugh A. Barton, Pfizer, Inc., Groton, CT	10
9:40 AM–10:00 AM	**Parameterization of Physiologically-Based Pharmacokinetic Models with Minimum Reliance on *In Vivo* Toxicokinetic Studies: Describing Average Person vs. Population** Lisa M. Sweeney, Naval Medical Research Unit Dayton, Wright Patterson AFB, Dayton, OH	24
10:30 AM–10:50 AM	**PART 1—Examples of the Use of Physiologically-Based Pharmacokinetic Models in Support of *In Vitro* Based Safety Assessment: Hands-On Demonstration of a Work Flow** Miyoung Yoon, ScitoVation, Research Triangle Park, NC	42
10:00 AM–10:30 AM	**BREAK**	
10:30 AM–10:50 AM	**PART 2—Examples of the Use of Physiologically-Based Pharmacokinetic Models in Support of *In Vitro* Based Safety Assessment: Hands-On Demonstration of a Work Flow** Miyoung Yoon, ScitoVation, Research Triangle Park, NC	42
10:50 AM–11:25 AM	**A Tiered Approach to Incorporate Exposure and Pharmacokinetics Consideration in *In Vitro*-Based Safety Assessment** Cecilia Tan, US EPA, Research Triangle Park, NC	58
11:25 AM–12:00 Noon	**Approaches for Evaluation of Non-Animal-Based Physiologically-Based Pharmacokinetic Models Including the Use of Human Biomonitoring Data** Jos Bessems, VITO, Mol, Belgium	73

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Physiologically-Based Pharmacokinetic Modeling to Support Modernized Chemical Safety Assessment

2018 SOT CE Course: AM07

Chair: Miyoung Yoon, ScitoVation, USA
Co-Chair: Alicia Paini, European Commission Joint Research, Italy

Course Objectives

This course will provide information on:

1. Basic principles of PBPK modeling with a special focus on supporting chemical risk assessment under the new toxicity testing paradigm.
2. Basic principles of model construct (parametrization).
3. Evaluation of model performance and reliability along with use of available human data.
4. Development and application of the PBPK models to support risk-based decisions in different tiers of risk assessment.
5. Demonstration using PLETHEM.

Course Schedule

8:15–8:30 am Introduction: Physiologically-Based Pharmacokinetic Models as a Critical Component in Moving Forward with the New Toxicity Testing Strategies Based on *In Vitro* and Computational Approaches. Alicia Paini.

8:30–9:05 am 1st Lecture: Physiologically-Based Pharmacokinetic Models for Risk and Safety Assessment: Basic Principles and Examples of the Applications in Traditional Risk Assessment. Hugh A. Barton.

9:05–9:40 am 2nd Lecture: Parameterization of Physiologically-Based Pharmacokinetic Models with Minimum Reliance on *In Vivo* Toxicokinetic Studies: Describing Average Person vs. Population. Lisa M. Sweeney.

09:40–10:00 am 3rd Lecture and Demo Part I: Examples of the Use of Physiologically-Based Pharmacokinetic Models in Support of *In Vitro*-Based Safety Assessment: Hands-On Demonstration of a Work Flow. Miyoung Yoon.

10:00–10:30 am Break

10:30–10:50 am 3rd Lecture and Demo Part II: Examples of the Use of Physiologically-Based Pharmacokinetic Models in Support of In Vitro Based Safety Assessment: Hands-On Demonstration of a Work Flow. Miyoung Yoon.

10:50–11:25 am 4th Lecture: A Tiered Approach to Incorporate Exposure and Pharmacokinetics Consideration in *In Vitro*-Based Safety Assessment. Cecilia Tan.

11:25–12:00 noon 5th Lecture: Approaches for Evaluation of Non-Animal-Based Physiologically-Based Pharmacokinetic Models Including the Use of Human Biomonitoring Data. Jos Bessems.

Introduction

Physiologically-Based Pharmacokinetic Models as a Critical Component in Moving Forward with the New Toxicity Testing Strategies Based on *In Vitro* and Computational Approaches

Alicia Paini, PhD, and Sandra Coecke, PhD
European Commission Joint Research Centre
Ispra, Italy

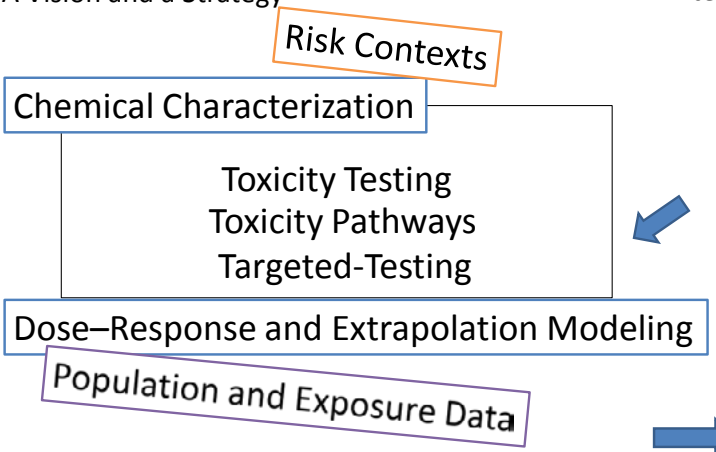
Conflict of Interest Statement

The contents of this presentation neither represent, nor necessarily reflect the position or policy of the author institution.

The author declares no conflict of interest.

New Toxicity Testing Strategies

Toxicity Testing in the 21st Century:
A Vision and a Strategy



Elements within IATA
Integrated Approaches to Testing and Assessment

Mechanism (AOP)

- QSARs
- Test Guidelines (*In Vitro/In Vivo*)
- Non-Standard Tests
- PD Models
- Categorization/Read-Across
- *In Vitro* Testing Strategies
- Uncertainty and Weight of Evidence

Exposure

- EXPOSURE
- ADME
- PBPK Model* (IVIVE)
- QIVIVE

*PBK models are synonymous to PBPK (physiologically-based pharmacokinetic), PBBK (physiologically-based biokinetic) and PBTK (physiologically-based toxicokinetic) models (Bessems *et al.*, 2014).

PBPK Model Applications

In USA

- Critical need for flexible kinetic modeling tools to support tiered testing strategies from prioritization to regulation.
- Development of open-source IVIVE and PBPK platforms to support the new safety assessment paradigm beyond traditional PBPK use areas.
- Meeting the needs for rapid and efficient risk-based decisions under the Toxic Substances Control Act (TSCA)

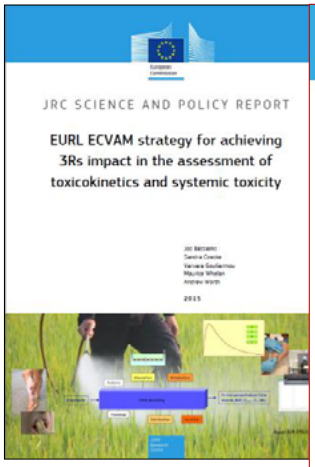
In EU

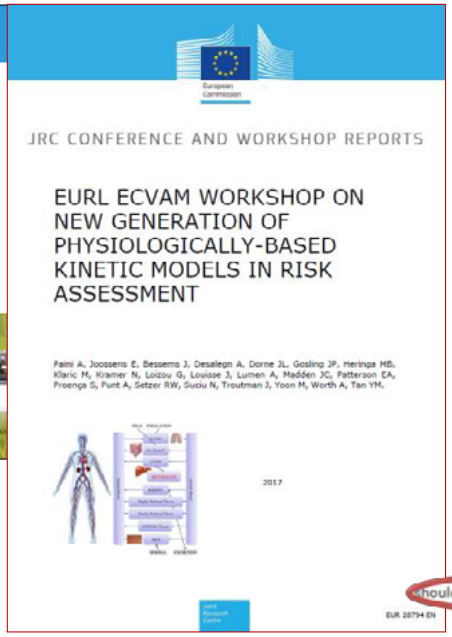
- EFSA evaluations on food
- EMA DDI dossier submission

An evaluation of the various risk evaluations reveals limited use of PBPK modeling approaches in risk evaluations of food chemicals and medicines. PBPK models were mainly used to support the evaluation of species differences. (BPA and Acrilamide; and DDI). (Punt et al., 2017)

Punt et al., 2017
https://encrypted.google.com/search?q=PBPK+model+predictionsi+in+EFSA+opinions&ei=LjY6WuqsH8T7kwW5uY84&start=10&sa=N&biw=1536&bih=779

Why PBPK Model Training?

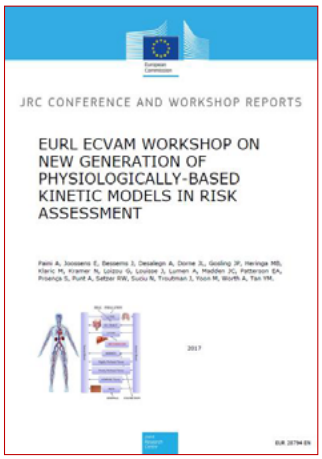


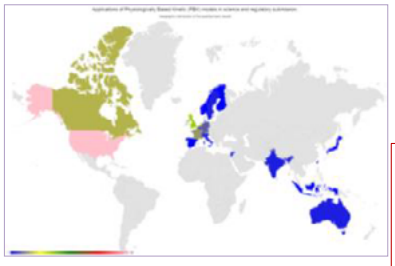


(i) identify current challenges in the application of PBPK modeling to support regulatory decision making;
(ii) discuss challenges in constructing models with no *in vivo* kinetic data and opportunities for estimating parameter values using *in vitro* and *in silico* methods;
(iii) present the challenges in assessing model credibility relying on non-animal data and address strengths, uncertainties and limitations in such an approach;
(iv) establish a *good kinetic modeling practice* workflow to serve as the foundation for guidance on the generation and use of *in vitro* and *in silico* data to construct PBK models designed to support regulatory decision making.

...should be an increase in **communication and training** of the regulatory community, ... risk assessors and risk managers. As reported in the EURL ECVAM TK ...

Why PBPK Model Training?



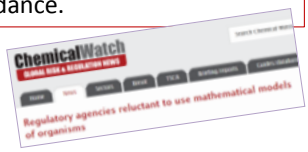


International effort
OECD GD on
New generation of
PBPK modeling

Five (5) main challenges highlighted in gaining regulatory acceptance*:

(i) Lack of expertise in PBPK modeling;
(ii) Validation of models needs to test various scenarios;
(iii) Lack of user friendly software;
(iv) Differences in acceptance criteria between agencies and countries;
(v) Lack of guidance.


A recommendation from the experts was to ***develop/refine/adapt good modelling practice,*** as well as ***to generate harmonized terminology/ontologies.*** This working group proposed the drafting of a guidance document for good modelling practice for PBK[12], which could be extended to other in silico biokinetic models. With the increasing demands for alternative methods within the risk assessment framework, the need to



*Paini et al., 2017 https://www.ncbi.nlm.nih.gov/pubmed/28866268
The survey results can be accessed at http://bolegweb.geof.unizg.hr/questionaire/pbk/
EURL ECVAM WS Report http://publications.jrc.ec.europa.eu/repository/bitstream/JRC108231/kjna28794enn.pdf

Physiologically Based Pharmacokinetic Models for Risk and Safety Assessment: Basic Principles and Examples of the Applications in Traditional Risk Assessment

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Conflict of Interest Disclosure

No conflict of interest. Employed by Pfizer and owns Pfizer stock and options.

Outline

- PKPD & Risk/Safety Assessment Context
- PBPK Model Basics
- Applications in Risk/Safety Assessment

Abbreviations

AUC: area under the concentration-time curve (mg/L*hr)

Cl: clearance from body (L/hr, ml/hr/kg)

Cl_d: distributional clearance (L/hr)

Cmax: maximum concentration (mg/L or M)

ka: absorption rate constant (1/hr)

Km: Michaelis-Menten constant (M)

PD: pharmacodynamics

PK: pharmacokinetic

TD: toxicodynamics

TK: toxicokinetics

V_i: volume of compartment I (L, ml/kg)

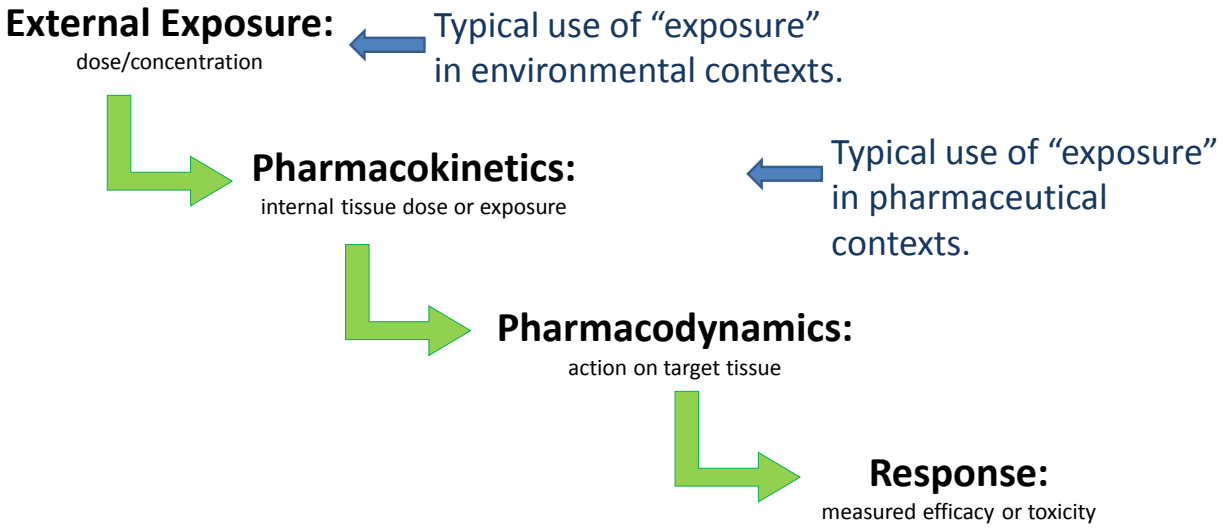
Vd: volume of distribution (L)

Vmax: maximal metabolism rate

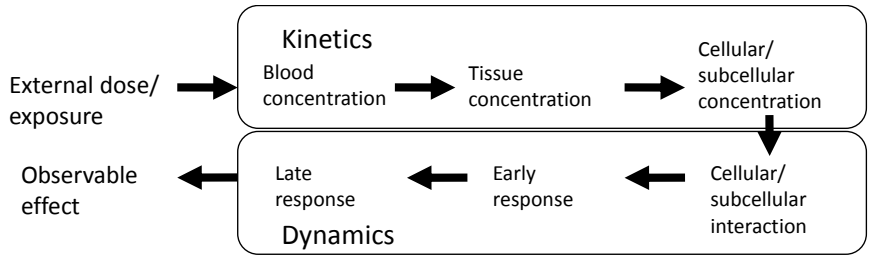
PKPD

- Pharmacokinetics = study of time-course of chemicals in plasma or tissues
  - What the body does to the drug
  - Absorption, Distribution, Metabolism, Excretion (ADME)
  - TK: toxicokinetics
- Pharmacodynamics = study of biological effects of chemicals
  - What the drug does to the body
  - TD: toxicodynamics

Dose/Exposure-Response



Role of PKPD in Risk and Safety Assessments



- Environmental Risk Assessment Extrapolations
  - Interspecies, human variability, route-to-route, high-to-low dose, characterize uncertainties
- Pharmaceutical Safety Assessments
  - Nonclinical (*in vitro* and *in vivo*) to clinical translation, human variability, dosing regimen, drug-drug interactions,
- Laboratory objectives
  - Improved compound selection
  - Improved toxicity or efficacy study designs

IPCS 2010

PK Analysis Methods

- **Noncompartmental analysis**
  - Direct analysis of time course data to obtain PK parameters, e.g., area under the curve for plasma concentration (AUC mole/liter*min), clearance (CL ml/min or ml/min/kg), volume of distribution (Vd ml or ml/kg), Cmax (mg/mL)
- **Compartmental PK analysis**
  - One, two, three compartment models fitted to time-course data (e.g., two compartment parameters: V1, V2, CL, CLd)
  - PK driver for PKPD modeling of effects (efficacy or safety)
- **Physiologically-based pharmacokinetic (PBPK) modeling**
  - Describes physiology (e.g., tissue volumes, blood flows)
  - Includes major biological processes determining PK (e.g., metabolism, transport, tissue distribution)
  - Systems of ordinary differential equations
  - Models available in general (e.g., acslX, Matlab, Berkeley Madonna, Nonmem) and/or specialized software (e.g., SimCYP, Gastroplus)

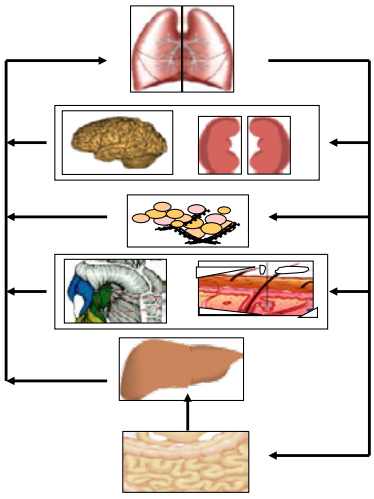
Rowland and Tozer 2010, Himmelstein & Lutz 1979, Peters 2012

PBPK Analysis

- Strengths
  - Captures biological processes and hypotheses explicitly
    - Varying degrees of detail or biological realism
    - Flexibility to reflect biology
  - Can incorporate changes in PK due to chemical (e.g., GSH depletion, protein induction), growth/aging
  - Facilitates analyses across species, doses and human population subgroups
- Limitations:
  - Greater requirements for *in vitro* or *in vivo* data than other methods
  - Statistical evaluation of uncertainty and variability more challenging
  - Model development and implementation requires appropriate expertise

PBPK Model Components

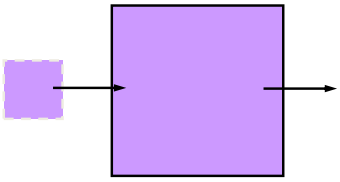
- Model Purpose/Goal
- Structural or Deterministic Model
  - Biological Hypotheses
  - Exposure conditions
  - Desired outputs
- Non-Deterministic Model (fitting to data)
  - Statistical model
  - Error model
- Data

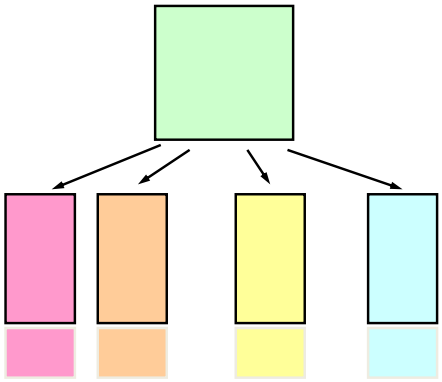


Models for Full Characterization of Variability and Uncertainty

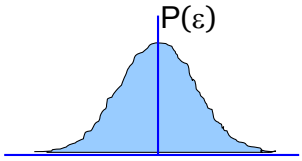
Hierarchical Statistical Model

PK and/or PD Model





Measurement Model



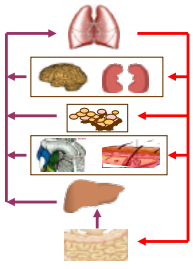
Measurement Error ε

Estimate all parameters simultaneously

PBPK Model Components

Physiological/Anatomical

- Tissue volumes
- Blood flow rates
- Lymphatic flow rates
- Cardiac output
- Glomerular filtration rate
- Alveolar ventilation rate
- Hematocrit
- Glutathione concentration



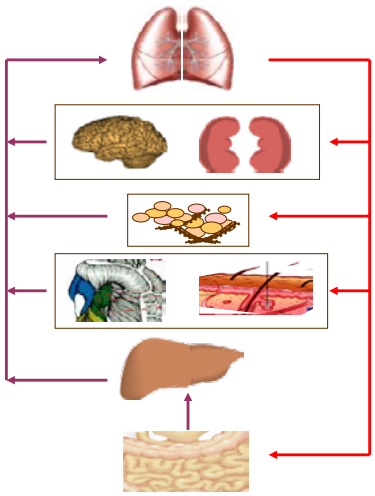
Biochemical/Physicochemical

- Tissue:blood partition coefficient
- Blood:air partition coefficient
- Enzymatic rate constants
- Transporter rate constants
- Equilibrium or rate constants for plasma protein binding

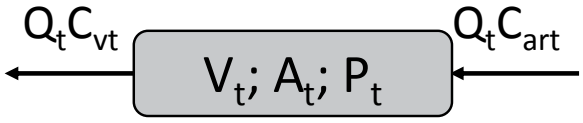
ADME

What Tissues (Compartments)?

- Absorption
  - Gastrointestinal tract
  - Lung
  - Skin
- Distribution
  - Storage (e.g., fat, bone, serum protein binding)
  - Distributional kinetics (e.g., total body water)
- Clearance
  - Metabolism
  - Excretion (e.g., urine, bile, hair)
- Target tissues
  - For efficacy or toxicity or surrogate (often blood)



Description for a Single Well-Mixed Tissue Compartment



TISSUE

TERMS

Q_t = tissue blood flow

C_{vt} = venous blood concentration

P_t = tissue blood partition coefficient

V_t = volume of tissue

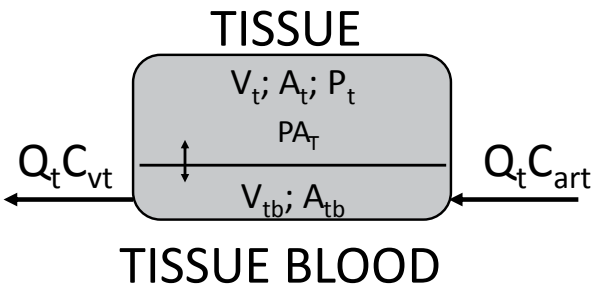
A_t = amount of chemical in tissue

mass-balance equation: $$\frac{dA_t}{dt} = V_t \frac{dC_t}{dt} = Q_t C_{art} - Q_t C_{vt}$$

venous equilibration assumption $$C_{vt} = C_t / P_t$$

Cvt = Ct,u: free concentration in tissue

Diffusion Limited Distribution



PA_T: permeability area cross-product for tissue

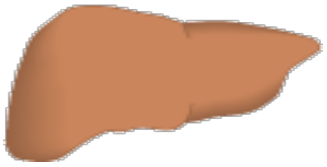
TISSUE BLOOD mass-balance equation:

$$\frac{dA_{tb}}{dt} = V_{tb}\frac{dC_{tb}}{dt} = Q_t(C_{art} - C_{vt}) + PA_t(C_t / P_t - C_{bt})$$

TISSUE mass-balance equation:

$$\frac{dA_t}{dt} = V_t\frac{dC_t}{dt} = PA_t(C_{tb} - C_t / P_t)$$

Liver Compartment



rate of change of amount in liver = rate of uptake in arterial blood - rate of loss in venous blood - rate of loss by metabolism

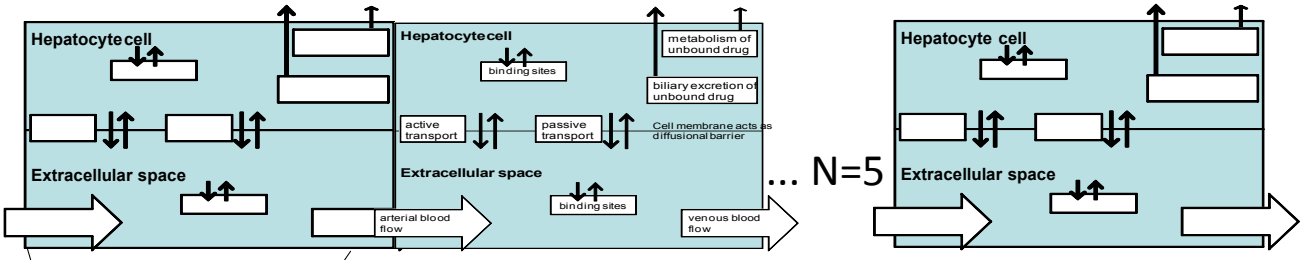
$$\frac{dA_l}{dt} = Q_l(C_a - C_{vl}) - \frac{V_m C_{vl}}{K_m + C_{vl}}$$

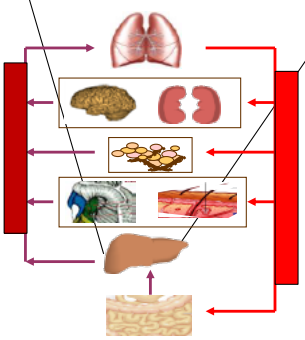
Flow limited metabolism: high intrinsic metabolism compared to blood flow

PBPK Models in Risk/Safety Assessment

- Pharmacological Agents
- Volatile Organic Compounds
- Mixtures/Drug-Drug Interactions
- Nasal Tract Toxicity
- Lifestages

Liver Transporter PBPK Model

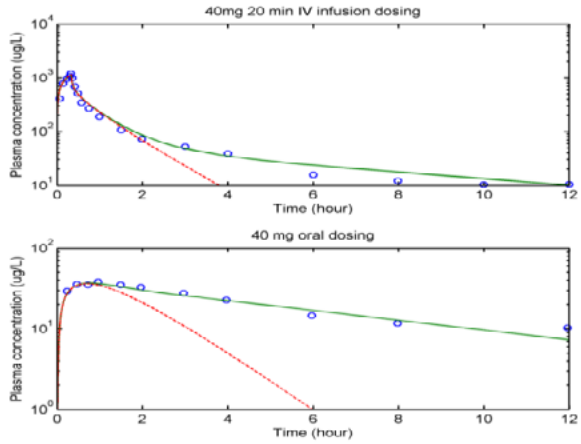




Metabolism of unbound drug–HLM CLint
Biliary excretion of unbound drug–SCHH
Active transport into hepatocyte cell–SCHH or HH suspensions
Passive transport–SCHH or HH suspensions in presence of uptake inhibitor
Active efflux–assumed negligable
Fu,plasma; Fu,liver
Kp values predicted (Rodgers & Rowland 2006) for well stirred tissues

Jones et al., 2012; Li et al., 2014a

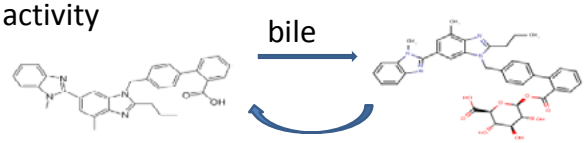
Enterohepatic Recirculation



Telmisartan with (green line) and without (red line) ER. Circles are data.

Li et al., 2014b; Stangier et al., 2000

- Compound excreted in bile into the GI tract can be reabsorbed substantially extending plasma exposure
- ER of parent compound or glucuronide metabolites that are subsequently cleaved by bacterial enzymes in the gut
- ER can extend exposure for hours to days, perhaps a week
- Substantial inter-subject variability due to differences in gut microflora and glucuronidase activity

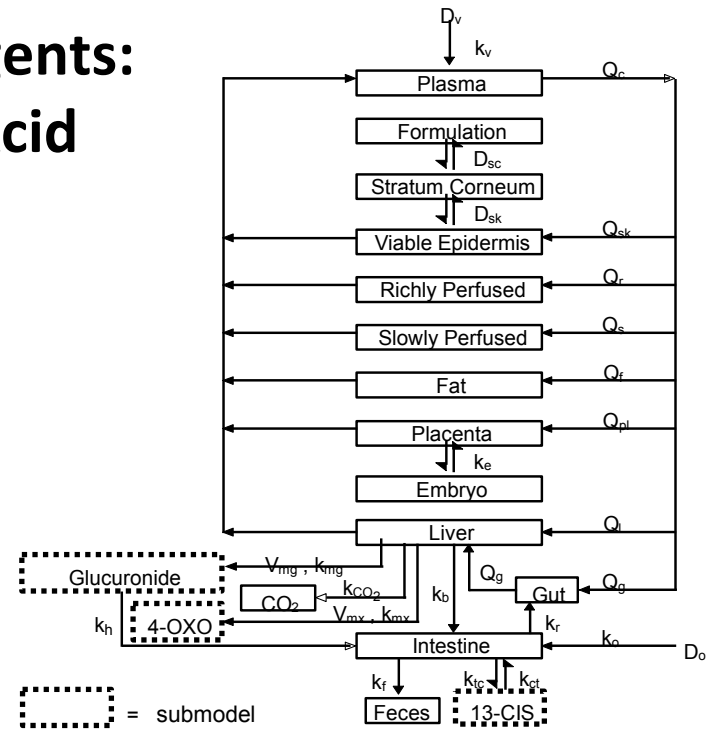


Gut glucuronidase cleavage & ER

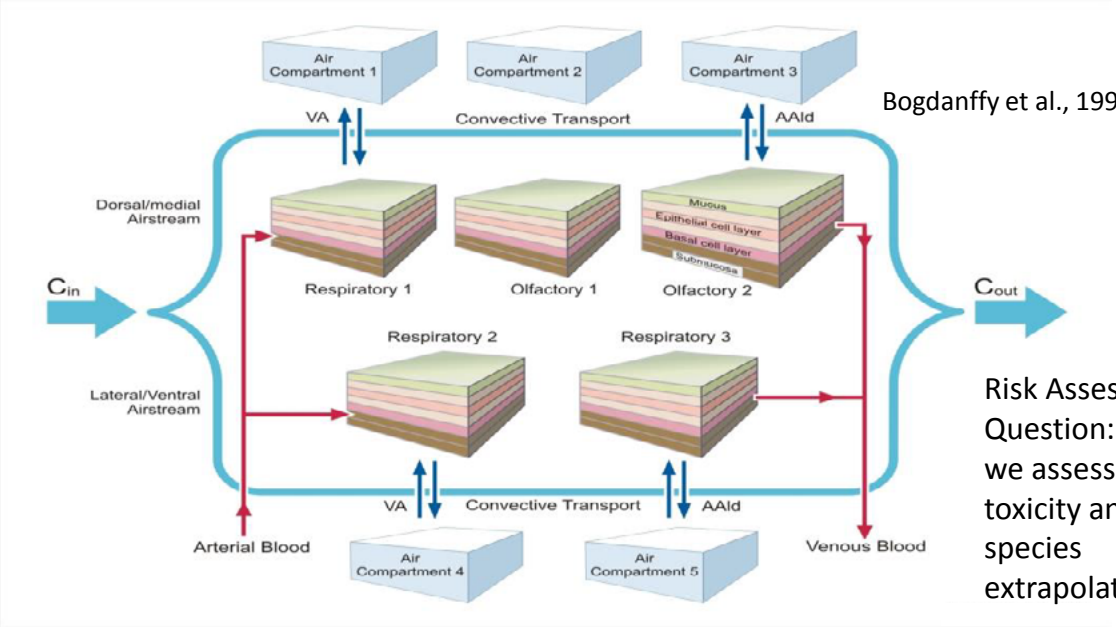
Pharmacological Agents: All-Trans Retinoic Acid

Clewell et al., 1997

Safety question: Would dermal application of ATRA cream have potential for developmental toxicity due to 13-CIS?



Nasal Toxicity: Vinyl Acetate



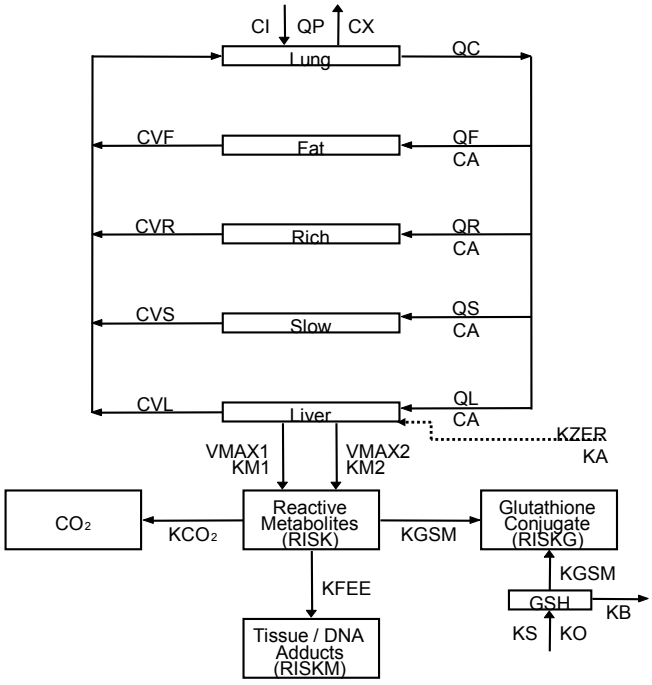
Bogdanffy et al., 1999

Risk Assessment Question: How do we assess nasal toxicity and cross-species extrapolation?

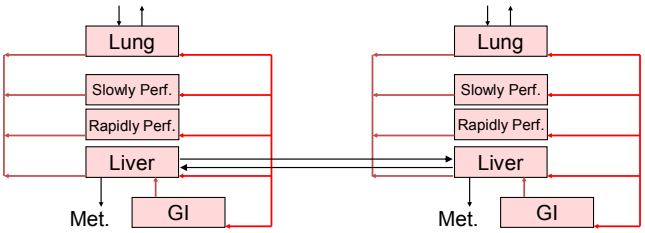
Volatile Organic Compounds: Vinyl Chloride

Clewell et al., 2001

Risk Assessment Question: How best to evaluate cancer risk of VC in humans and other species?



VOCs: Mixtures



$$\frac{dA_l}{dt} = Q_l(C_a - C_{vl}) - \frac{V_m C_{vl}}{K_m[1 + I / K_i] + C_{vl}}$$

Risk/Safety Question: How do we assess PK impacts of exposures to multiple compounds?

Barton et al., 1995

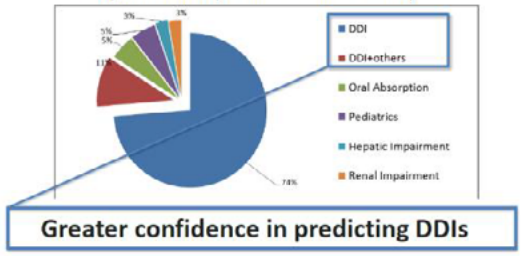
Drug-Drug Interactions

PBPK submissions to the FDA since 2004

As of June, 2014	As of Aug, 2016
n = 96 (60% DDI)	n = 217 (60% DDI)
Sinha, MHRA Workshop, 2014	Zhao, EMA Workshop, 2016

DDIs: Drug-drug Interactions

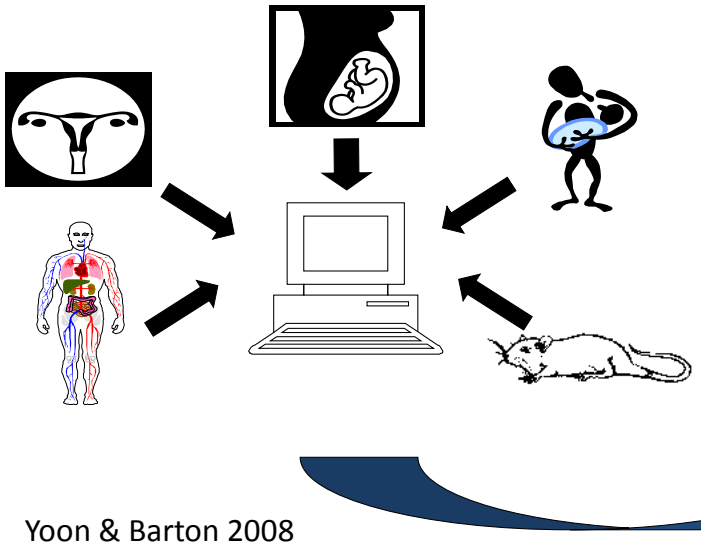
PBPK supporting dosing recommendations in US prescribing information (38 cases 2009-2016)

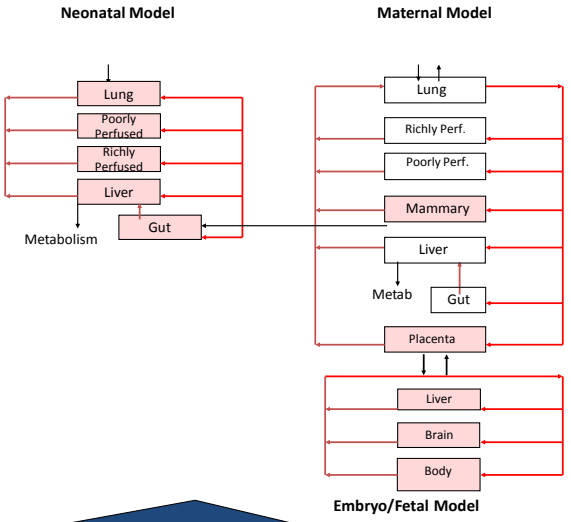


Greater confidence in predicting DDIs

https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AdvisoryCommitteeforPharmaceuticalScienceandClinicalPharmacology/UCM550577.pdf

Life Stage and Species Extrapolations





Yoon & Barton 2008

Characterizing the Level of Confidence in PBPK Models for Use in Risk/Safety Assessment

BIOLOGICAL BASIS of model structure and parameters

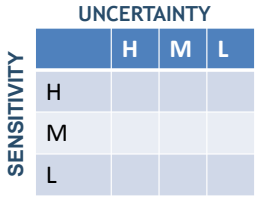
- Do the model structure and parameters have a reasonable biological basis, consistent with integrated evidence on mode of action?

PERFORMANCE of model: Model simulations *vs. experimental data*

- How well does the PBPK model reproduce the chemical-specific TK data
  - Various conditions?
  - within 2 fold

RELIABILITY of model predictions of dose metrics relevant to risk assessment

- Which parameters most influence outcome (sensitivity)
- "Uncertainty" (variability) in parameter estimation



Clewell et al., 2002, IPCS 2010

Summary

- Pharmacokinetics (PK)
  - Analysis methods: non-compartmental (NCA), classical compartmental, PBPK
- Physiologically-based pharmacokinetic (PBPK) modeling
  - Separates biology of system (e.g., species, tissues, blood or lymphatic flows) from compound-specific characteristics (e.g., metabolism or transport rates, plasma protein binding, tissue distribution).
  - Model structure should address planned application or need (e.g., target-site for efficacy or toxicity) and relevant biological determinants of PK (e.g., sites of metabolism or transport), but can be quite varied due to different needs and biology
- Risk/Safety Assessment Applications
  - Interspecies extrapolation or nonclinical-to-clinical translation, human variability including life stages, route-to-route extrapolation, mixtures or drug-drug interactions, dosing regimen evaluation, characterize uncertainties
  - Confidence in model for application: biological basis, performance or evaluation, reliability/uncertainty

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Parameterization of Physiologically-Based Pharmacokinetic Models with Minimum Reliance on *In Vivo* Toxicokinetic Studies: Describing Average Person vs. Population

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Conflict of Interest Statement

Neither I nor any of the members of my immediate family have any financial interest in or affiliation with a commercial organization that has a direct or indirect interest in the subject matter of my presentation.

Disclaimers/Acknowledgements

The views expressed in this presentation are those of the author and not her employer or the US Government.

Abbreviations

ADME: absorption, distribution, metabolism, and excretion

BW: Body weight

CYP: cytochrome P450

EFH: Exposure Factors Handbook

US EPA: US Environmental Protection Agency

HERO: Health and Environmental Research Online

ICRP: International Commission on Radiological Protection

Abbreviations

IPVI: Interpopulation Variability Index

ISVI: Intrasubpopulation Variability Index

KM: Michaelis constant (enzyme affinity)

PBPK: Physiologically-based pharmacokinetic

P3M: Physiological Parameters for PBPK Modeling

UGT: UDP-glucuronosyl transferase

VmaxC: BW normalized maximum metabolism

Presentation Outline

- What do we mean by “parameterization” and “minimum reliance on *in vivo* toxicokinetic studies?”
- Types and sources of data pertinent to physiologically-based pharmacokinetic (PBPK) modeling
  - Exposure factors/biological/physicochemical
  - Physiological/anatomical, genetic, biochemical
  - Chemical specific (physicochemical)
- Modeling of variability
  - Categories and distributions
  - Monte Carlo Simulation

Why Pursue Minimum Reliance on *In Vivo* Toxicokinetic Data?

- Increased use of *in vitro* data and *in silico* predictions may be faster, cheaper, and better than traditional whole-animal approaches
  - Typically easier to generate additional *in vitro* data or *in silico* predictions than *in vivo* data
  - New approach facilitates extrapolation and descriptions of variability
    - Variability due to genetic (e.g., ethnic) or hormonal (sex/gender/life stage) diversity
  - New approach allows *in vivo* data to be saved for **validation** instead of being used for **calibration**, potentially increasing model **confidence**
    - Trade-offs exist regarding accuracy

What Is "Parameterizaton" and Why Does Parameterization Matter?

- Parameterization is selecting the "numbers" that go into the model
- Appropriate structure and parameterization are essential characteristics of any predictive model
  - To assess whether *in vitro*- and *in silico*-based models are fit-for-purpose, decision makers must understand both the specific assessment's requirements and the models' limitations.
- *In vitro*- and *in silico*-derived models tend to have more parameters

Parameters for PBPK Models

- Chemical-independent parameters
  - Exposure factors
  - Physiological/anatomical
  - Scaling information for *in vitro* or *in silico* data (addressed in next talk)
- Chemical specific *in vivo, in vitro*, or *in silico* pharmacokinetic data
  - Absorption
  - Metabolism
  - Distribution/elimination (e.g., transporters, binding proteins, partitioning)

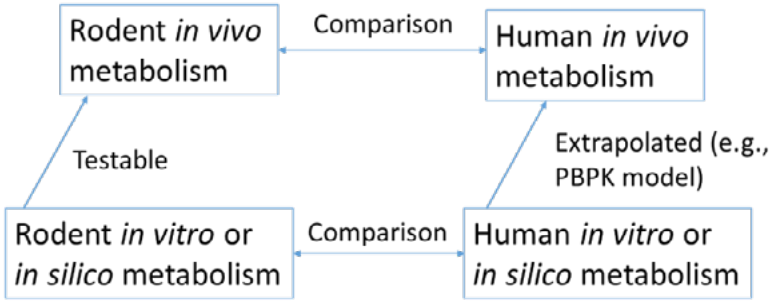
Example of *In Vivo* vs. *In Vitro* or *In Silico* Parameterization of Metabolism

- *In vivo*
  - Parameters
    - Body weight (BW)
    - BW normalized maximum metabolism (VmaxC)
    - Michaelis constant (KM; enzyme affinity)
  - Derivation
    - Measurement (BW)
    - Optimization of fit to *in vivo* data (VmaxC, KM)
- *In vitro* measurement or *in silico* prediction
  - Parameters
    - Maximum metabolism, normalized to enzyme amount, cell number, etc.
    - Michaelis constant(s)
    - Scaling factors to compute whole-organ metabolism
    - Tissue mass as fraction of BW for multiple tissues
    - BW
  - Derivation
    - Measurement (BW, BW normalized tissue weights, scaling factors)
    - Optimization of fit to *in vitro* data (Vmax and KM for enzyme) or prediction

Historical Context for Reliance on a Hybrid of *In Vitro* and *In Vivo* Data

- *In vitro* data for partition coefficients (Gargas et al., 1989) and *in vivo* data used to calibrate metabolic parameters

- *In vitro* metabolism data used to extrapolate (e.g., ratios for interspecies); parallelogram approach (Reitz et al., 1996)



Exposure Factors

- Choice of exposure factors will be related to model purpose
  - Population
    - Age
    - Health status/life stage
    - Level of activity
  - Descriptive vs. protective

Exposure Factors

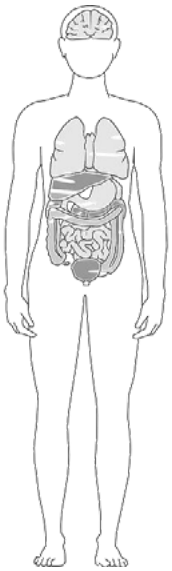
- US Environmental Protection Agency (US EPA) "ExpoBox" (www.epa.gov/expobox), based on the Exposure Factors Handbook (EFH; US EPA, 2011)
  - Water ingestion rates
    - For pregnant and lactating women
    - For high-activity levels/hot climates
  - Dust and soil and food ingestion rates
  - Inhalation rates
  - Dermal exposure factors (body surface area, adherence)
  - Body weight
  - Activity factors (e.g., time spent in locations, doing types of tasks)

Exposure Factors

- ExpoBox is a series of tool sets organized by Approaches (e.g., indirect estimation, exposure reconstruction), Media, Routes, Tiers, and Types (screening level/refined, deterministic/probabilistic), Life stages and Populations, and Chemical Classes
- Enhancements available in ExpoBox vs. EFH
  - Downloadable Excel spreadsheets of recommended values and data tables
  - Links to 700 references in US EPA's Health and Environmental Research Online (HERO) database
  - Links to related information (e.g., European Union, Japanese, and Australian exposure factors efforts)

Physiological and Anatomical Parameters

- Body weight
- Tissue weight
- Inhalation rate (total vs. alveolar)
- Cardiac output
- Regional blood flow
- Urine production
- Glomerular filtration



Sources of Physiological/Anatomical Parameters

- ExpoBox/EFH (US EPA, 2011) (humans only; BW, ventilation rate, surface area)
- Brown et al., (1997) (humans and common laboratory species)
- Mirfazaelian and Fisher (2007) (maturing male Sprague-Dawley rat)
- International Commission on Radiological Protection (ICRP) (2002) (human)
- PopGen (virtual human population generator) (McNally et al., 2014)
- Physiological Parameters for PBPK Modeling (P3M) (Price et al., 2003) (human)
- Urinary flow rate (ml/hr or ml/hr/kg BW) by age, sex, race/ethnicity, and body mass index from National Health and Nutrition Examination Survey (2009-2012) data (Hays et al., 2015)

Scaling *In Vitro* or *In Silico* Data

- Types of data
  - Hepatocytes/g liver
  - Subcellular fractions (total protein, cytosolic protein, microsomal protein)
  - Enzyme/transporter abundance
  - Tissue/biological fluid composition (water, neutral lipids, lipoprotein, protein)

Sources of Parameters for Scaling *In Vitro* or *In Silico* Data

- SimCyp® (Jamei et al., 2009)
  - Human liver, kidney and intestinal microsomal protein
  - Human hepatocytes
  - Recombinant cytochrome P450 (CYP) and UDP-glucuronosyl transferase (UGT) enzymes
- Ontogeny of human drug-metabolizing enzymes (McCarver and Hines, 2002; Hines and McCarver, 2002; Upreti and Wahlstrom, 2016)
- Human liver microsomal protein content and CYP activity (Zhang et al., 2015)
- Rat and human hepatocyte number and microsomal protein yield, human liver CYP content (Lipscomb and Poet, 2008)

Sources of Parameters for Scaling *In Vitro* or *In Silico* Data

- Tissue/bodily fluid composition (e.g., for partition coefficients)
  - Poulin and Haddad (2012) and references therein
  - Duck (1990) *Physical Properties of Tissue*.
    - Tissue and bio-fluid water, ash, lipid, and protein percentages for humans; percentage water content of animal tissues

Chemical-Specific ADME Predictions *In Silico*

- Simcyp® (Jamei et al., 2009)
  - Solubility, absorption rate, pKa, permeability, fraction unbound in plasma
- Poulin and co-workers (Poulin and Haddad, 2012 and references therein)
  - Tissue:plasma partition coefficients

Simulation of Variability

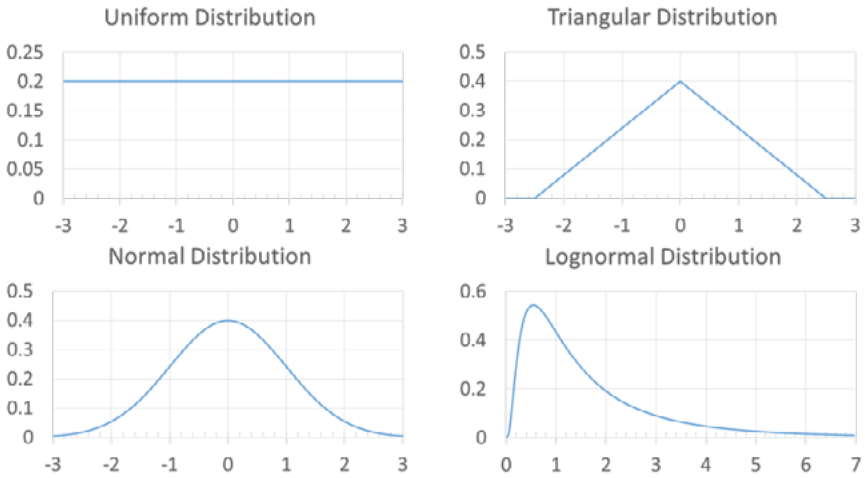
- Why
  - Simulation of special subpopulations
  - General population
    - Predict variation in internal dosimetry for the same exposure
    - Predict a range of exposure estimates via reverse dosimetry
- How
  - Deterministic
    - Central tendency parameters for different life stages, etc.
  - Monte Carlo simulation (parameter distributions)

Monte Carlo Simulation

- Rely on random number generators to select values across distributions
- Number of runs required depends on level of precision needed (e.g., 95^{th} vs. 99^{th} percentile)
- Model structure and parameterization considerations
  - Mass balance for regional (organ) blood flows
  - Ventilation/Perfusion ratios
  - Covariates
    - Age/body weight with organ weights, ventilation rates, cardiac output
    - Sex/gender with body weight, body composition, enzyme abundance

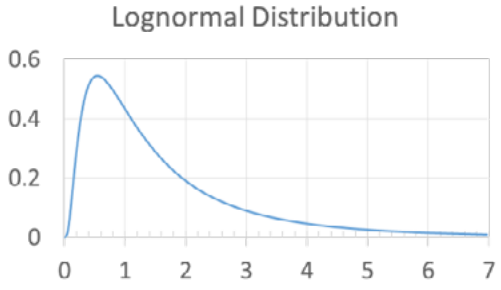
Monte Carlo Simulation

- Parameter distributions
  - When possible, should reflect known shape for the population
  - Genetic polymorphisms could be multimodal



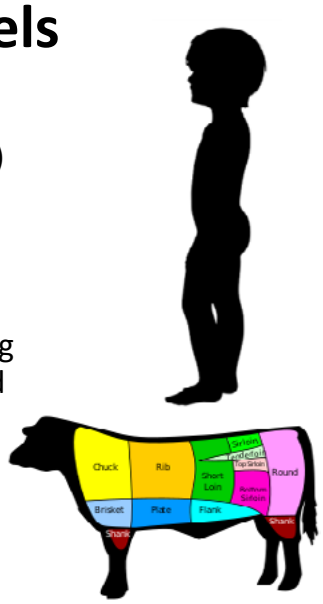
Monte Carlo Simulation

- Parameter distributions
  - Truncation
    - Particularly important for maintaining mass/flow balance



Examples of Applications of Monte Carlo Simulation of PBPK Models

- Inter-individual variability (numerous examples)
  - Identification of sensitive subgroups (e.g., young children)
  - Comparison to default inter-individual uncertainty factors
  - Comparison to variability in experimental data
- Food safety assessment
  - Prediction withdrawal times to reduce 99th percentile drug concentrations (penicillin G) in edible tissues of swine and cattle to established tolerances (Li et al., 2017)

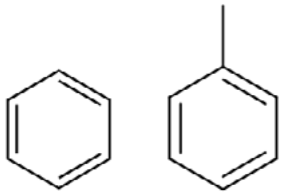


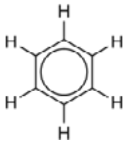
Examples of Applications of Monte Carlo Simulation of PBPK Models

- Uncertainty reduction in chemical incident exposure reconstruction
  - Quantified how prompt collection of biomonitoring data and individual physiological data reduce uncertainty in external exposure estimates (Huizer et al., 2014)
- Selection of optimal sampling times
  - Sampling times in drug pharmacokinetic study design were selected to minimize relative standard errors for parameters of interest (Dumont et al., 2013)
  - Guidance was provided for selection of sampling times for optimal interpretation of biomarkers of time-varying occupational exposure and workload (Verner et al., 2012)

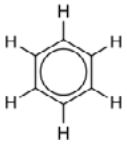
Monte Carlo Simulation Example: Internal Dose Metric Variability

- We will consider a subset of the many scenarios considered by Valcke and Haddad (2015)
- Four life-stages: Adults (18-64 yrs), neonates (0-30 days), toddlers (1-3 yrs), and pregnant women (15-44 yrs)
- Benzene exposure: 0.094 ppm
  - Without (baseline) or with co-exposure to 50 ppm toluene (competitive inhibition)



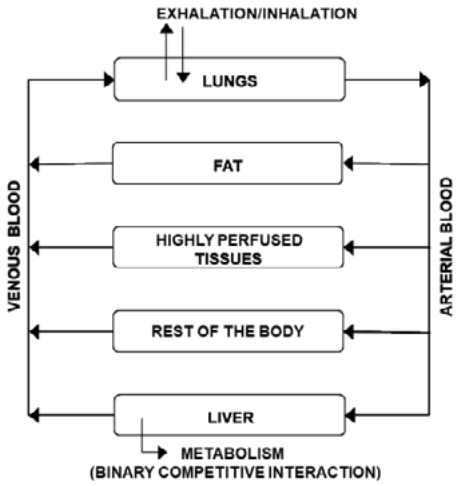


Monte Carlo Simulation Example: Internal Dose Metric Variability



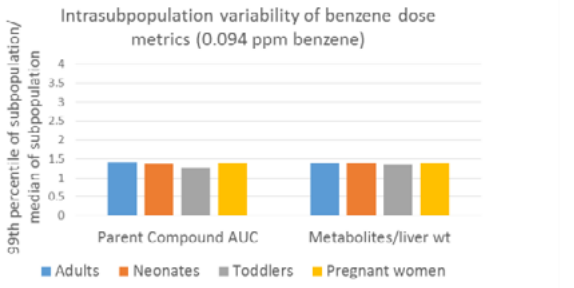
- Different benzene dose metrics
  - Parent compound
    - 24 hr area under the concentration vs. time curve for arterial
  - Metabolites
    - Amount metabolized in 24 hrs, normalized to liver volume
- Two measures of variability
  - Intra-subpopulation Variability Index (ISVI): subpopulation 99th percentile value/subpopulation median
  - Inter-population Variability Index (IPVI): subpopulation 99th percentile value/adult population median

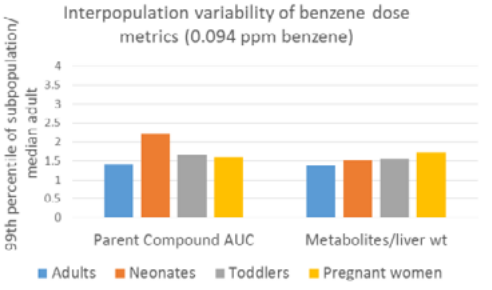
Monte Carlo Simulation Example: Internal Dose Metric Variability



- Subpopulation specific fixed data
  - Median age
  - Relative CYP2E1 activity
- Parameters varied for Monte Carlo simulations
  - Body height and weight distributions from P^3M (60% correlated)
  - Adult CYP2E1 variability (pmol/mg liver microsomal protein)
  - Alveolar ventilation and blood flow variability (13%; normal distribution)
  - Liver volume variability (19%; normal distribution)

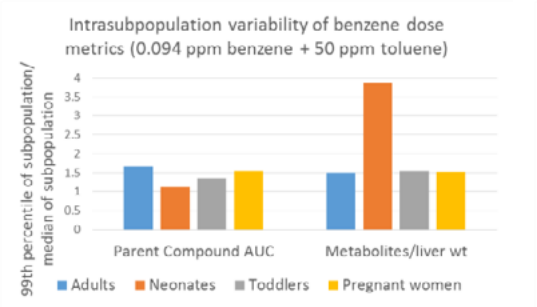
Monte Carlo Simulation Example: Internal Dose Metric Variability for Benzene Alone

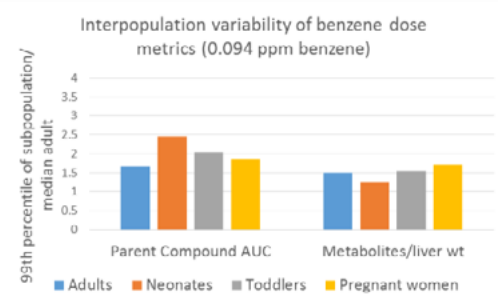


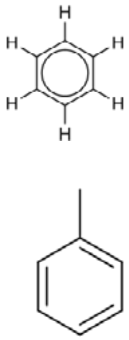


- Variability within subpopulations: similar across age groups and for dose metrics
- Key difference: 99th percentile neonate has elevated parent compound in blood

Monte Carlo Simulation Example: Internal Dose Metric Variability for Benzene with Toluene







- Variability within subpopulations: metabolites in neonates highly variable
- Key difference: in the presence of high toluene, benzene metabolism is inhibited, most notably in neonates

Conclusions

- Application-specific “fit for purpose assessments” are increasingly conducted with minimal reliance on *in vivo* toxicokinetic data
- Assessment quality will be dependent on the suitability and quality of databases of exposure factors, anatomical data, physiological data, biochemical data (for scaling), and chemical-specific absorption, distribution, metabolism, and excretion (ADME) data.

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Examples of the Use of Physiologically-Based Pharmacokinetic Models in Support of *In Vitro* Based Safety Assessment

Hands-On Demonstration of a Work Flow

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Conflict of Interest Statement

The author declares no conflict of interest.

Abbreviations

PBPK: physiologically-based pharmacokinetic modeling

IVIVE: *in vitro* to *in vivo* extrapolation

QIVIVE: quantitative *in vitro* to *in vivo* extrapolation

POD: point of departure

Vmax: maximal metabolism rate

Km: affinity constant

Clint: intrinsic clearance

DDI: drug-drug interaction

HTS: high-throughput screening

MOE: margin of exposure

MOA: mode-of-action

MPPGL: microsomal protein per-gram liver

CPPGL: cytosolic protein per-gram liver

HPGL: hepatocyte cellularity per-gram liver

ISEF: inter-system extrapolation factor

TSCA: The Toxic Substances Control Act

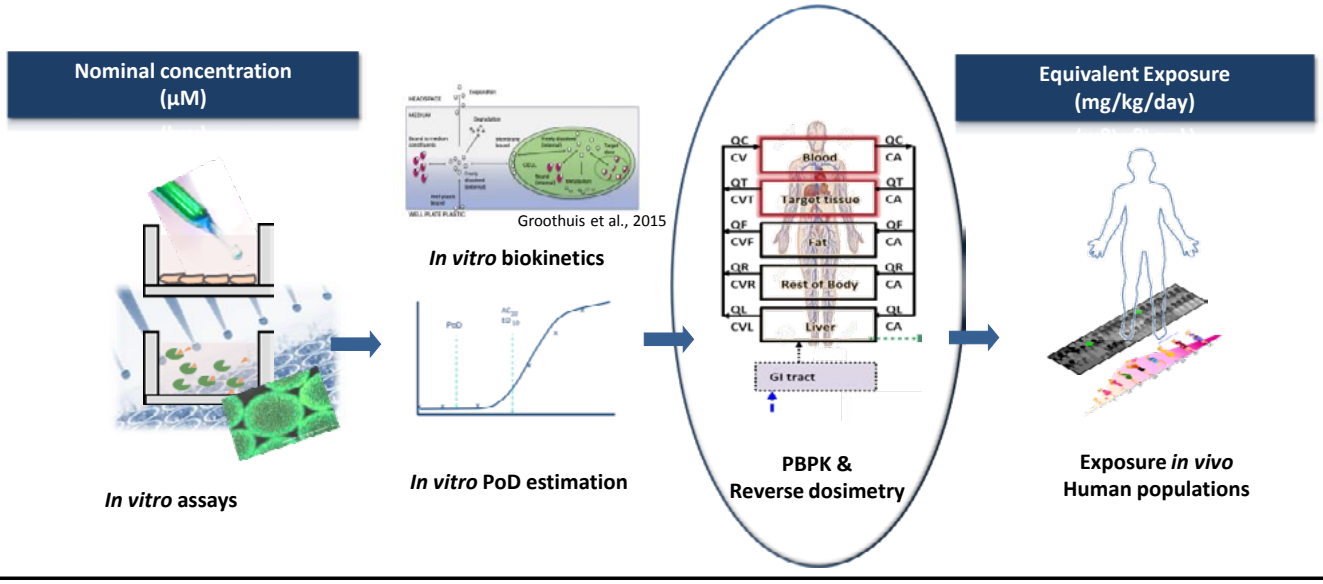
Outline

- Introduction to IVIVE as a parameterization approach
- Fundamental basis of IVIVE for kinetics
- Demonstration of IVIVE work flows
- Application examples
  - MoE analysis using HTS data
  - Quantitative interpretation of *in vitro* testing results (QIVIVE)
  - Early-life sensitivity for sensitive population risk assessment

PBPK Modeling Is a Key Component in *In Vitro*-Based Safety Assessment

- ***In vivo*** toxicity is affected by **pharmacokinetic factors**, but they are not appropriately reflected in ***in vitro*** toxicity tests
- Data on kinetic processes—absorption, distribution, metabolism and excretion (ADME)—in humans can be generated using *in vitro* and *in silico* methods
- PBPK modeling serves as an integration and translation tool as a quantitative bridge from *in vitro* to *in vivo* for safety decisions

Translation of *In Vitro* Toxicity Testing Results to Safe Human Exposure



In Vitro to *In Vivo* Extrapolation (IVIVE)—Predicting *In Vivo* Kinetics Based on *In Vitro* Data

- Drug discovery
  - DDI
  - Model-based drug development
- Quantitative risk assessment
  - Early-life sensitivity
  - Cross-species extrapolation
- Safety assessment based on alternative approach-based tiered testing strategies
  - Quantitative interpretation of HTS assay results (MoE analysis)
  - QIVIVE to estimate safe human exposure from *in vitro* dose-response studies

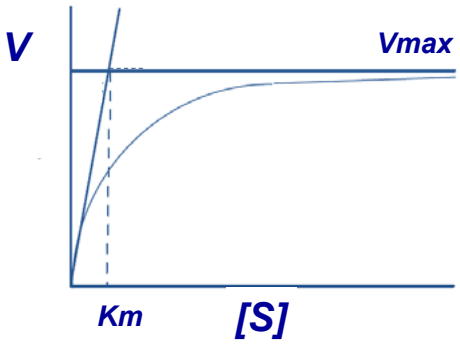
PBPK Model Parameterization with IVIVE

- The bottom-up PBPK modeling has been used in environmental chemical risk assessment started with volatiles and later on for others including pesticides (Clewell, 1993; Clewell and Andersen, 1996; Kedderis and Lipscomb, 2001; Hinderliter et al., 2011; reviewed in Yoon et al., 2012)
- The *in vitro*-based parameterization strategy has been well accepted for generic PBPK modeling for pharmaceutical compounds (e.g., Simcyp, Simulation Plus, PKSim etc.)
- Because of a broader range of chemical properties for environmental chemicals compared to pharmaceuticals, the applicability of IVIVE has been evaluated specifically for environmental chemicals (Rotroff et al., 2010; Wetmore et al., 2013; Yoon et al., 2014)

Basis for Extrapolation of *In Vitro* Metabolic Constants to *In Vivo*

- Rate of enzyme reaction is directly proportional to the total amount of enzyme present in the system
- Data generated with an *in vitro* system therefore can be extrapolated to *in vivo* by relating:
  - the total amount of enzyme present in the system (capacity of metabolism)
  - the available substrate concentration for enzyme reaction (affinity of metabolism)
- Physiological factors affecting metabolism (blood flow and tissue volume) are already accounted for by the PBPK model

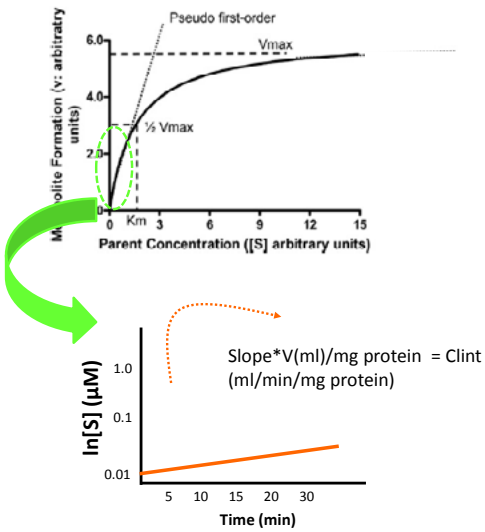
Kinetics of Metabolism



$$V = \frac{Vmax[S]}{(K_M+[S])}$$

Most chemical metabolism is explained by saturable M-M kinetics

Determining Metabolism Parameters *In Vitro*



- **Vmax and K_M** from full enzyme kinetic assays to simulate
  - high-dose conditions (e.g., animal toxicity studies)
  - Parent and metabolite kinetics
- **Clint** by measuring parent chemical disappearance over time (assuming [S]<<Km) to simulate
  - typical environmental exposure conditions in humans
  - parent compound kinetics only

At low [S], M-M eq becomes

$$V = \frac{Vmax}{K_M}[S]$$

Cl_{int}

Extrapolation of *In Vitro* Metabolic Constants

Vmax (Capacity of metabolism)

- Specific activity = $\frac{enzyme\ activity}{unit\ enzyme\ content}$

  e.g., nmol/min/mg protein
- Total activity in the system =

  specific activity * total enzyme content in the system

  same — different

  Scaling required!

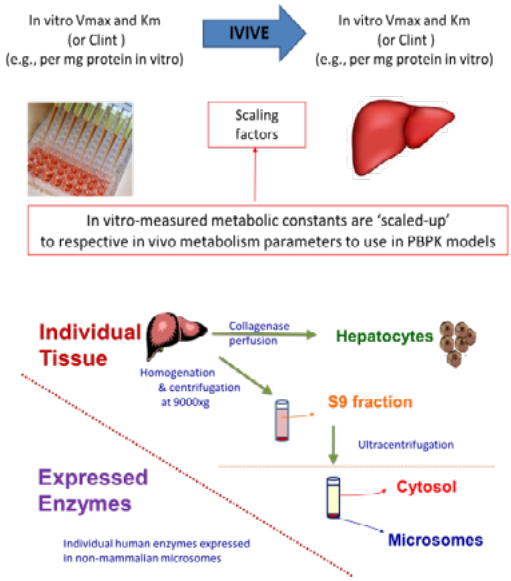
  between in vivo and in vitro

Km (Affinity of metabolism)

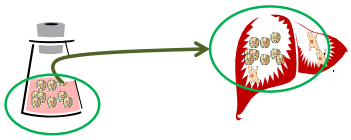
- Only free compounds are available for enzyme reactions regardless of the system
  - Effects of *in vitro* kinetic factors (Groothuis et al., 2015)
  - Effects of system differences in substrate availability to enzymes, e.g., microsomal binding vs. plasma protein binding etc. (Teeguarden and Barton, 2004;Yoon et al., 2012;2013)
- Also applies to Clint scaling as Clint=Vmax/Km, this also applies to scaling of Clint

Biological Scaling for Different *In Vitro* Systems

System	Unit of Vmax or Clint in the System	Scaling factors to scale up to whole liver
Microsomes	nmol/min /mg protein	MPPGL x Liver weight
Cytosol	nmol/min /mg protein	CPPGL x Liver weight
Expressed Enzyme	µl/min /pmol enzyme	(ISEF x$Enz_{abundance}$) x MPPGL x Liver weight
Hepatocyte	µl/min /10^6 cells	HPGL x Liver weight
Liver	nmol/min/g liver	Liver weight
Whole Body	nmol/min/whole liver	



IVIVE of Metabolic Constants from Primary Hepatocytes



Hepatocellularity in intact liver

HPGL = # of hepatocytes/g liver

$Vmax_{in\ vivo}$ (µmol/hr) =

$Vmax_{hepatocyte}$ (µmol/hr /# of cells)
x **HPGL (# of cells/g liver)**
x **Liver weight (g)**

Calculation step	Unit	
19.0	nmol/hr/10^6 cells	$Vmax_{\ in\ vitro}$
x 137	10^6cells/g liver	HPGL (Arias et al., 1982)
x 0.026 x 70 x 10^3	g	Liver weight 2.6 % of BW, BW=70kg
/ 10^3	µmol/nmol	nmol to µmol
= 4737	**µmol/hr**	**$Vmax_{\ in\ vivo}$**

Example: Oxidation of furan to cis-2-butene-1,4-dial in human hepatocytes

(Kedderis & Held, 1996)

Keeping Up with New Technologies

- The promise of advanced cell culture models and multi-organ cultures in toxicity testing to increase predictivity of *in vitro* models for potential health effects (e.g., fit-for-purpose assays for higher tier safety decisions)

- To generate meaningful outputs for extrapolation to humans, these advanced cultures (e.g., body-on-chips) would need to be designed with consideration of biologically relevant scaling of each component in the system as well as whole entity

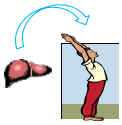
Extrapolation to *In Vivo*

Animals

Allometric scaling
physiological and biochemical parameters scaled by function of body size

Traditional *in vitro* systems

Biological scaling (IVIVE)
Relating intrinsic functions by accounting for inter system scale and microenvironment differences



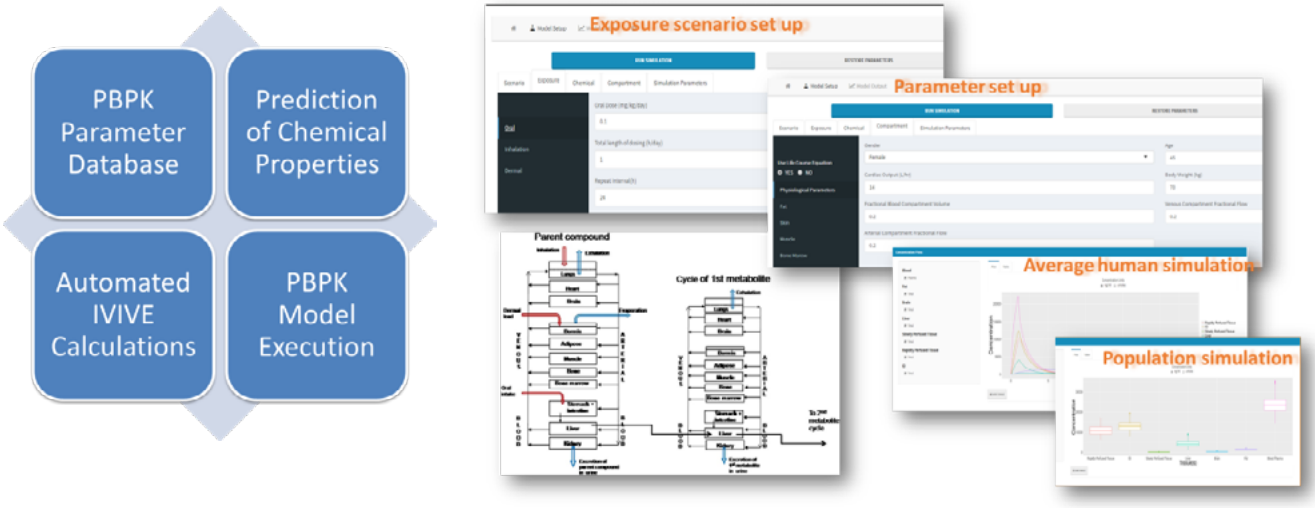
Organotypic culture, tissue-chips, bioreactor etc.

What would be the best way to scale?

Improving the 'Throughput' of PBPK Modeling

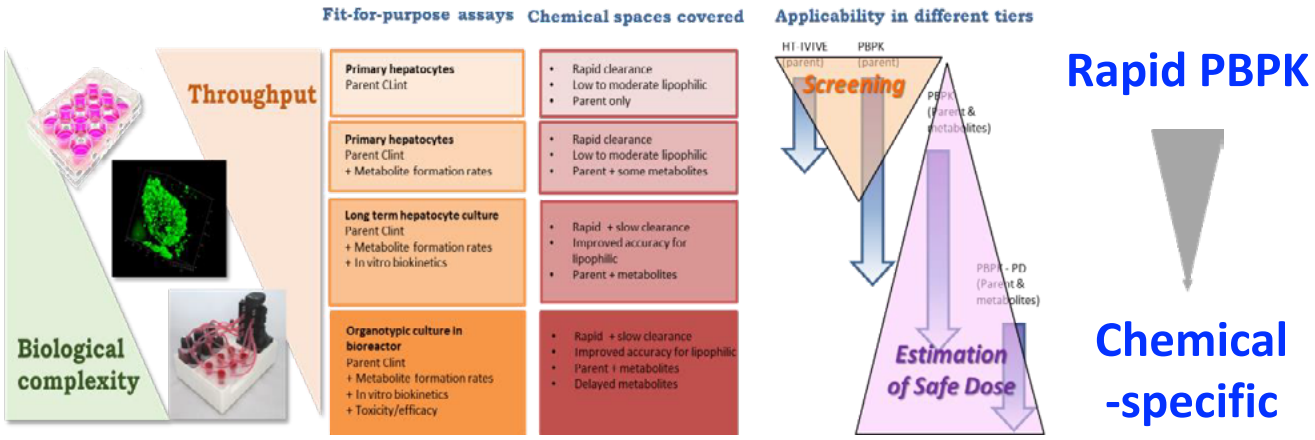
- Development of PBPK models is regarded as expensive and time/resource intensive task
- Advances with *in silico*/*in vitro*-based tools, particularly those for distribution and hepatic clearance enabled the emergence of 'ready to use' or 'generic' PBPK software tools (e.g., SimCyp, GastroPlus for drugs)
- A significant surge in the development of generic PBPK platforms for environmental chemicals in support of new toxicity testing and regulatory needs (e.g., new TSCA)

Generic PBPK Platform Components and Capabilities Population Life-Course Exposure to Health Effect Modeling Platform (PLETHEM) as an Example



Jongeneelen and ten Berge, 2011, extended in PLETHEM

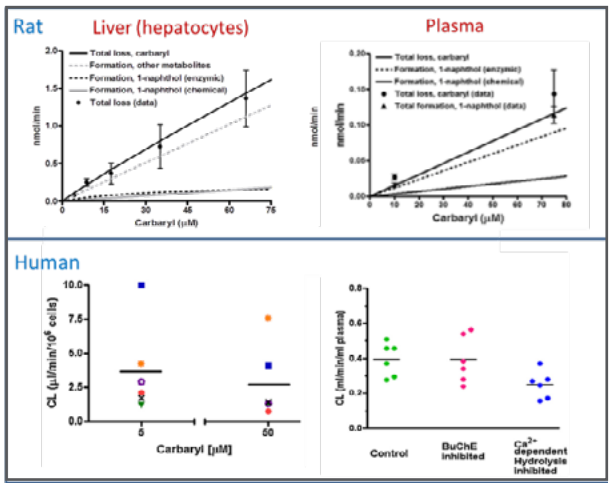
Domains of Applicability Driven by *In Vitro* Data—Serving Different Purposes for Safety Assessment

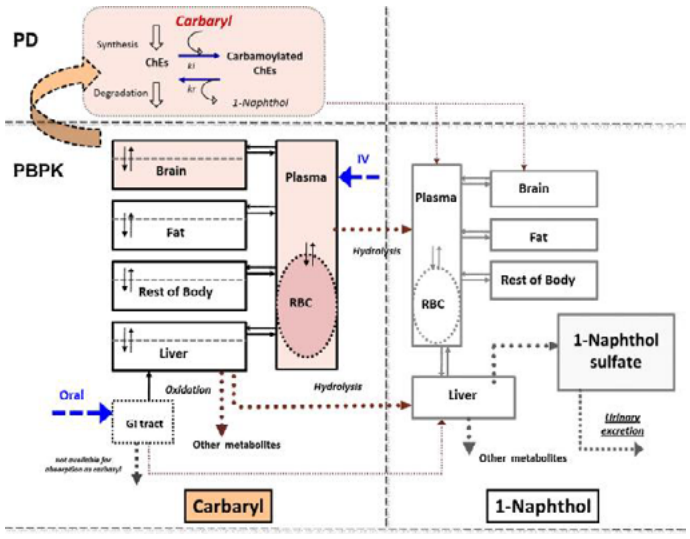


IVIVE for PBPK Model Parameterization

Carbaryl PBPK/PD Model

In vitro data provides key PBPK parameters of metabolism





(Yoon et al., 2011 and 2015a)

IVIVE for PBPK Model Parameterization

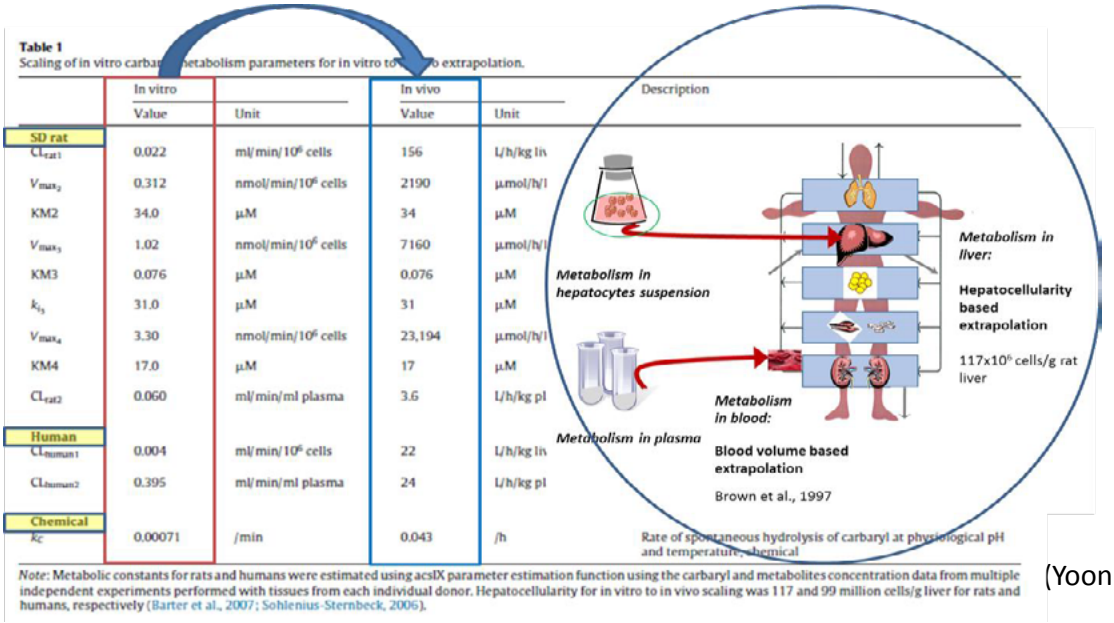


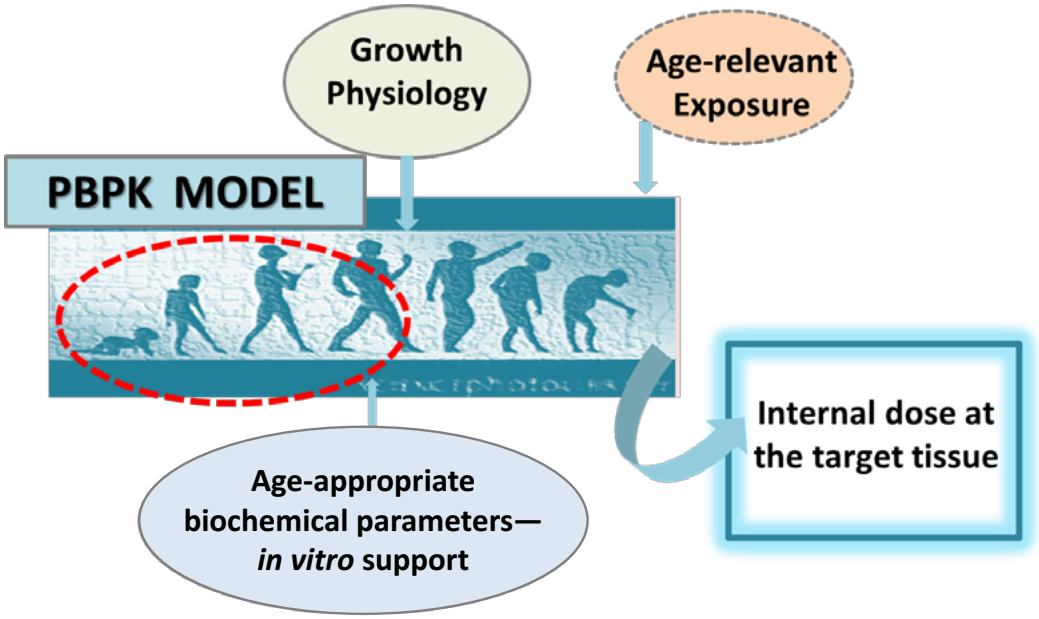
Table 1
Scaling of in vitro carba[illegible]metabolism parameters for in vitro to [illegible]o extrapolation.

	In vitro Value	In vitro Unit	In vivo Value	In vivo Unit	Description
SD rat					
Cl_{rat1}	0.022	ml/min/10^6 cells	156	L/h/kg liv	
V_{max_2}	0.312	nmol/min/10^6 cells	2190	µmol/h/l	
KM2	34.0	µM	34	µM	
V_{max_3}	1.02	nmol/min/10^6 cells	7160	µmol/h/l	
KM3	0.076	µM	0.076	µM	
k_{i_3}	31.0	µM	31	µM	
V_{max_4}	3.30	nmol/min/10^6 cells	23,194	µmol/h/l	
KM4	17.0	µM	17	µM	
Cl_{rat2}	0.060	ml/min/ml plasma	3.6	L/h/kg pl	
Human					
Cl_{human1}	0.004	ml/min/10^6 cells	22	L/h/kg liv	
Cl_{human2}	0.395	ml/min/ml plasma	24	L/h/kg pl	
Chemical					
k_C	0.00071	/min	0.043	/h	Rate of spontaneous hydrolysis of carbaryl at physiological pH and temperature; chemical

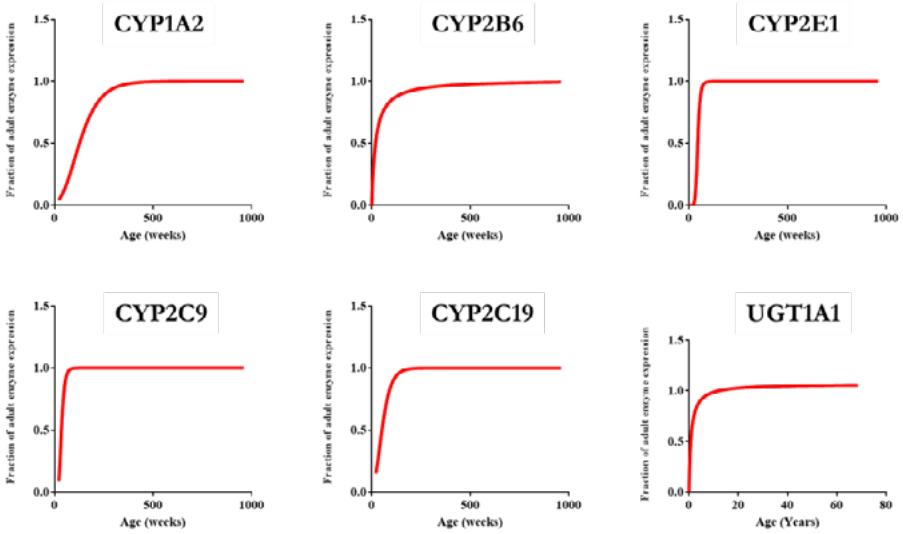
Note: Metabolic constants for rats and humans were estimated using acslX parameter estimation function using the carbaryl and metabolites concentration data from multiple independent experiments performed with tissues from each individual donor. Hepatocellularity for in vitro to in vivo scaling was 117 and 99 million cells/g liver for rats and humans, respectively (Barter et al., 2007; Sohlenius-Sternbeck, 2006).

(Yoon et al., 2015a)

IVIVE and PBPK for Early-Life Risk Assessment



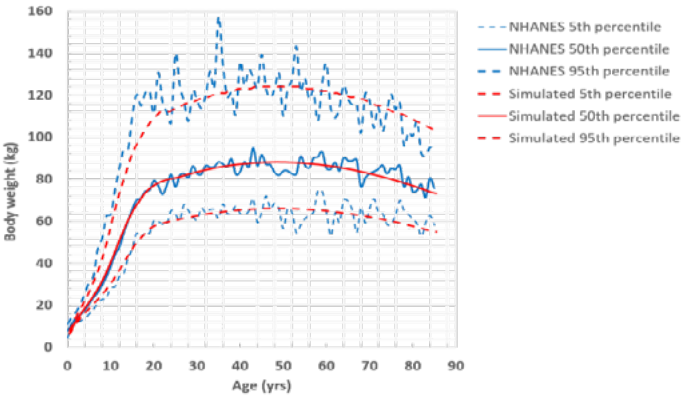
Using Enzyme Ontogeny to Define Age-Dependent Metabolism Parameters



Data for the shown examples are from McCarver et al., 2017

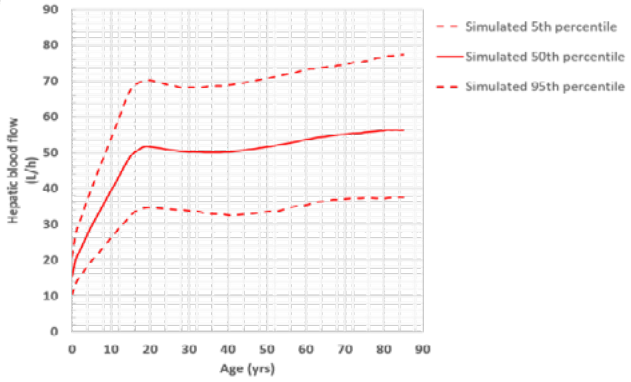
Incorporating Age-Dependent Changes in Physiology

Body Weight



(Human life stage-specific physiological parameter database used in PLETHEM)

Liver Blood Flow



Expressed Enzyme-IVIVE for Early-Life PBPK Modeling

- Reducing reliance on pediatric tissue samples
  - Even more limited availability
  - Questionable quality
  - Large donor-to-donor variability
- An efficient and reliable alternative to estimate *in vivo* metabolic parameters for early life PBPK models when combined with enzyme ontogeny (age-related changes in abundance) information

IVIVE Workflow with Expressed Enzymes

1. **Screening active enzymes** to a given compound metabolism using CYP and other enzyme panels
2. **Measuring Clint** *in vitro* for each involved enzyme
3. **Extrapolation to *in vivo* adult Clint** and then across ages using appropriate scaling factors

   Abundance in adult + Ontogeny (as fraction of adult)

   ↓ Relative expression levels of each enzyme in adult liver

   ↓ Age-dependent changes in each enzyme expression and its relative abundance
4. **Integration to obtain total liver Clint for a given age** in a PBPK model

IVIVE Predicts *In Vivo* Metabolic Clearance across Ages

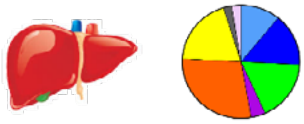
Expressed enzyme Clint

CYP2C9
CYP2B6
CYP2C19
CYP3A4
CES1-m
CES1-c
CES2-m
CES2-c

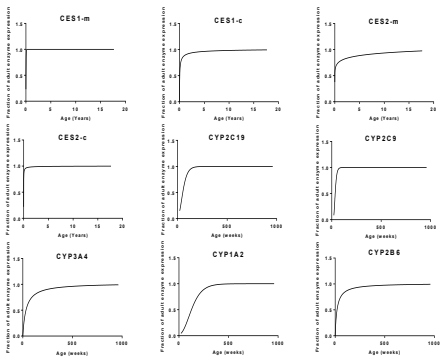
ISEF × MPPGL × Liver Weight × Enzyme abundance

↓ IVIVE

Adult hepatic Clint

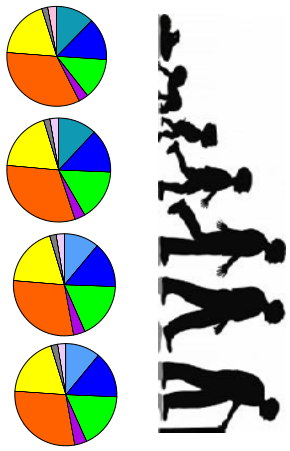


Enzyme ontogeny



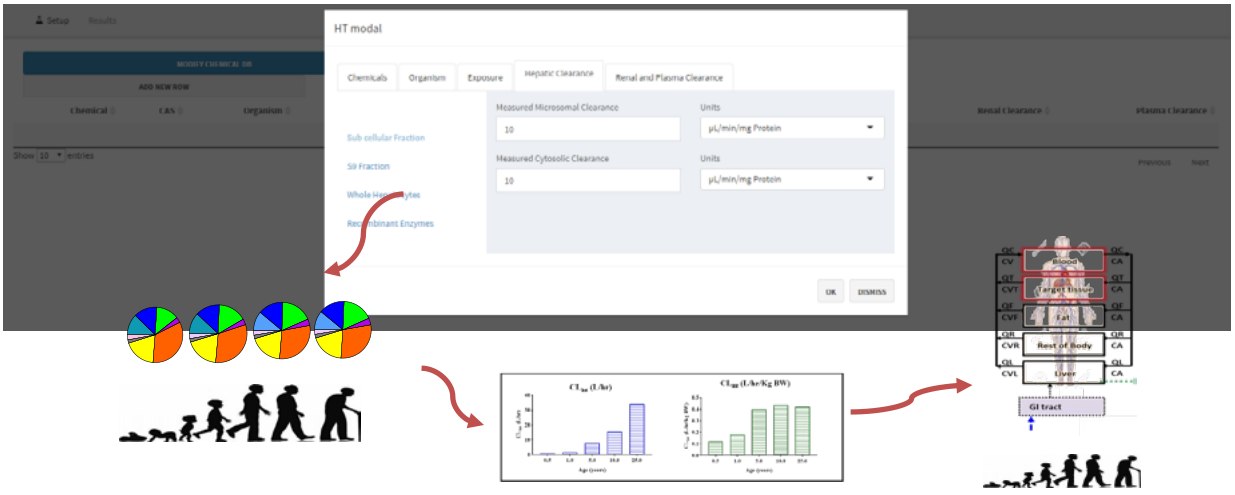
(McCarver et al., 2017)

Life-stage hepatic Clint



*This example does not represent a specific compound

IVIVE Module Generates Life-Stage *In Vivo* Metabolism Parameters for Early-Life PBPK Modeling



*This example does not represent a specific compound

Supporting Risk Assessment for Sensitive Populations

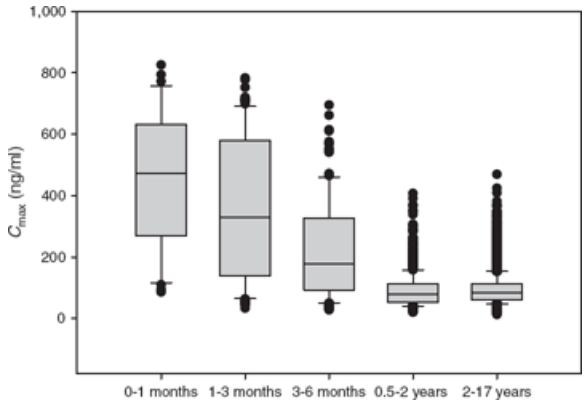


Figure from Maharaj and Edginton, 2014

Derivation of a Chemical-Specific Adjustment Factor (CSAF)for early age PK difference

$$CSAF_{age\_PK} = \frac{\boldsymbol{Juvenile\ Cmax}_{\mathbf{50\%\ percentile}}}{\boldsymbol{Adult\ Cmax}_{\mathbf{50\%\ percentile}}}$$

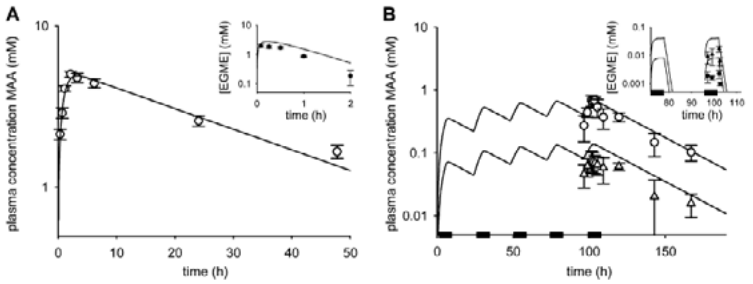
Examples of QIVIVE Applications

Predicting *In Vivo* Dose-Response for Glycol Ethers Based on *In Vitro D*ose-Response

In vitro measured developmental toxicity of active metabolites

PBPK model predicted both parent and metabolite kinetics

QIVIVE-PBPK used to predict *in vivo* dose response from *in vitro* dose-response



Ethylene glycol monomethyl ether (EGME) and its metabolite, methoxyacetic acid (MAA) kinetics (adapted from Figure 2. in Louisse et al., 2010)

(Examples from Louisse et al., 2010,2012)

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A Tiered Approach to Incorporate Exposure and Pharmacokinetic Considerations in *In Vitro*-Based Safety Assessment

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Conflict of Interest Statement

Neither myself nor any of my coauthors, including members of our immediate families, have any financial interest or affiliation with a commercial organization that has a direct or indirect interest in the subject matter of my presentation.

Disclaimer

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the US EPA.

Abbreviations

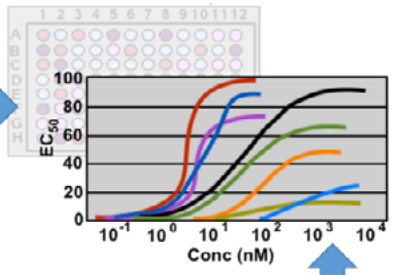
PK: Pharmacokinetic
PBPK: Physiologically-Based Pharmacokinetic
IVIVE: *in vitro* to *in vivo* extrapolation
PD: Pharmacodynamic
ADME: Absorption, distribution, metabolism, excretion
AChE: Acetylcholinesterase
TPO: Thyroid peroxidase
TSH: Thyroid-stimulating hormone
TRH: Thyrotropin-releasing hormone
T3: triiodothyronine
T4: thyroxine

Course Objectives/Outline

- Integrating exposure, PK, and hazard considerations in current risk assessment strategies
  - Comparing exposures in different systems
  - Converting *in vitro* exposure to *in vivo* exposure
- Demonstrating a tiered approach for incorporating PK properties in safety assessment approaches
  - Example 1: qualitative screening approach
  - Example 2: quantitative ranking approach
  - Example 3: metabolism consideration

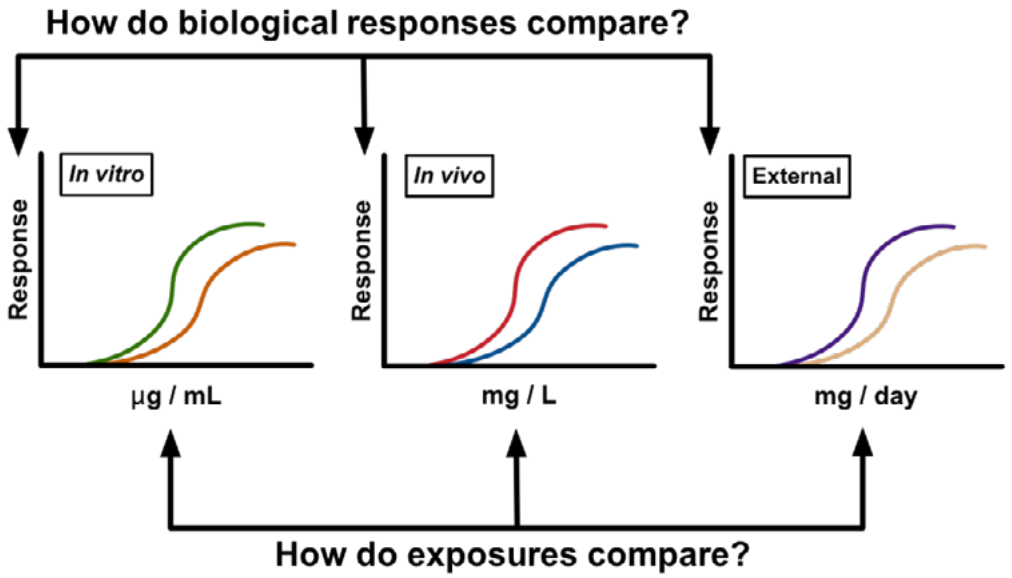
A Shift in Risk Assessment towards the Use of Alternative Test Methods

in vitro perturbations should be interpreted in terms of *in vivo* molecular interactions, which causally link to the resulting health outcomes in a whole organism or a population



in vitro doses should be converted to *in vivo* concentrations at the target tissue, or at the external level for comparison to exposure levels in a population

Exposures in Different Systems



Typical Applications of PBPK Modeling

- Predict dose metric under new or inaccessible conditions
  - High- to low-dose extrapolation
  - Route to route extrapolation
  - Animal to human extrapolation
  - Average to sensitive population extrapolation
  - Additional exposure scenarios
- Quantify variability and uncertainty in physiology and PK
- Interpret biomonitoring data

High Confidence in Models Is Required for Typical Risk Assessment Applications

- "PBPK models intended for use in risk assessment should be evaluated to ensure they provide simulations of pharmacokinetic profiles consistent with the experimental data" (US EPA 2006)
- "The level of confidence in PBPK models intended for specific end use in risk assessment should be characterized on the basis of comparison of model simulations with experimental data" (WHO 2010)

Increasing Use of *In Vitro*-Based PBPK Models

As risk assessment increases its focus on alternative testing, PBPK models are parameterized using *in vitro*/*in silico* data for data-poor chemicals

***In vivo*-based PBPK models**	***In vitro*-based PBPK models**
Mode-of-action, dose metric, and toxic moiety are often known from *in vivo* studies	Mode-of-action, dose metric, and toxic moiety are often challenging to interpret from *in vitro* studies
Model structure reflects a balance between the principles of parsimony and plausibility	Model structure reflects mechanistic understanding of physiology and biokinetics
Model calibration often relies on *in vivo* data	Model parameterization relies on *in vitro*/*in silico* data
Confidence in a model depends on the model's ability to reproduce independent *in vivo* data	Confidence in a model depends on the basis of scientific principles and the quality of input parameters
Primary application is extrapolating from species/conditions with *in vivo* data to species/conditions without *in vivo* data	Primary application is linking external exposures to internal exposures

Expanding Applications of PBPK Modeling

- Organize mechanistic data and present state of knowledge
- Identify data gaps and suggest new experiments
- Provide quantitative characterization of PK to be integrated with *in silico*/*in vitro*-based exposure and hazard data for chemical screening and prioritization (Berggren et al., 2017)

In Vitro to *In Vivo* Extrapolation (IVIVE)

- IVIVE often refers to approaches for estimating PK parameters (Chen et al., 2012; Cho et al., 2014; Jamei et al., 2014; Yoon et al., 2016)
  - scaling *in vitro* intrinsic clearance or enzyme kinetic data to *in vivo* intrinsic clearance
  - converting *in vitro* transporter kinetics to *in vivo* transporter effects
  - estimating *in vivo* absorption rates from *in vitro* systems
- IVIVE is also defined as an approach that uses *in vitro* data to predict *in vivo* phenomena (Yoon et al., 2015; Bell et al., 2017)
  1. Using an *in vitro* system to estimate the active concentration
  2. Relating an equivalent blood or tissue concentration to an external dose using PBPK or PK models

Available Data Drive Modeling Approaches—PBPK Models Are Not Required All the Time

- Qualitative screening–investigating the potential for a chemical to reach an *in vivo* target
- Quantitative ranking–predicting *in vivo* bioactivity based on exposure, PK, and *in vitro* bioactivity
- Considering metabolism–metabolism relates to both clearance of a parent and potential activation of a metabolite

Example 1—Qualitative Screening

- Identify false positives from *in vitro* active chemicals: chemicals that have no exposure potential to access the molecular target
- Identify false negatives from *in vitro* inactive chemicals: bioactive chemicals that are not detected in an *in vitro* system, or inactive parent compounds of active metabolites

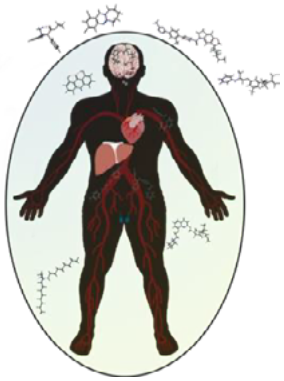
Example 1—Identifying False Positives

Exposure
1) Literature search
2) Database
3) Predictions

Absorption
1) Physicochemical properties
2) 100% absorbed

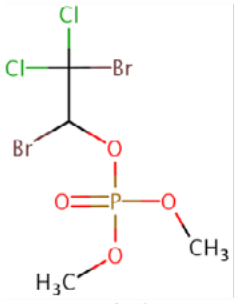
Distribution
1) Physicochemical properties
2) Biological barriers

High Priority



Phillips, Leonard et al., (2016)

Example 1—Identifying False Negatives



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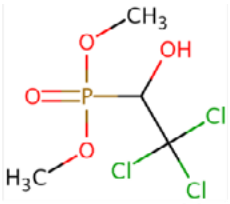
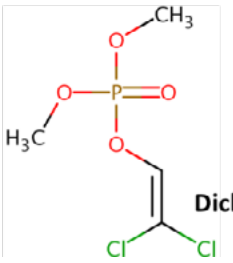


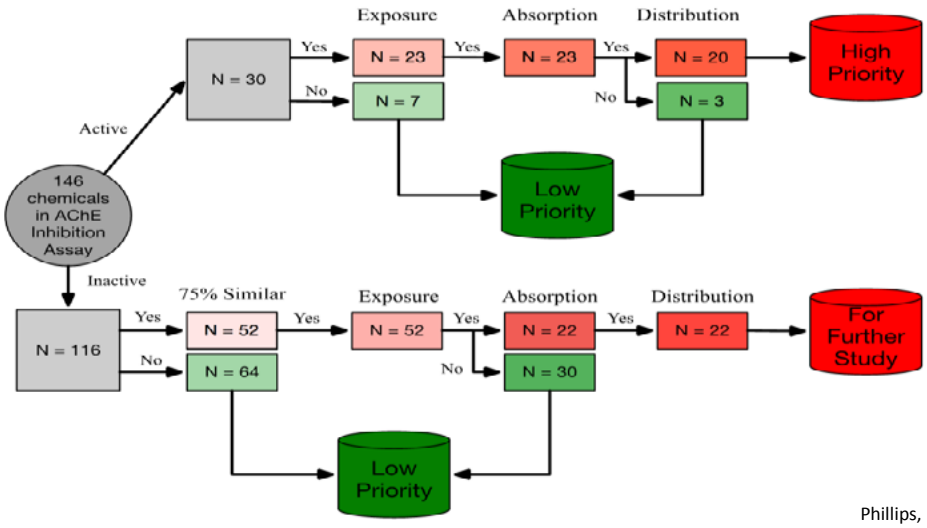
Trichlorfon (86% similar)



Dichlorvos (83% similar)

Phillips, Leonard et al., (2016)

Example 1—Qualitative Screening of Acetylcholinesterase Inhibitors

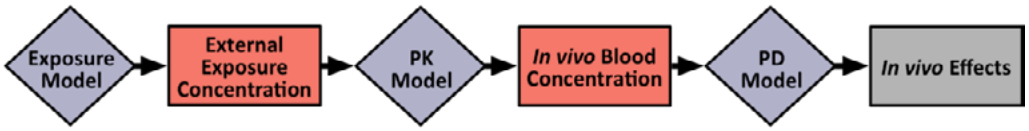


Phillips, Leonard et al., (2016)

Example 2—Quantitative Ranking

- Data-poor chemicals can be ranked according to their estimated *in vivo* bioactivity by incorporating exposure and PK predictions, along with *in vitro* bioactivity data
- A PK/PD model or a PBPK/PD model may be used depending on data availability and study purpose
- Sensitivity of model parameters should be examined across possible ranges of all parameters to better characterize the impact of their uncertainty on the model outputs

Example 2—A PK/PD Model for Acetylcholinesterase Inhibiting Chemicals



$$\frac{dC_P}{dt} = \frac{1}{V_b}\left[Dose_P - \frac{Q_l\ Cl_{int}\ C_P}{Q_l + Cl_{int}} - (K_e\ C_P)\right]$$

$$\frac{dC_M}{dt} = \frac{1}{V_b}\left[Dose_M + Stoich_M\ \frac{Q_l\ Cl_{int}\ C_P}{Q_l + Cl_{int}} - (Cl_M\ C_M)\right]$$

$$A = \left[\frac{E_{Max(P)}\ C_{Avg(P)}}{EC_{50(P)} + C_{Avg(P)}}\right] + \left[\frac{E_{Max(M)}\ C_{Avg(M)}}{EC_{50(M)} + C_{Avg(M)}}\right]$$

- C_P: blood concentration of parent chemical (mg/L)
- C_M: blood concentration of metabolite (mg/L)
- V_b: blood volume (L)
- $Dose_P$: absorbed dose of parent chemical (mg/h)
- $Dose_M$: absorbed dose of metabolite (mg/h)
- Q_l: liver blood flow rate (L/h)
- Cl_{int}: intrinsic hepatic clearance rate of parent (L/h)
- K_e: renal clearance rate (L/h)
- $Stoich_M$: % active metabolite generated from overall metabolism
- C_{avg}: average plasma concentration of parent or metabolite after integrating over time
- E_{max}: half-maximal effective concentration of parent or metabolite
- A: biological activity

Leonard et al., (2016b)

Example 2—Sensitivity of a Parameter Depends on Values of Other Parameters

$$\frac{dC_P}{dt} = \frac{1}{V_b}\left[Dose_P - \frac{Q_l\ Cl_{int}\ C_P}{Q_l + Cl_{int}} - (K_e\ C_P)\right]$$

$$\frac{dC_M}{dt} = \frac{1}{V_b}\left[Dose_M + Stoich_M\ \frac{Q_l\ Cl_{int}\ C_P}{Q_l + Cl_{int}} - (Cl_M\ C_M)\right]$$

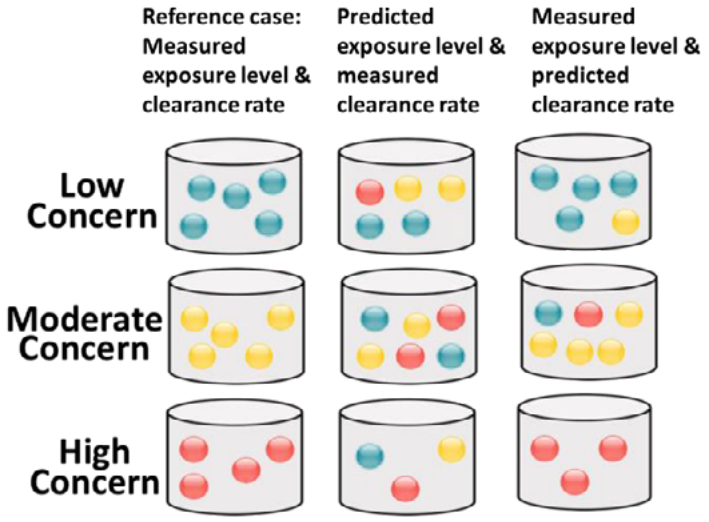
$$A = \left[\frac{E_{Max(P)}\ C_{Avg(P)}}{EC_{50(P)} + C_{Avg(P)}}\right] + \left[\frac{E_{Max(M)}\ C_{Avg(M)}}{EC_{50(M)} + C_{Avg(M)}}\right]$$

When intrinsic clearance >> liver blood flow, clearance is determined by liver blood flow

When average plasma concentrations >> EC_{50}, the bioactivity approaches E_{Max}

Leonard et al., (2016b)

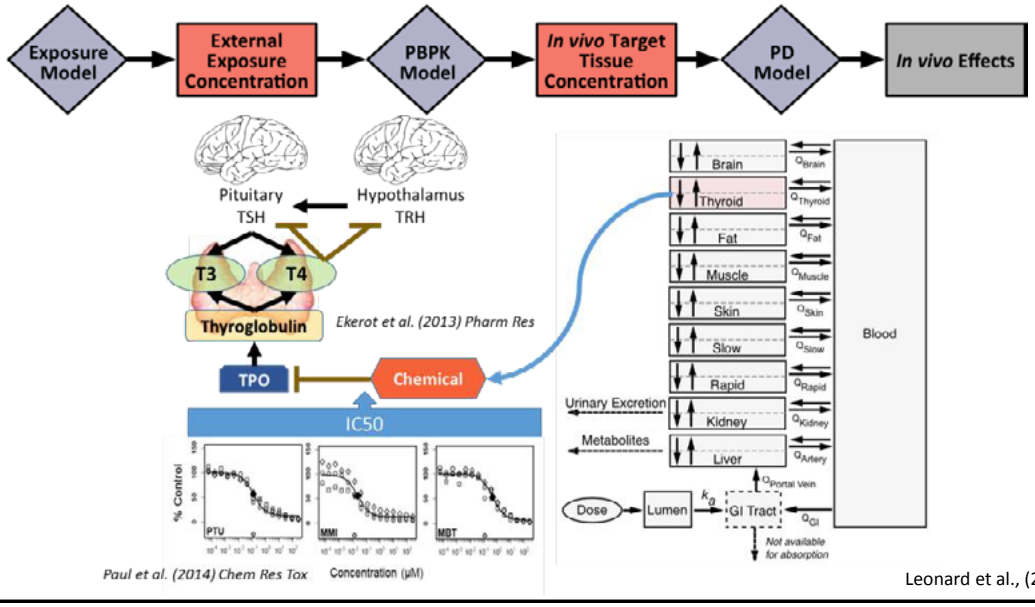
Example 2—Chemical Ranking



- A case study placed 30 AChE inhibiting chemicals into priority bins using a PK/PD model
- Using predicted exposure levels resulted in nearly twice the number of bin misassignment than when using predicted clearance rates
  - Clearance rate is constrained to physiological boundaries
  - Greater uncertainty with exposure estimates due to large differences in product use, human behaviors, etc.

Leonard et al., (2016b)

Example 2—A PBPK Model for Thyroid Peroxidase Inhibiting Chemicals



Leonard et al., (2016a)

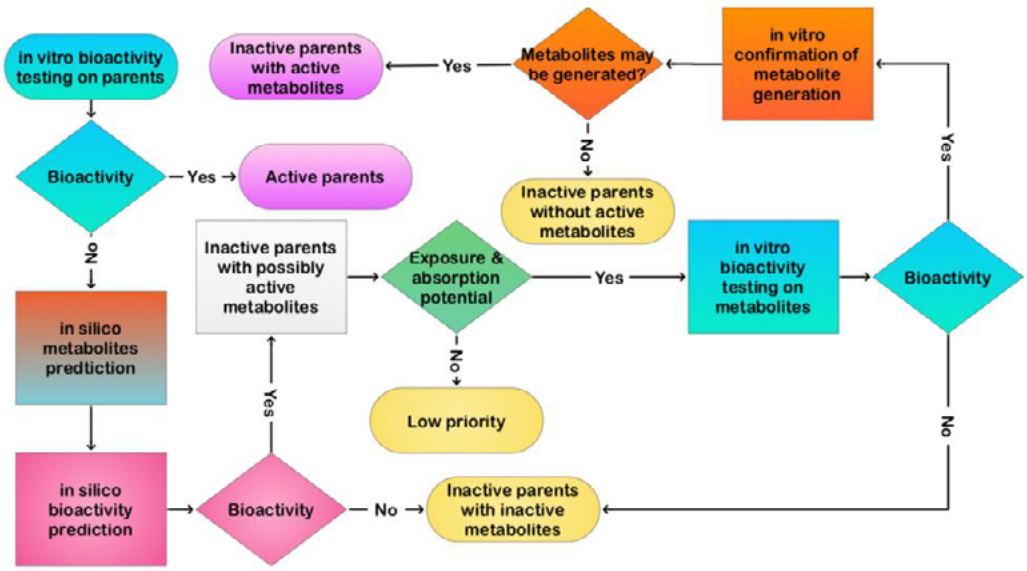
Example 2—Risk-Based Screening and Ranking Requires Consideration of Exposure, PK, and Potency

	Potency	Exposure	Exposure + PK	Exposure + PK + Potency
Methimazole				
6-Propylthiouracil				
Benzophenone-2				
2-Mercaptobenzothiazole				
Triclosan				
Resorcinol				

	High priority
	Medium priority
	Low priority

Leonard et al., (2016a)

Example 3—Considering Metabolism



Leonard et al., (2017)

Example 3—Identifying Inactive Parents That Are Likely to Have Active Metabolites

in vitro bioactivity Parent	*in silico* bioactivity Parent	*in silico* bioactivity Metabolite	*in vitro* bioactivity Metabolite	
+	+			Parent is likely to be active
+	—			Parent may be active
—	—	—		Parent/Metabolite likely to be inactive
—	+	—		Parent/Metabolite may be inactive
—	+	+	+ / —	Metabolite may be active
—	—	+	+ / —	Metabolite may be active

Leonard et al., (2017)

Take Home Messages

- As risk assessment increases its focus on alternative testing, *in vitro*-based PBPK models are being developed for data-poor chemicals
- Typical applications of PBPK models require evaluating model predictions against *in vivo* data; applications of *in vitro*-based PBPK models depend upon the degree of uncertainty allowed for a study purpose
- Incorporating PK with exposure estimates and potency data is essential for risk assessment; PBPK models are important tools but not always necessary, depending upon data availability

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Approaches for Evaluation of Non-Animal-Based Physiologically-Based Pharmacokinetic Models Including the Use of Human Biomonitoring Data

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Disclaimer and Acknowledgement

The views expressed in this presentation are those of the author and do not necessarily reflect the official policy or position of VITO

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Abbreviations

AH: animal-to-human

HBM: human biomonitoring

HVS: human volunteer study

NAB: non-animal-based

PBK: **human** physiologically-based kinetic (modeling)

RA: risk assessment

TK: toxicokinetics

k_a: absorption rate constant

K_m: Michaelis-Menten constant

V_{max}: maximal metabolism rate

Objectives

- What do we have?
- What are the difficulties?
- What is important?

Outline

- **Human PBK Modelling Recap**
  - Reparametrized animal-to-human (AH) **VERSUS** bottom-up non-animal-based (NAB)
- **Human Data**
  - occupational: HBM **VERSUS** HVS (mimicking occupational exposure)
  - general population: HBM **VERSUS** HVS (mimicking general population exposure)
- **Human Use**
  - to evaluate and improve NAB PBK models
  - to assess complex external exposure scenarios

Human PBK Models

Classical "animal-based"

versus

21st Century "non-animal-based" (NAB)

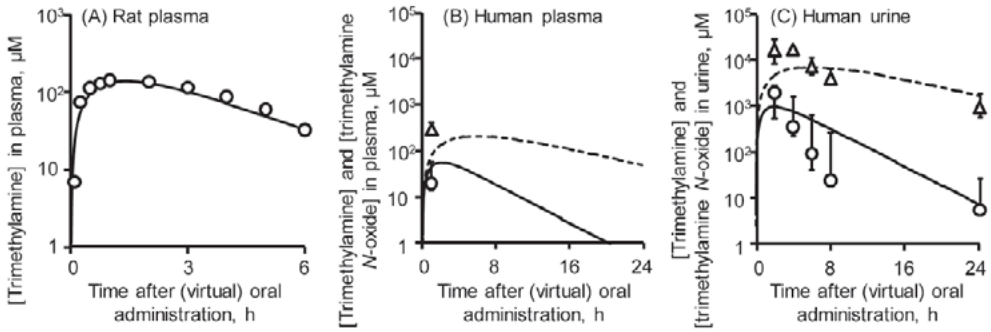
Classical Approach

Reliable animal PBK model

Re-parametrization

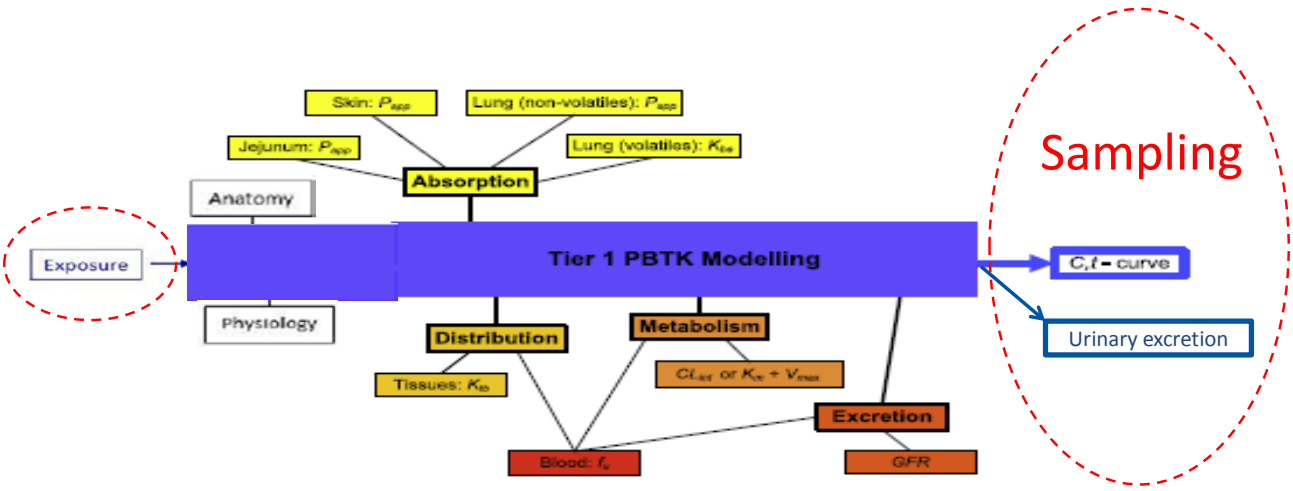
Human PBK model

- Anatomy
- Physiology
- Passive processes
  - identical
  - *in vitro*
  - *in silico*
- Active processes
  - scaling (to BW)
  - *in vitro*



Shimizu and Yamazaki (2017)

21st Century Approach



Adapted from Bessems et al., (2014, 2015)

In Silico/In Vitro Parametrization

Test method output parameters and conversion to input parameters in a PBTK model[a].

Process	Specification	Test result	PBTK rearrangement	Unit
Absorption	Oral	P_{app}[b]		cm h^{-1}
			$J = P_{app} \cdot SA \cdot C$	mmol h^{-1}
	Dermal	P_{app} ($K_{p,app}$)[c]		cm h^{-1}
			$J = P_{app} \cdot SA \cdot C$	mmol h^{-1}
	Inhalation	P_{app}		cm h^{-1}
			$J = P_{app} \cdot SA \cdot C$	mmol h^{-1}
		$K_{b:a}$[e]		Dimensionless
Distribution	Partitioning	$K_{t:b}$		Dimensionless
Metabolism	Only linear	CL_{int}		mL h^{-1}
			$Rate = CL_{int} \cdot C_u$	mmol h^{-1}
	(Non-)linear	K_m		mmol mL^{-1}
	(Non-)linear	V_{max}		mmol h^{-1}
			$Rate = (V_{max} \cdot C_u)/(K_m + C_u)$	mmol h^{-1}
Excretion	Exhalation	$K_{b:a}$		Dimensionless
	Renal excretion		$Rate = C \cdot f_u \cdot GFR$	mmol h^{-1}
General	Protein binding	f_u		Dimensionless

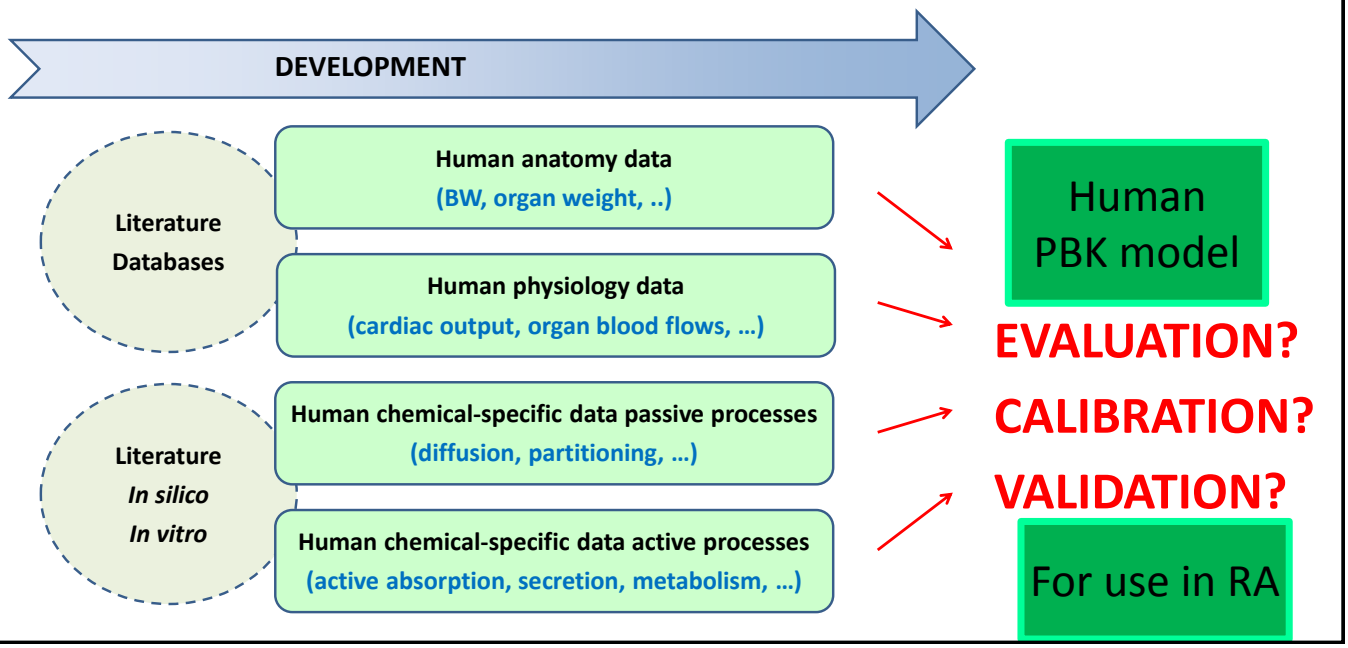
Bessems et al., (2014)

An Example

Zhuang and Lu (2016)

Parameter	Unit convert to	*In vitro* test system
Molecular weight	g/mol	Physicochemistry property measurement, less prefer an *in silico* prediction
log*P*		Octanol:water partition coefficient
pK_a (s)		Physicochemistry property measurement, less prefer to use an *in silico* prediction
Compound type	Base, acid, neutral	Based on the chemical structure or pH-dependent solubility test
pH-dependent solubility	μg/mL	Measured in buffer with different pH
Plasma protein binding	f_u	*In vitro* in human plasma (pay attention to whether compound binds to AGP)
Blood–plasma partitioning	B:P	*In vitro* in human blood
Apparent permeability	10^{-6} cm/s	Caco-2 , MDCK
Intrinsic clearance in microsomes, or S9, or hepatocytes, or rhCYP	μL/min/mg for microsomes and S9, uL/min/million cells for hepatocytes, uL/min/pmol for rhCYP	*In vitro* assay, or use *in vivo* clearance if available
Protein concentration in *in vitro* test	mg/mL	*In vitro* assay for intrinsic clearance
In vitro test matrix binding	f_u	Measure the free fraction using the same protein concentration in the *in vitro* test system

Overview



But, Does NAB PBK Model Make Sense?

To evaluate NAB PBK …..

NEED!
Reliable exposure data

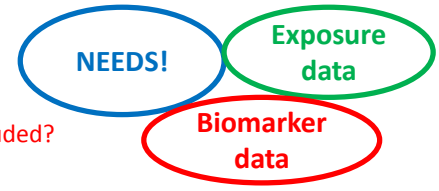
→

NEED!
Human blood data

Same individuals: external exposure + internal exposure data (TK)

- occupational exposure → occupational HBM + HVS
- general population exposure → general population HBM + HVS

Confidence in NAB PBK Models for Use in Human RA Depends on



BIOLOGICAL BASIS of model structure and parameters

- Do model structure and parameters have reasonable biological basis?
- Relevant biomarker(s) of exposure in the most relevant biological matrix included?

PERFORMANCE of model: Model simulations *vs. human data*

- How well does the PBK model reproduce the chemical-specific *in vivo* human TK data?
  - Under various conditions: within a factor of 2
  - Shape of the curve more relevant than absolute predictions
  - Accuracy considering human variability (exposure, genetic polymorphism etc.)

RELIABILITY of model predictions and RELEVANCE to risk assessment

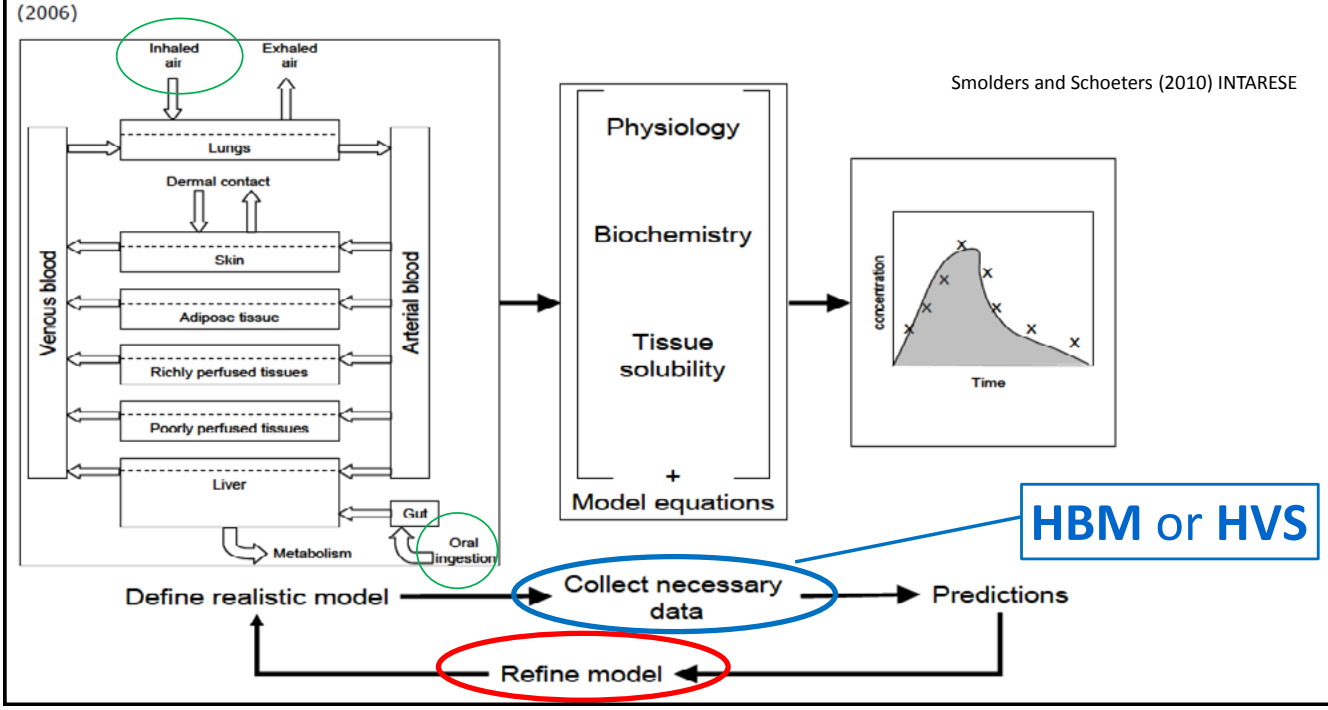
- Which parameters most influence outcome (sensitivity analysis)
- Uncertainty analysis (including on variability) in parameter estimation

Modified from WHO IPCS (2010)

SENSITIVITY \ UNCERTAINTY	H	M	L
H			
M			
L			

Figure 2.1 An idealized approach for a PBPK model for simulation diffusion-limited tissue uptake and multi-route exposures. Dottet lines represent the separation of cellular matrix and tissue blood components (redrawn from Andersen et al (2003) and USEPA (2006)

Smolders and Schoeters (2010) INTARESE

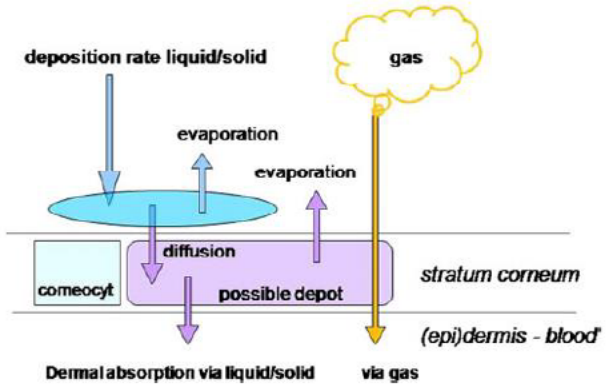


Examples: Occupational Settings

- ❑ Workplace air monitoring
  - ✓ Provides exposure information for input PBK model to be evaluated with **HBM** data
  - ✓ Provides exposure information to set up **HVS** mimicking occupational exposure

- ❑ Basically
  - ✓ Retrospective occupational **HBM**
  - ✓ Prospective occupational **HVS**

IndusChemFate PBK Model in Excel



Jongeneelen and Ten Berge (2011)

Table 1. The human physiological parameters of the PBTK model

Parameter	Symbol	At rest	Light work
Cardiac output (l h^{-1})	CardOutp	390	640
Alveolar ventilation (l h^{-1})	AlvVent	530	1350
Fraction of cardiac output to adipose tissue	FrAdip	0.053	0.0417
Fraction of cardiac output to bone tissue	FrBone	0.021	0.0128
Fraction of cardiac output to brain tissue	FrBrain	0.12	0.0731

$$PCtb = \frac{0.133 \times K_{OW}^{0.48} + 0.775}{0.0056 \times K_{OW}^{0.48} + 0.830} - 0.21$$

Jongeneelen and Ten Berge (2011)

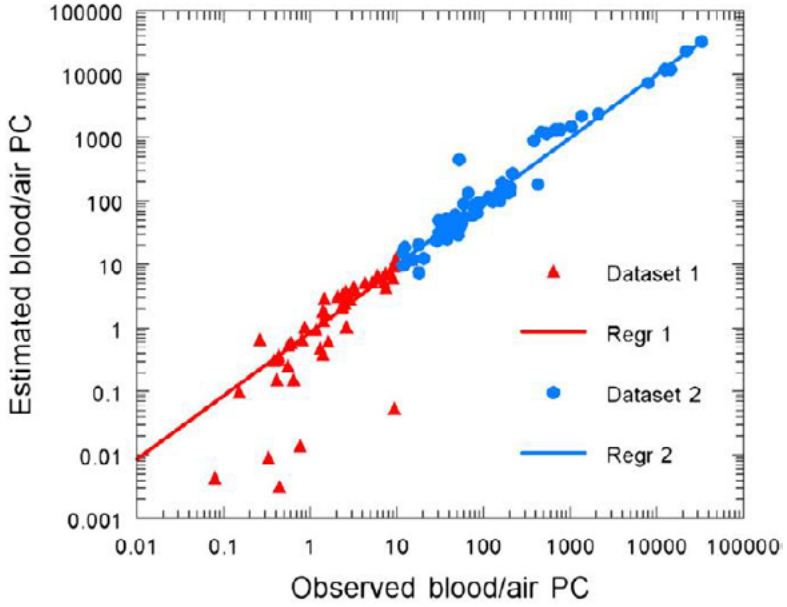
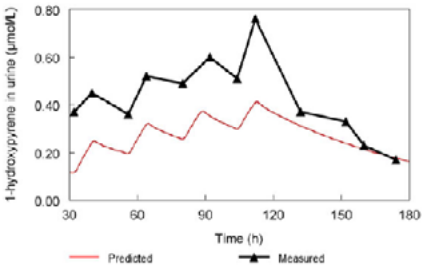


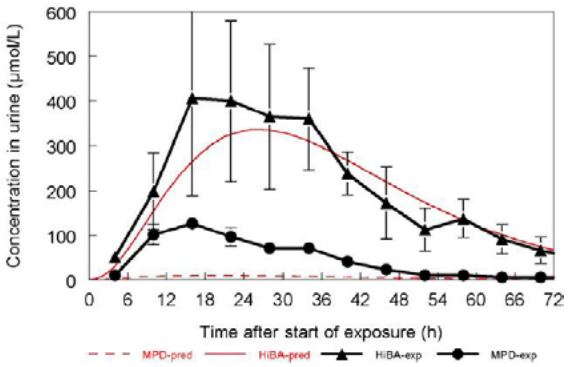
Table 3. Input data for the simulation of pyrene, 1-OHP and 1-OHP-gluc in the PBTK model

Compound / Property	Pyrene	1-OHP[a]
CAS reg. Nr.	129-00-0	5315-79-7
Density (g/L)	1270	1000
Molecular weight	202.26	218.28
Vapour Pressure (Pa)	0.0106	0.000022
Log(K_{ow}) at skin pH 5.5	4.88	
Log(K_{ow}) at blood pH 7.4	4.88	4.45
Water solubility (mg/L)	0.135	2.77
Resorption tubuli (y/n/?)	y	y
Enterohepatic removal (relative to liver venous blood)	0	0
V_{max} liver of removal (µmol/(kg tissue * h))	360 (Removal of pyrene, estimate)	6,900
k_M liver of removal (µmol/L)	4.5 (Removal of pyrene, estimate)	7.7
V_{max} liver of metabolite production (µmol/(kg tissue * h))	180[c] (Formation 1-OHP; Jongeneelen et al, 1987b)	6900[d] (Formation 1-OHP-gluc; Luukkanen et al, 2001)

HBM - Pyrene

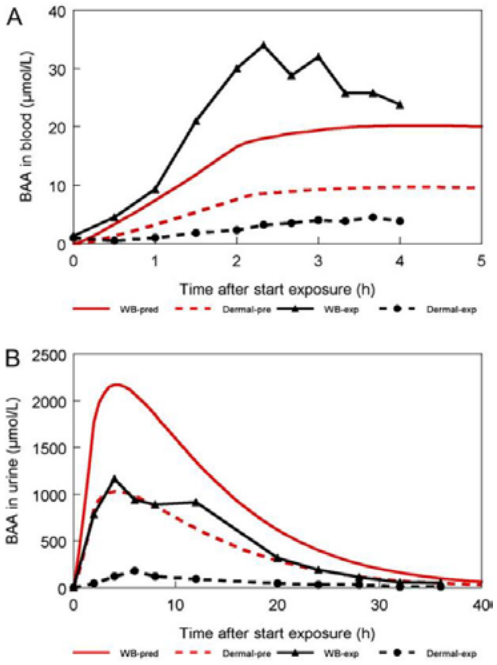


HVS - MTBE

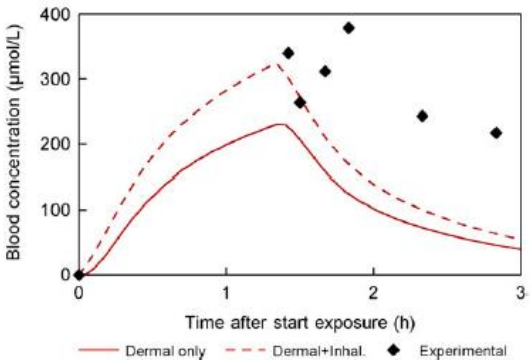


Jongeneelen and Ten Berge (2011)

HVS – 2-BAA whole body and dermal



HVS – Hand rubbing EtOH

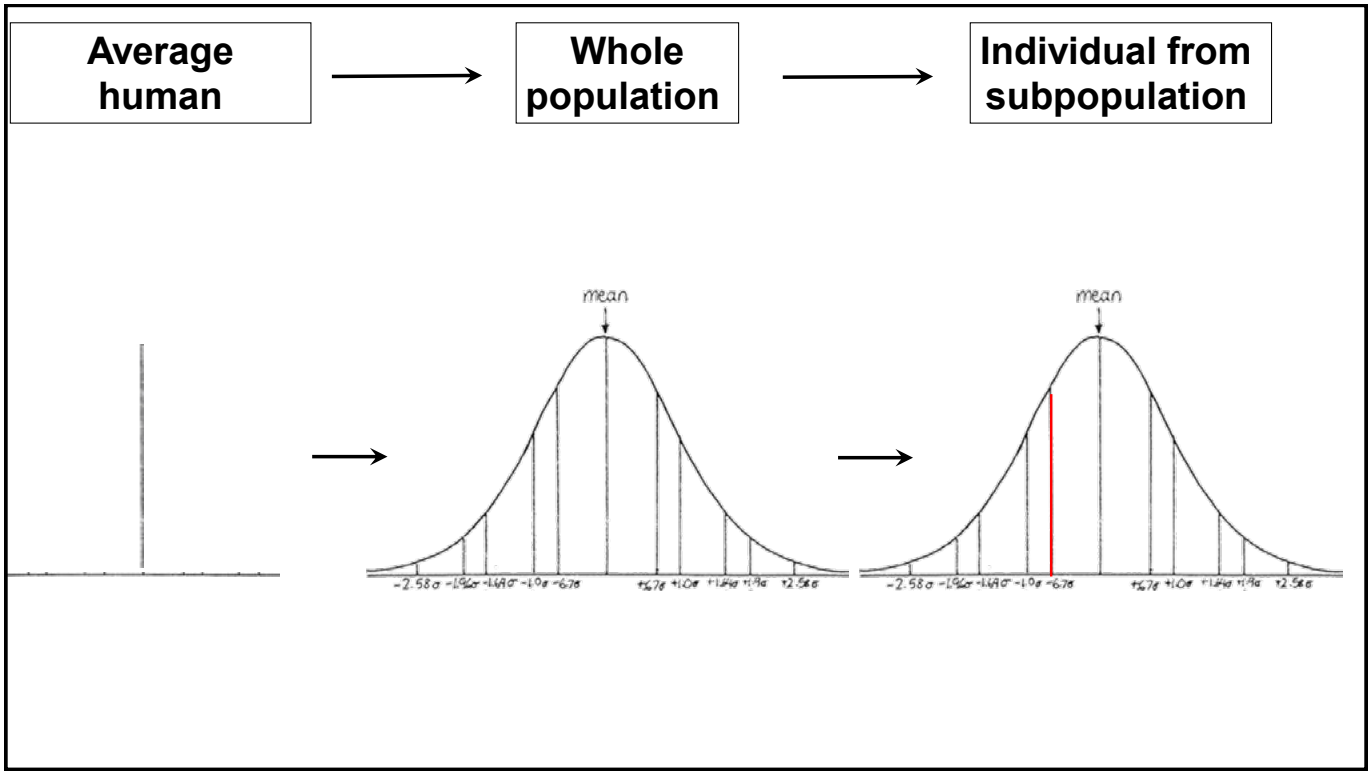


Jongeneelen and Ten Berge (2011)

Examples: General Population HBM

✓ Retrospective **HBM**

UBA Website



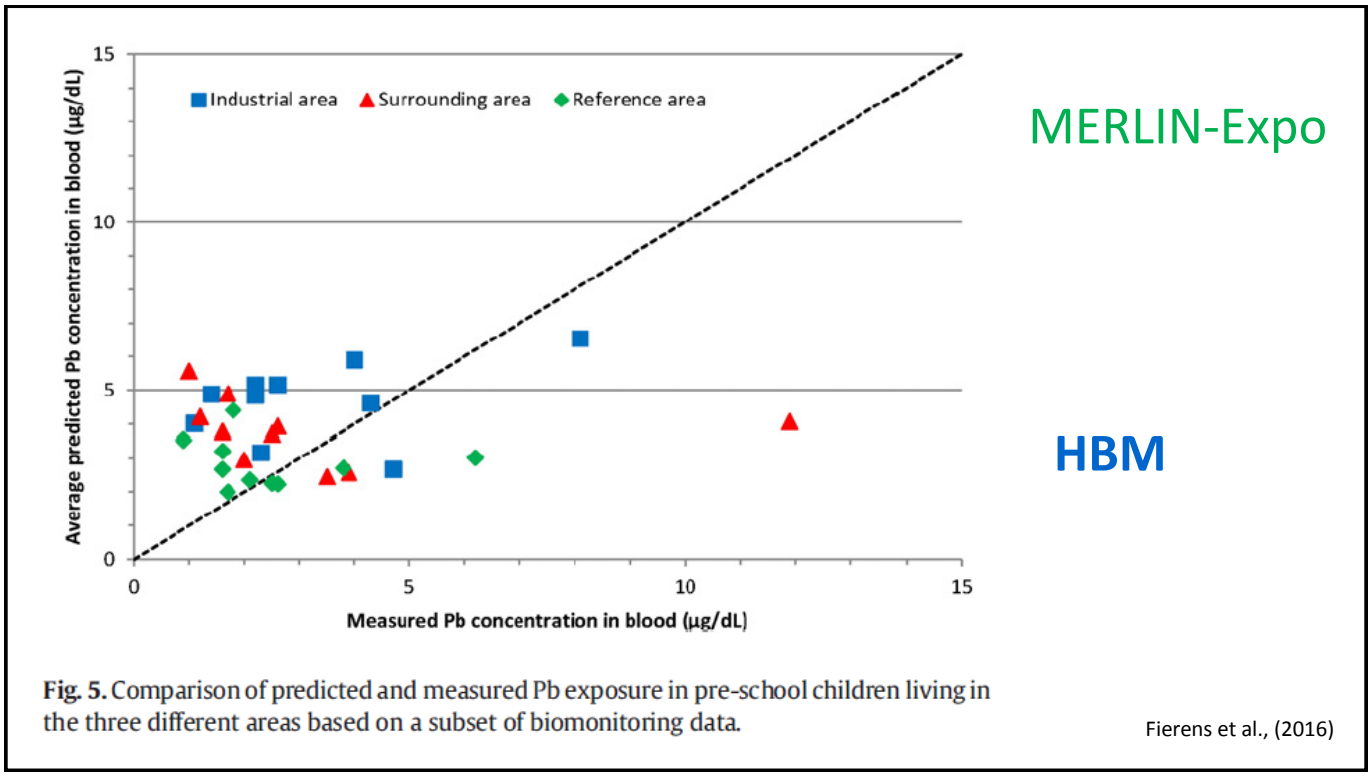
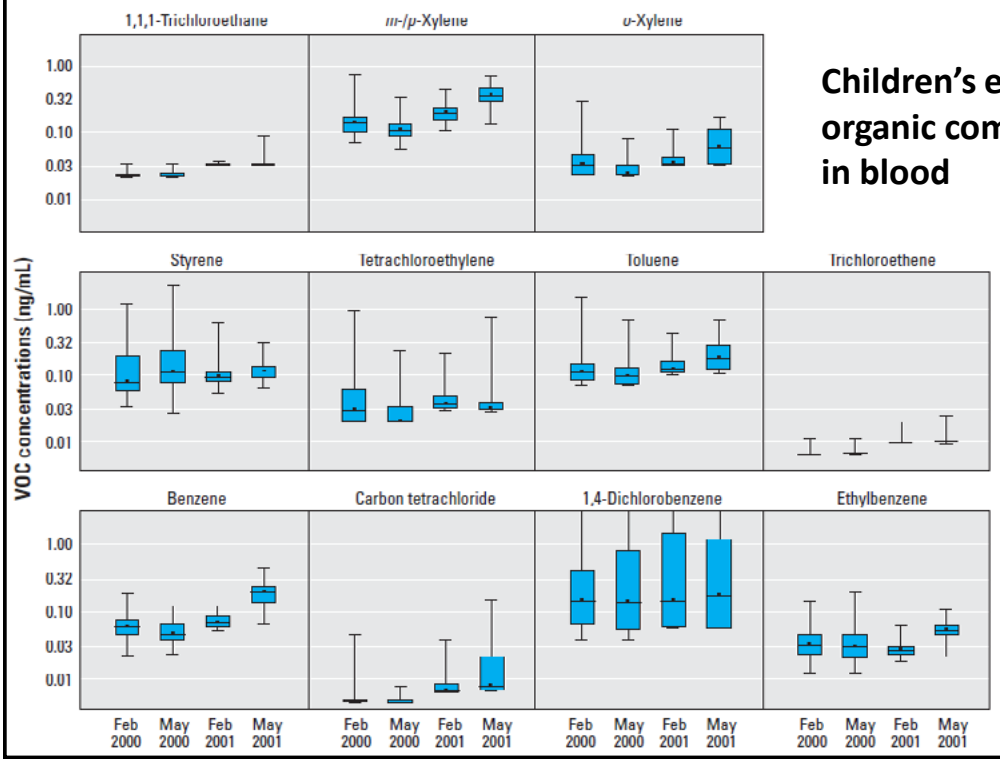


Fig. 5. Comparison of predicted and measured Pb exposure in pre-school children living in the three different areas based on a subset of biomonitoring data.

Fierens et al., (2016)

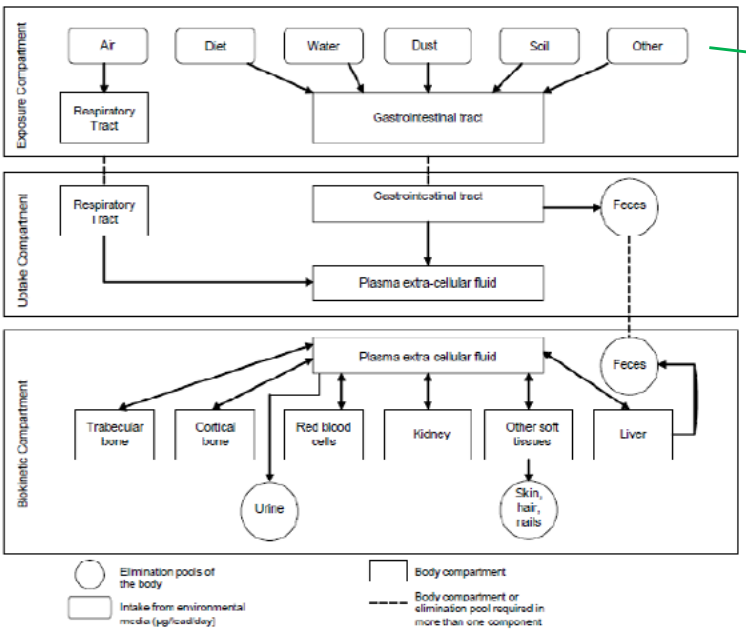


Children's exposure to volatile organic compounds measured in blood

Considerable interindividual variation

Sexton et al., (2005)

Use Example: Exposure Assessment



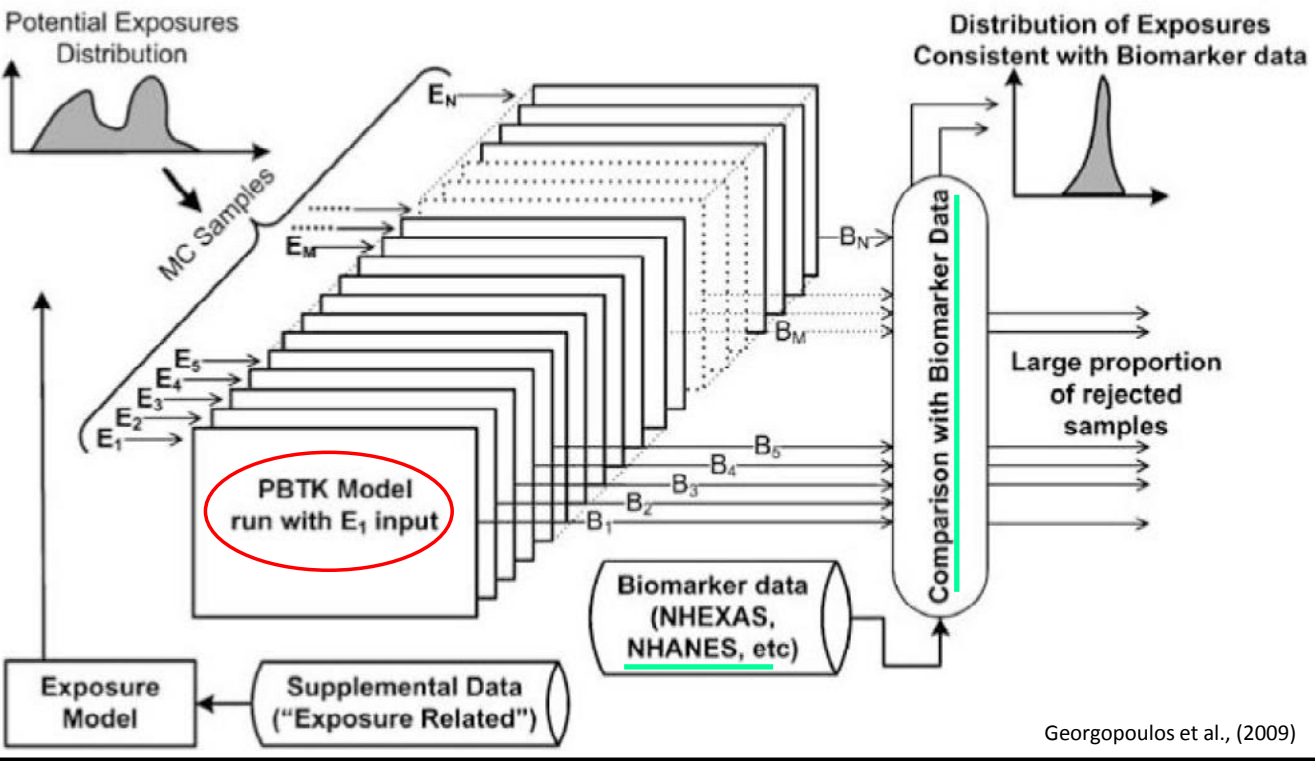
NEED! Reliable exposure data

Biomonitoring of lead and cadmium
Preliminary study on the added value for human exposure and effect assessment

RIVM Letter report 2016-0215
B. Tiesjema | M. Mengelers

Tiesjema and Mengelers (2017)

Figure 3. Schematic for integrated lead-exposure-kinetics model in which simulated multi-media exposures are linked to simulations of lead uptake, tissue distribution and excretion



Georgopoulos et al., (2009)

Example: General Population HVS

✓ **Prospective HVS**

Chemical Research in Toxicology

Article

pubs.acs.org/crt

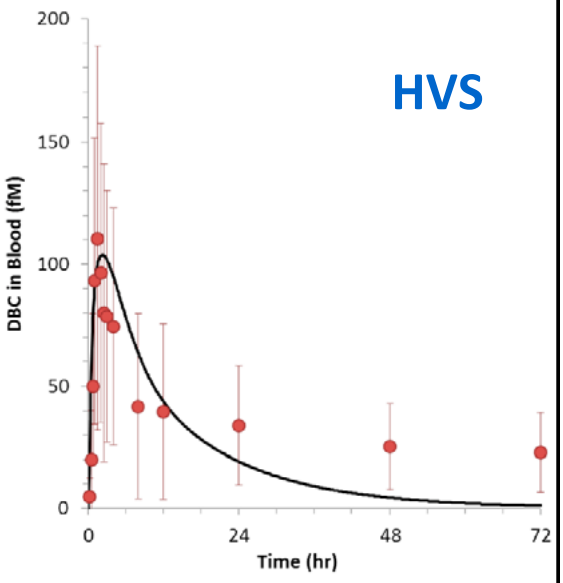
Human *in Vivo* Pharmacokinetics of [^{14}C]Dibenzo[*def*,*p*]chrysene by Accelerator Mass Spectrometry Following Oral Microdosing

Erin Madeen,[†,‡] Richard A. Corley,[‡,⊥] Susan Crowell,[‡,⊥] Kenneth Turteltaub,[#] Ted Ognibene,[¶] Mike Malfatti,[#] Tammie J. McQuistan,[§] Mary Garrard,[‡,§,∥] Dan Sudakin,[†,‡,∥] and David E. Williams*[,†,‡,§,∥]

Mimicking Food-Borne Exposure in the General Population

- ✓ **Mimicking** everyday exposure (controlled prospective)
- ✓ i.v. followed by oral

- oral micro-dosing (29 ng, 5 nCi) of [^{14}C]-DBC
- PBK model: **metabolism scaled** from rat model



Madeen et al., (2015)

Checklist of Characteristics for Selecting PBPK Models for Use in Risk Assessment

Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment

IPCS

Biological basis
- Major ADME included?
- Sum total organ flows equal cardiac output?
- Allometric scaling done appropriately?
- …..

Model simulation of data
- Model evaluated various relevant conditions?
- Reproduction trend of data (peaks, valleys, saturation)?
- Predicted levels not too far 'off'?

Reliability (uncertainty, sensitivity)
- Uncertainty addressed for relevant dose metric?
- What is reliability of measured data?
- Sensitivity dose metric to change in input tested?
- …..

Applicability
- Relevance species, sex, life-stage
- Exposure routes (model, human exposure)
- Model tested for relevant scenario's?
- …..

WHO IPCS (2010)

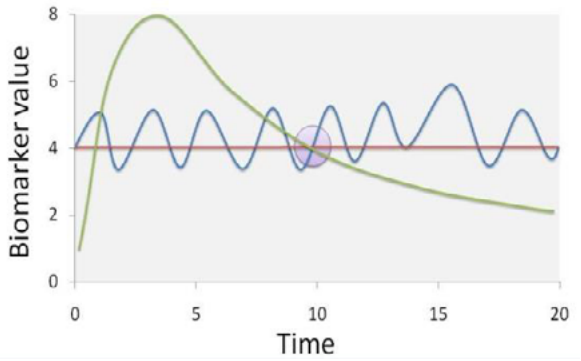
HBM—Critical Issues

- **How to increase quality of the data set?**
  - Add more sampling times in each individual?
  - Or expand the number of individuals sampled?
- **Which biomarker?**
  - Parent compound
    - Maybe easier to reconstruct in PBK model
    - But less abundant because of rapid biotransformation
  - Metabolite
    - Maybe easier to analyse
    - May also be metabolite of another chemical substance (relevant for mixture exposure assessment)

Depends on biomarker half-life and exposure characteristics, but also intrusiveness issues and costs

HBM—Critical Issues

- **Matrix to be sampled**
  - Blood
  - Urine
  - Exhaled air
  - ….
- **Sampling time**
  - Fixed:
    - Morning or evening?
    - Pre-shift or post-shift?
  - At random during the day
  - One spot urine sample versus 24 h urine



Smolders and Schoeters (2010) INTARESE

Improving Interpretation of HBM Data through Modeling

Requirements and Needs

Smolders and Schoeters (2010) INTARESE

- Accommodate inter-individual variability in
  - ADME
  - time-activity patterns
  - life-style
- Accommodate dynamic exposure profiles
  - temporal and spatial variability
  - HBM value is just a point estimate of a whole profile
- Generic application:
  - accommodate several chemical classes
  - easy computation
  - user-friendly interface
- Mechanistic information
  - on sources of contaminant exposure
  - have relationship with health effects if possible
  - exposure change reflected in internal dose

HBM versus HVS

- **HBM** (human biomonitoring)
  - fewer ethical issues
  - exposure variability and uncertainties (exposure history vs. chemical's half-life)
  - organisational challenge and costly
  - human variability: age groups, sex differences, genetic polymorphism, non-healthy persons, confounders such as smoking (e.g., next to occupational Cd exposure)

- **HVS** (human volunteer studies)
  - exposure conditions well-controlled
  - ethical issues
  - national and regulatory differences in acceptance

Evaluation NAB PBK Modeling Overall

Take home

❑ **Strengths**

- ✓ Automatically more focus on typical human species issues
  - ❑ Human specificities, e.g., slow excretion per- and polyfluorinated alkyl substances (PFAS, PFOA)
  - ❑ Human variability (e.g., individual donor microsomes) such as between human subpopulations (sex, life-stage, smoking, weight, health status etc.)
  - ❑ Real life, e.g., often induced biotransformation enzymes, complex exposure scenarios
- ✓ Can capture TK interaction with combined exposure (e.g., volatiles, CYP2E1)

❑ **Limitations**

- ✓ Huge needs for *in silico, in vitro,* and finally human *in vivo* data
- ✓ Statistical evaluation of uncertainty and variability more challenging
- ✓ **HBM** approach: estimating exposure far more challenging than animal dosing

→ **HVS** approach might be the only option in critical cases

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