In Silico Toxicology 101

Applications and Case Studies: Part I

Aswani Unnikrishnan, MS; Xiaoqing Chang, PhD

Inotiv

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Outline

- Learning objectives
- Case studies
  - Quantitative structure-activity relationship (QSAR)
  - Read-across
  - Physiologically-based pharmacokinetic (PBPK) modeling
  - *In vitro* to *in vivo* extrapolation (IVIVE)
- Q&A

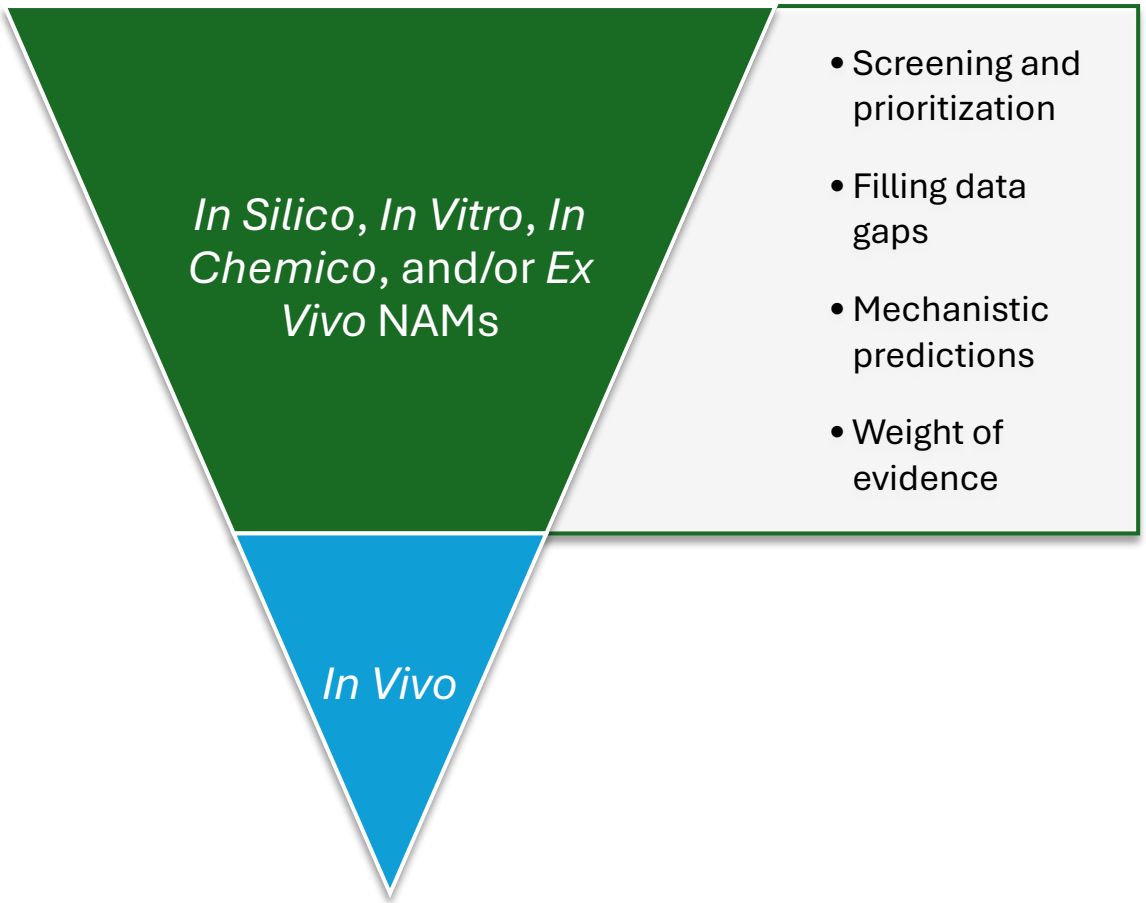
Learning Objectives

- Explain what is involved in developing, implementing, applying, and verifying *in silico* NAMs.
- Describe several ways in which an *in silico* NAM can be evaluated and validated.
- Summarize the drawbacks and benefits of the usage of *in silico* NAMs.
- Give examples of the types of data needed to support the development and testing of various types of *in silico* NAMs.
- Identify the strengths and weaknesses of *in silico* approaches relative to *in vivo* methods for the case studies presented (applicability, chemical space).
- Recognize some software used in the development and evaluation of NAMs.

Review

https://www.thepsci.eu/in-silico-tools-webinars/

YouTube



New Approach Methodologies (NAMs) is a broad term (often synonymous with Non-Animal Methods) referring to any technology used to assess chemical hazard without using intact animals.

Featured *In Silico* Methods:

- Quantitative structure-activity relationship (QSAR)
- Read-across
- Physiologically-based pharmacokinetic (PBPK) modeling
- *In vitro* to *in vivo* extrapolation (IVIVE)
- Quantitative adverse outcome pathway (qAOP)
- Molecular modeling
- Artificial intelligence/Machine learning/Deep learning

Note: Words that are **bolded in orange** are defined in the webinar glossary.

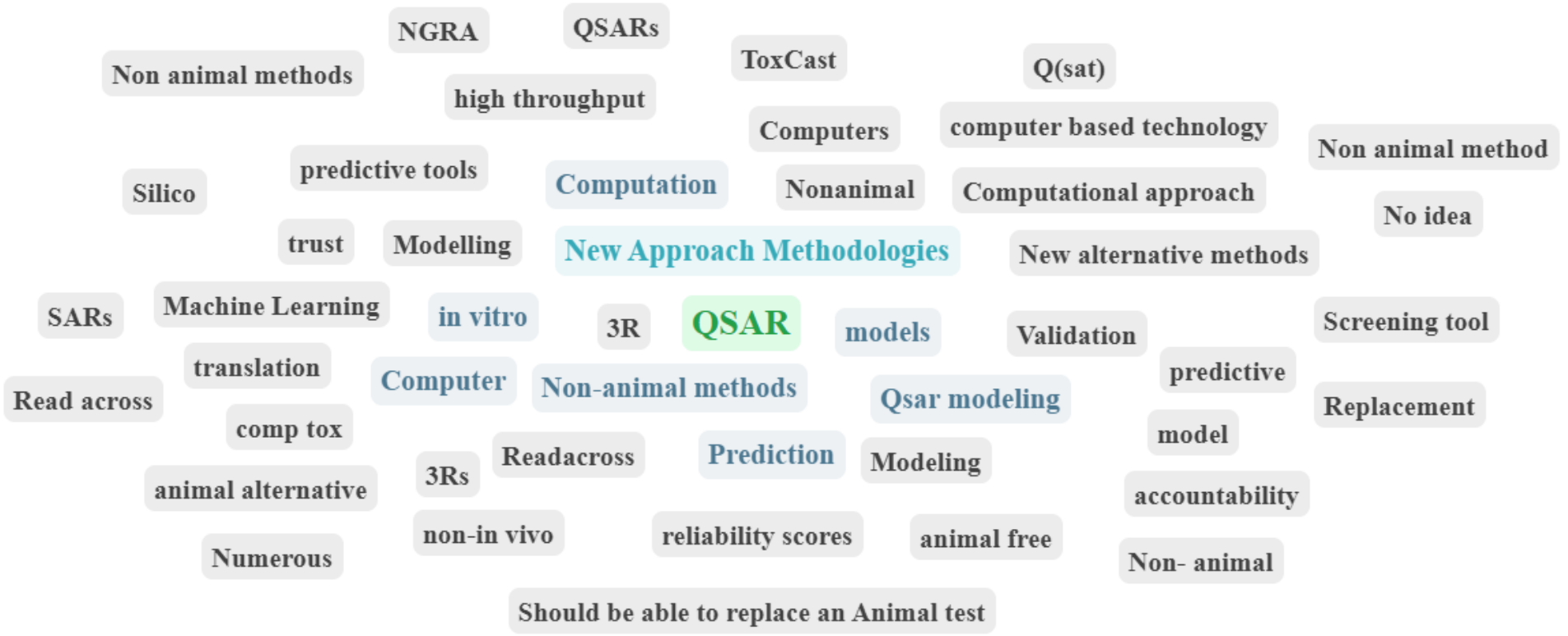
Review

- Not every method or model works for every purpose!
- **Context of use** is the manner and purpose of use for a particular method, approach, or application.
  - What toxicological endpoint is the method testing for?
  - What, if any, regulatory need does the method address?
- **Applicability domain** is the chemical or biological space in which the model's predictions are considered accurate.

QSAR

Review

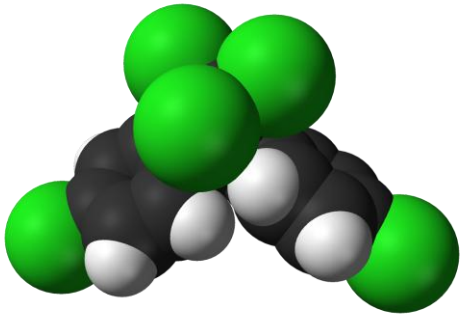
What word or phrase do you associate with "*in silico*" and/or "NAMs"?



QSAR

- Will the chemical be toxic to a specific endpoint (*e.g.*, mutagenicity)?
- What is the predicted potency or severity of an effect?
- How does structural modification affect toxicity?

Purpose: **Quantitative structure-activity relationships** (QSARs) relate structural properties of a molecule to its biological activity.



Molecular descriptors

molecular weight
polar surface area
number of H-bond donors
dipole moment
LUMO
number of aromatic rings
…

QSAR →

Biological activity

LD_{50}, IC_{50}
K_i, K_d
active vs. inactive
toxic vs. non-toxic
low, medium, high toxicity
…

Quantitative structure property relationships (QSPRs) is a similar technique, where the molecular structure is related to some chemical property, like solubility.

What do you think is a big benefit of using QSAR?

QSAR Case Study

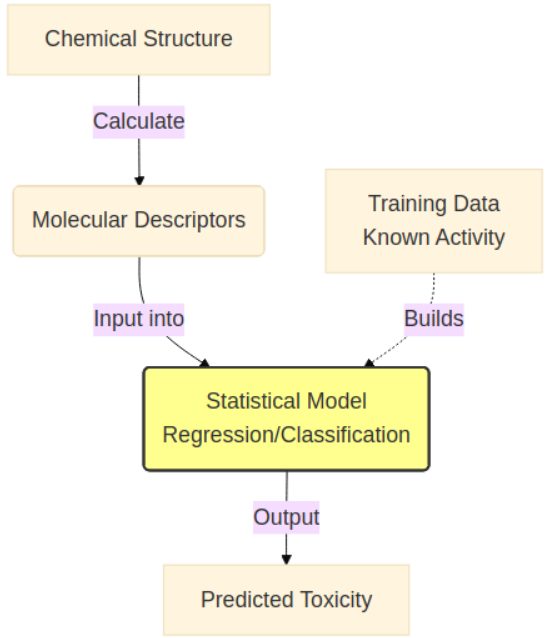
SAR and QSAR modeling of a large collection of LD_{50} rat acute oral toxicity data

Gadaleta et al., 2019

Journal of Cheminformatics

doi: 10.1186/s13321-019-0383-2

Are QSAR/SAR models reliable NAMs for predicting rat acute oral toxicity (LD_{50})?



NICEATM & NCCT curated a dataset of ~12,000 rat oral LD_{50} values.

75% Training
8994 Chemicals

25% Validation
2895 Chemicals

Endpoints considered for modeling:

LD_{50} single point estimates

"Very toxic" (vT) binary classification

"Non-toxic" (nT) binary classification

EPA 4-category hazard classification

GHS 5-category hazard classification

QSAR Case Study

- Used comprehensive training set and **external validation**
- Individual models for both **regression** and **classification** endpoints
- Chemical structures represented using molecular descriptors
- Applicability domain
- Integrated modeling approach

Method	Software/tool	Applicability domain	LD50 point estimate	vT	nT	EPA	GHS
Balanced random forest/regression random forest (**BRF/rRF**)	KNIME (free/open) Indigo toolkit (open) Dragon descriptors (proprietary – Talete)	"Error" model Confidence Similarity	✓	✓	✓	✓	✓
Ab initio QSAR (**aiQSAR**)	R (free/open) Dragon descriptors (proprietary – Talete)	Applicability domain measure	✓	✓	✓	✓	✓
k-Nearest Neighbors (**istKNN**)	istKNN (commercial)	Similarity/activity-based thresholds	✓				
SARpy software	SARpy software (free/open)	Presence/absence of structural alerts		✓	✓		
Hyper-parameter tuning random forest (**HPT-RF**)	R (Caret; free/open)	Minor matrix Isolation forest	✓			✓	✓
Generalized linear model (**GLM**)	R (H20; free/open)	NA			✓		

Adapted from Gadaleta et al., 2019 (Table 2)

QSAR Case Study

Main Results:

- Integrated classification models achieved higher accuracy than individual models.
- Integrated regression model showed improved performance on the external set, compared to the best individual models.
- Significantly improved predictive performance for compounds that fall within the defined **applicability domain** (AD).

Comparison of experimental $logLD_{50}$ values with integrated model predictions

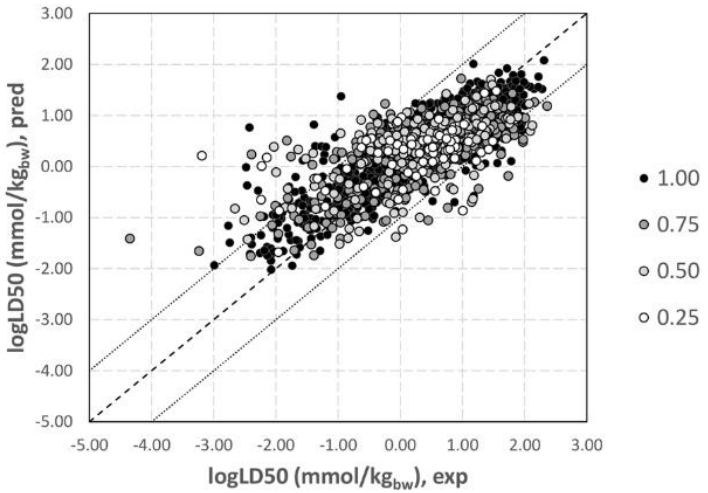


Table 8 External performance of continuous models for predicting classification endpoints (vT, nT, EPA, GHS)

	SEN	SPE	MCC	BA	#AD	%AD	CS
nT	0.794	0.796	0.587	0.795	2665	0.924	1
	0.840	0.841	0.677	0.840	2182	0.757	2
	0.878	0.858	0.733	0.868	1704	0.591	3
	0.913	0.883	0.794	0.898	1222	0.424	4
vT	0.743	0.938	0.577	0.840	2742	0.949	1
	0.796	0.976	0.737	0.886	2316	0.802	2
	0.890	0.978	0.820	0.934	1556	0.539	3
EPA	0.602	0.856	0.439	0.729	2653	0.928	1
	0.701	0.885	0.550	0.793	1731	0.605	2
	0.739	0.898	0.600	0.819	1200	0.420	3
GHS	0.567	0.894	0.461	0.731	1561	0.542	1
	0.644	0.911	0.541	0.777	908	0.315	2
	0.676	0.916	0.573	0.796	617	0.214	3

For each model, the sensitivity (SEN), the specificity (SPE), the balanced accuracy (BA), the Matthew's correlation coefficient (MCC) the number (#AD) and the percentage (%AD) of predictions in AD are reported, with respect to the CS threshold for defining predictions in AD. For multi-category endpoints (EPA and GHS), SEN and SPE are the average of sensitivities/specificities computed separately for each class, while BA is the arithmetic mean of the average SEN and SPE

QSAR Case Study

To what extent was the research question answered?

Are QSAR/SAR models reliable NAMs for predicting rat acute oral toxicity (LD_{50})?

- Established QSAR's feasibility for predicting acute oral toxicity.
- Integrated QSAR models proved to be a robust NAM for LD_{50} hazard classification and regulatory screening, contingent on use within the AD.

QSAR Case Study

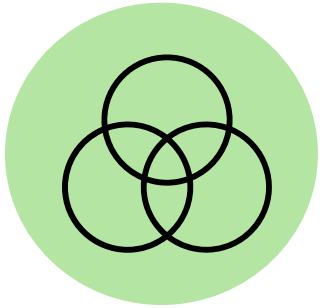
Strengths

- Large, high-quality dataset
- Diverse modeling strategies
- Integrated modeling
- Attention to AD

Weaknesses

- Use proprietary descriptors (Dragon)
- Commercial tool dependency (istKNN)
- Integrated models increased accuracy but decreased chemical space coverage
- No modeling of biological mechanisms

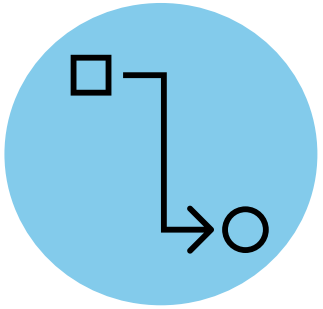
QSAR Case Study: Summary



Applies multiple QSAR methods and compares their performance against experimental data

Demonstrates real-world considerations vital for regulatory acceptance:

- Utilizing integrated modeling
- Defining the AD



Illustrates how QSAR models are built, validated, compared, and integrated to meaningfully support hazard classification and regulatory screening

Select an important metric for assessing a QSAR model's reliability and applicability

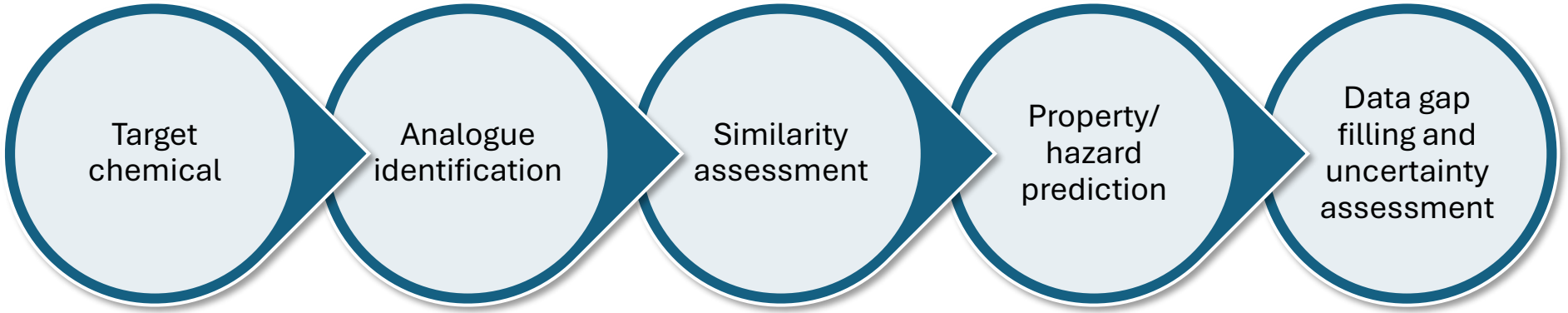
Read-across

Read-across

?

- What is the likely toxicity of a data-poor chemical based on that of similar chemicals?
- Can we fill data gaps for our compounds of interest without additional testing?
- What is the rationale for similarity between chemicals in the application of interest?

Purpose: **Read-across** fills experimental data gaps by transferring toxicity information from similar chemicals (**chemical analogues**).



Read-across Case Study

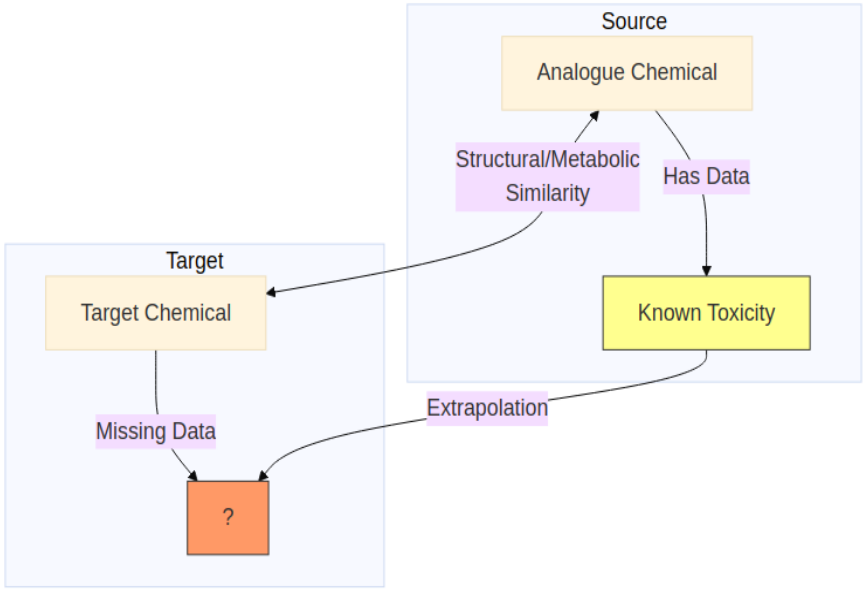
In silico identification of protein targets for chemical neurotoxins using ToxCast *in vitro* data and read-across within the QSAR toolbox

Chushak et al., 2018

Toxicology Research

doi: 10.1039/c7tx00268h

Can we effectively and efficiently use high-throughput screening (HTS) data combined with read-across to predict specific targets of chemical neurotoxins?



Data	Description
ToxCast *in vitro* HTS data	• 1050 chemicals covering neurological targets • Data from 216 biochemical and cell-based assays targeting 123 proteins identified as having neurological functions (via the Gene Ontology database) • Quantitative and qualitative bioactivity data
Chemical descriptors	• Structural properties from OECD QSAR Toolbox
Validation data	• DrugBank Database • Ki Database • Comparative Toxicogenomics Database

Read-across Case Study

Details

Target Chemicals	12 Pyrethroids Data Gap: Predict neurological protein target(s) each chemical interacts with and the potency of that interaction
Analogue Identification	OECD QSAR Toolbox Based on structural similarity Five nearest neighbors
Prediction	Mechanistic similarity Quantitative (Potency - AC_{50}) Qualitative (Classification - Active/Inactive)
Validation	**Internal validation**

Software

Data source	**U.S. EPA's ToxCast program** The data used (ToxCast HTS assay results) is public and freely available.
Data processing and management	**SQLite v.3 SQL database engine** A free, open-source, public domain database engine.
Chemical grouping and read-across	**OECD QSAR Toolbox v. 3.5** Freely available. The QSAR Toolbox is a public domain software developed by the OECD and ECHA for regulatory data gap filling.

Read-across Case Study

Main Results

- Neurological targets identification: 123 proteins related to neurological function
- Target activity analysis: skewed distribution
- Target prediction for pyrethroids: novel targets like retinoic acid receptors and dopamine transporters
- Prediction accuracy:
  - Classification prediction: 79% accuracy
  - Potency prediction: more variability and less reliability

Assays	Protein	N active	Cyfluthrin		Permethrin		Cypermethrin		Fenpropathrin		Tetramethrin		Resmethrin		Prallethrin		S-Bioallethrin		Allethrin		Tefluthrin		Esfenvalerate		Bifenthrin	
			ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted	ToxCast	Predicted
ATG_DR5_CIS	RARA, RARB, RARG	10	Active	Active	Active	Active	Active	Active	Active	Active	Inactive	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Inactive	Active
ATG_ERE_CIS	ESR1	5	Inactive	Active	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive
ATG_ERa_TRANS	ESR1	7	Inactive	Inactive	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active	Active	Active	Active
ATG_RARa_TRANS	RARA	6	Inactive	Active	Inactive	Active	Active	Active	Active	Inactive	Inactive	Active	Inactive	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive	Active	Inactive	Active	Inactive	Inactive	Inactive
BSK_3C_HLADR	HLA-DRA	9	Inactive	Inactive	Active	Active	Inactive	Active	Inactive	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Inactive	Active	Active
BSK_3C_MCP1	CCL2	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active	Inactive	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
BSK_4H_MCP1	CCL2	5	Active	Active	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Inactive	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
BSK_SAg_MCP1	CCL2	6	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active	Inactive	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
NVS_ENZ_hBACE	BACE1	7	Active	Active	Active	Active	Inactive	Inactive	Active	Active	Active	Inactive	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active	Active	Inactive	Active
NVS_GPCR_h5HT7	HTR7	5	Inactive	Active	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active	Inactive	Active
NVS_GPCR_hDRD	DRD1	5	Inactive	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Active	Inactive	Inactive	Inactive	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
NVS_GPCR_mCCK	CCKAR	5	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Active	Active	Inactive	Active	Inactive	Inactive	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
NVS_TR_hDAT	SLC6A3	9	Active	Active	Inactive	Active	Active	Active	Active	Active	Inactive	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Inactive	Active
NVS_TR_gDAT	SLC6A3	5	Inactive	Inactive	Inactive	Inactive	Active	Inactive	Active	Active	Inactive	Active	Inactive	Inactive	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

Read-across Case Study

To what extent was the research question answered?

Can we effectively and efficiently use high-throughput screening (HTS) data combined with read-across to predict specific targets of chemical neurotoxins?

Table 3 Summary of the classification prediction of pyrethroid activity

	Predicted		
ToxCast	Active	Inactive	Hit rate
Active	73	16	83%
Inactive	19	60	76%
Accuracy	79%	79%	

- Demonstrates ability to fill data gaps and classify several pyrethroid-protein interactions by using existing mechanistic data within a read-across framework.
- It leverages HTS data to predict and identify novel neurological targets for pyrethroids.

What do you see as a potential strength of this case study read-across approach?

Read-across Case Study

Strengths

Data Leverage: Effectively utilizes publicly available ToxCast HTS data, turning high-throughput screening results into predictive *in silico* models.

Regulatory Tool Integration: Demonstrates the practical use of a regulatory tool (OECD QSAR Toolbox) for a complex toxicological endpoint (neurotoxicity).

Classification Accuracy: 79% accuracy in predicting Active/Inactive status is good for initial hazard screening and prioritization.

Weaknesses

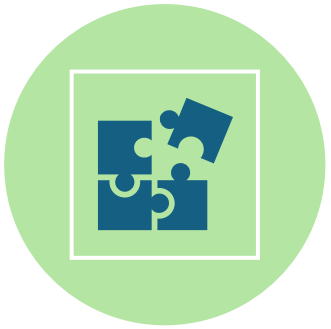
Potency Prediction: The prediction of potency AC_{50} values showed mixed accuracy, limiting the method's reliability for precise quantitative risk assessment.

Applicability Domain Limitation: The predictability for certain neurological proteins is limited by the low number of active chemicals for those specific targets.

Difficulty predicting activity for structural outliers.

Missing Targets: Neurotoxicity may involve many targets not yet covered by the 123 proteins, leading to potential false negatives.

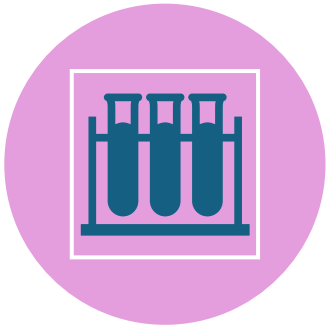
Read-across Case Study: Summary



The study demonstrated an *in silico* read-across method to identify potential neurological protein targets for neurotoxins by using public ToxCast high-throughput screening data.

The method achieved approximately 79% accuracy in classifying chemicals as active or inactive, demonstrating its utility for hazard identification.

Data from 123 neurological protein assays was processed within the QSAR Toolbox, where the read-across technique was employed to predict the activity profile of target compounds like pyrethroids.



The paper shows that integrating HTS data with read-across is an effective, resource-saving strategy to prioritize chemicals for further *in vivo* neurotoxicity testing.

PBPK

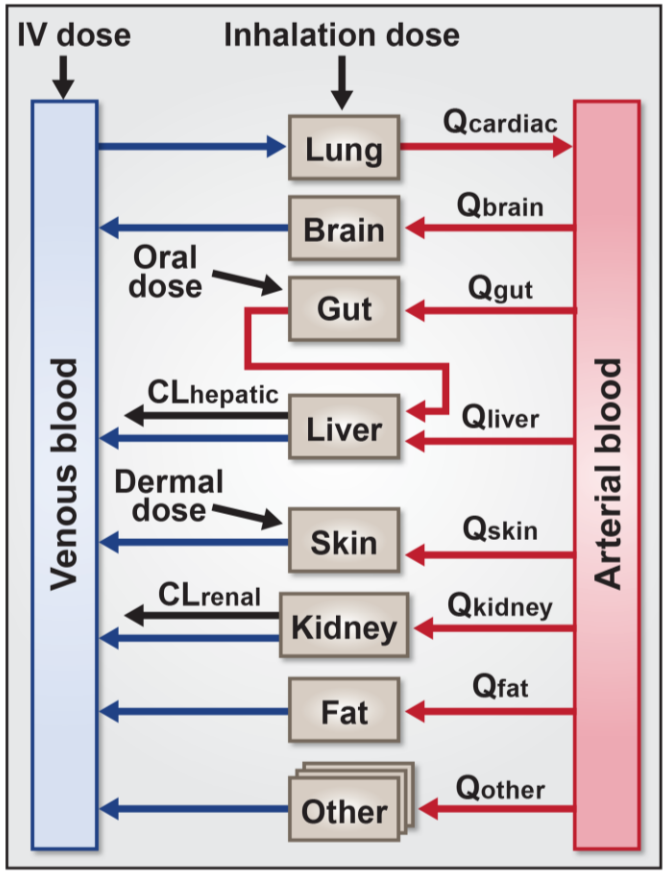
PBPK

?

- What are the internal tissue concentrations over time?
- How does exposure route affect target organ dose?
- Can we extrapolate between species, doses, or exposure scenarios?
- What is the relationship between external exposure and internal dose?

Purpose: Physiologically-based pharmacokinetic (PBPK) models predict chemical concentrations over time in specific tissue compartments for a given exposure scenario.

- The body is represented by relevant tissue compartments connected by major blood flows.
- Cross-species and exposure route extrapolations are possible.
- Mechanistic relationships can be included.



CL: clearance
Q: flow

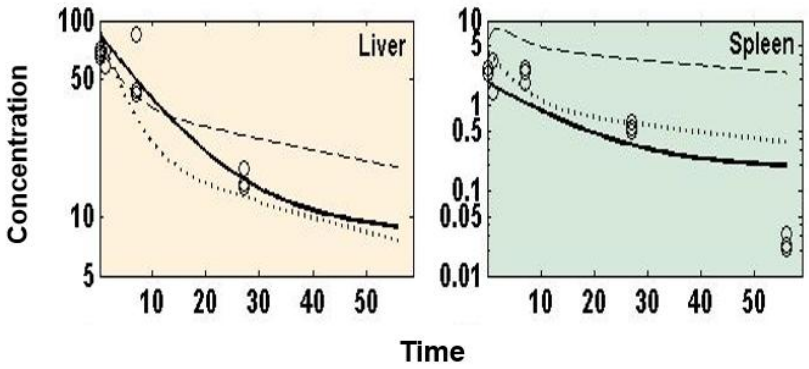
PBPK

Inputs

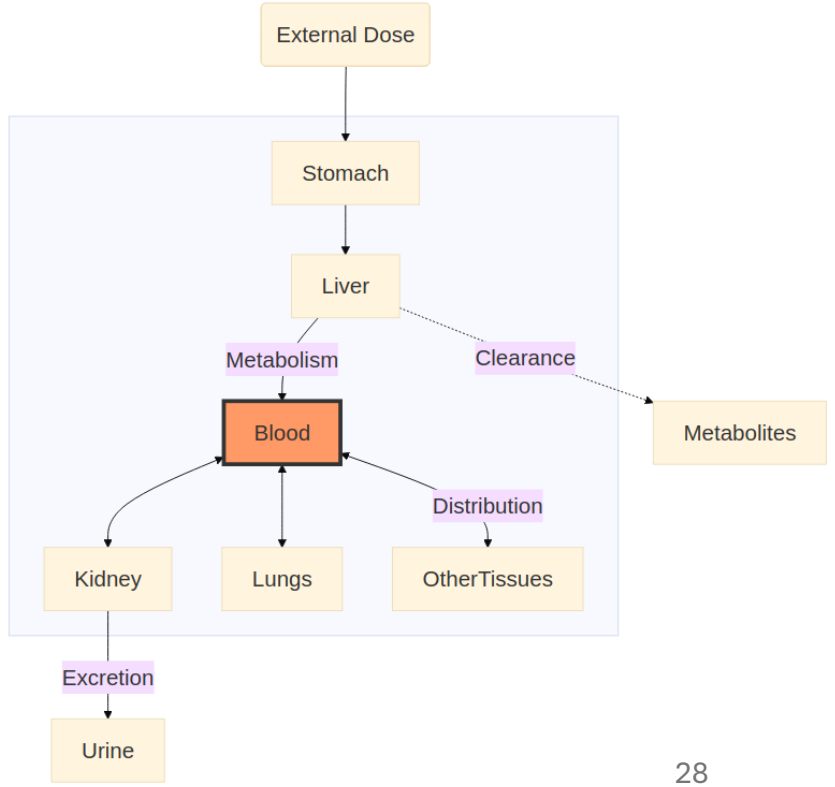
- Physiological parameters (*e.g.*, blood flow rates)
- Chemical-specific physicochemical (*e.g.*, logP) and **ADME** parameters (*e.g.*, intrinsic clearance)

Outputs

- Predicted concentration-time profiles of the chemical species in each compartment for a given dosing scenario



Information Flow



PBPK Case Study

The use of *in vitro* metabolic parameters and physiologically based pharmacokinetic (PBPK) modeling to explore the risk assessment of trichloroethylene

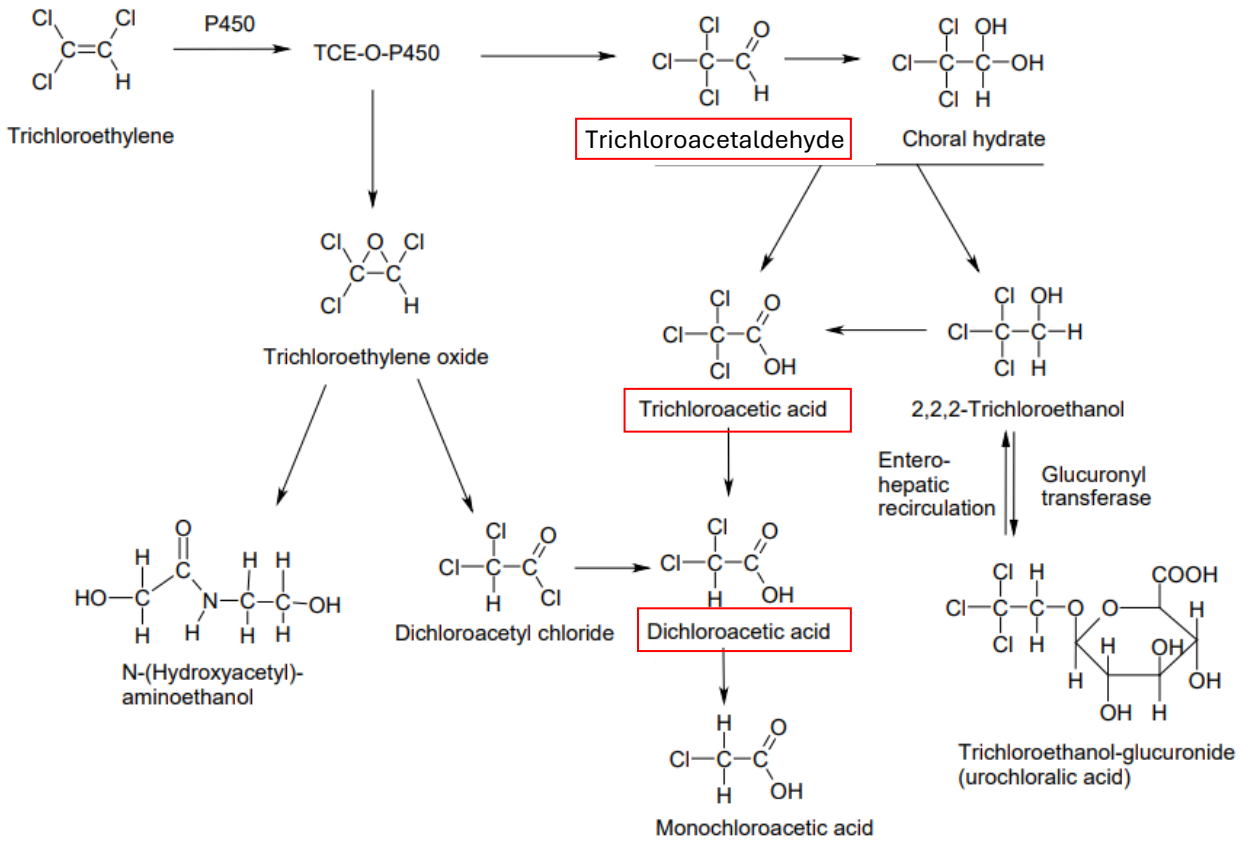
Hissink et al., 2002

Environmental Toxicology and Pharmacology

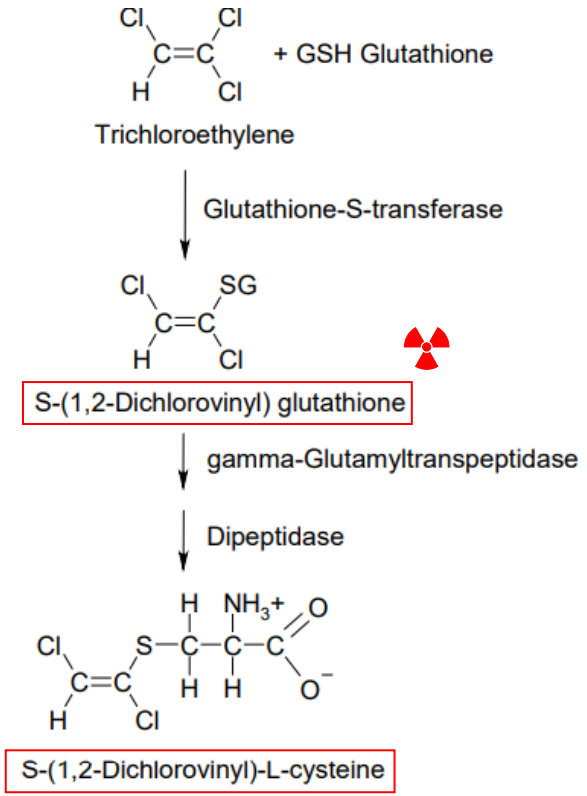
doi: 10.1016/S1382-6689(02)00019-4

- **Trichloroethylene (TCE)** is a synthetic volatile organic compound.
- Experimental results show species differences exist in organ-specific toxicities.
- Most **toxic effects** are due to TCE **metabolites.**
  - **Nephrotoxicity (rats: glutathione route)**
  - Lung tumors (mice: trichloroacetaldehyde)
  - Liver tumors (mice: trichloroacetic acid)

Cytochrome P450-oxidation pathway (liver and lung as target organs)



Glutathione (GSH) conjugation pathway (kidney as target organ)



Figures adapted from ATSDR. 2019. Toxicological profile for Trichloroethylene (TCE). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; https://archive.cdc.gov/www_atsdr_cdc_gov/csem/trichloroethylene/biological_fate.html

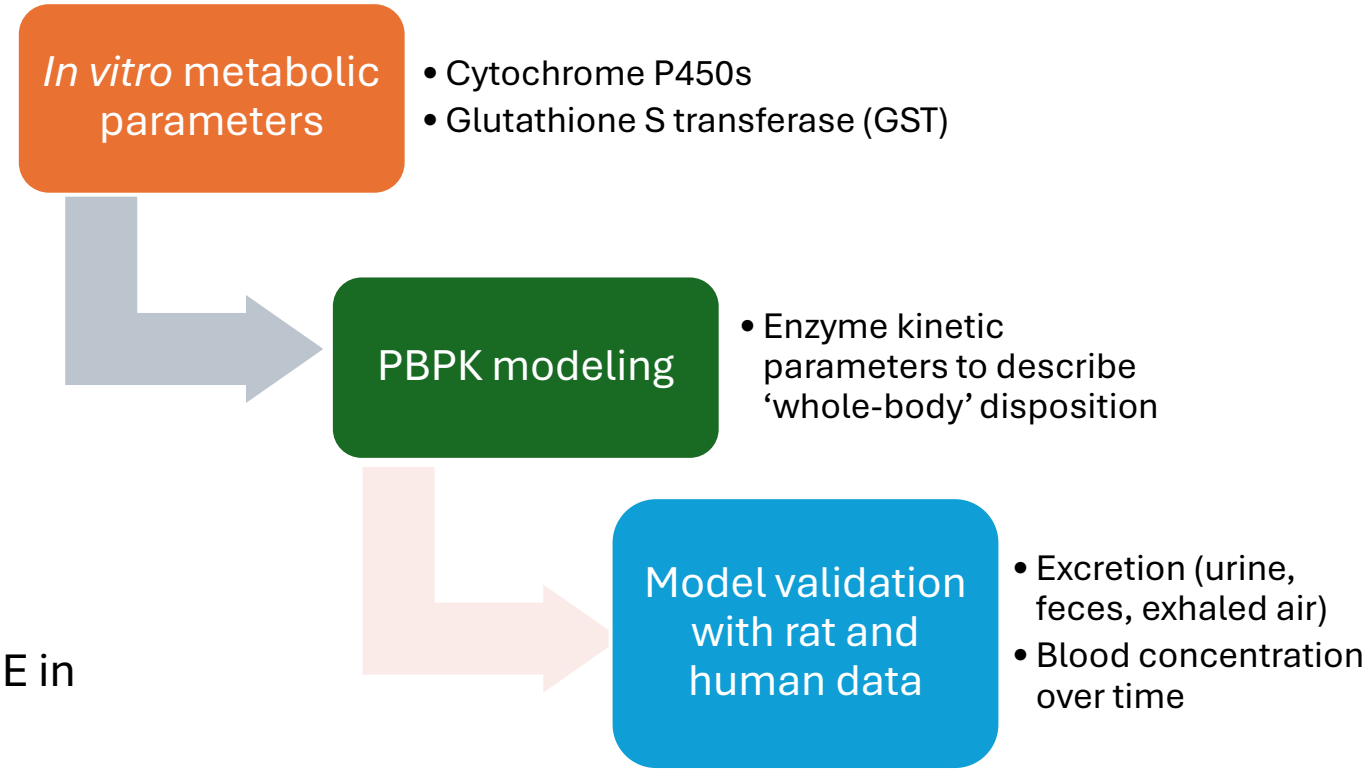
PBPK Case Study

Risk assessment challenges for TCE:

- How do species differences in metabolism influence toxicity?
- How does nephrotoxicity observed in rats translate to humans?
- How does interindividual variability in key enzymes (CYP2E1, GSTs) alter internal dose and toxic effects?

Overarching questions:

- Can ***in vitro* enzyme-specific metabolic parameters** be used in PBPK modeling for TCE in rats and humans, and subsequently improve human health risk assessment?



PBPK Case Study: PBPK Model Input

- Physiological parameters for rats and humans were extracted from 12 papers
- *In vitro* assays were used to quantify the enzymatic activity of cytochrome P450 enzymes and glutathione S-transferase (GST) isoenzymes on trichloroethylene
  - Enzyme kinetic parameters (Km, Vmax, Ki) were estimated using EZ-Fit™ v4.13 (proprietary software)
  - Mass balance equations were used to describe whole-body metabolism

Enzymatic activities of purified rat and human glutathione S-transferases towards trichloroethylene (concentration 250 μM)

Enzyme	Activity (pmol/mg GST/h)
Rat GST 3-3	915
Rat GST 4-4	51.8
Rat GST 7-7	10
Human GST M1-1	13
Human GST P1-1	5.2

highest activity

- No saturation was observed
- Rate of metabolism is described by pseudo-first-order kinetics (instead of using Michaelis- Menten kinetics)

Enzymatic activities of rat and human cytochrome P450 enzymes, and calculated enzyme kinetic parameters towards trichloroethylene

Enzyme (CYP450)	V_{max} (nmol/nmol CYP/h)	K_m (μM)
Rat CYP2E1	198	4.18
Human CYP2E1	84.3	4.12
Human CYP2A6	0.72 (at 100 μM)	C
Human CYP1A2	0.16 (at 100 μM)	–

The activities were too low to measure

PBPK Case Study: Model Overview

- **Whole-body PBPK models for rats and humans**
  - Include lung, liver, kidney, GI tract, fat, rapidly perfused tissues, and slowly perfused tissues
- **Assumptions:**
  - First-order uptake of the dose from the stomach into the GI tract
  - GST-mediated conjugation occurs in the liver, lungs and kidneys in both rats and humans
  - CYP2E1-mediated oxidation occurs in the liver, lungs and kidneys in rats, and in the liver only in humans
- **Proprietary software**
  - ACSL (Advanced Continuous Simulation Language)
- **Key mechanistic features**
  - Enzyme-specific kinetics incorporated directly from *in vitro* data
  - For human **CYP2E1** and **GST M1-1** metabolism, a range of activities was used based on interindividual variation described in literature
- **Parameter optimization**
  - Performed by searching for the best visual fits to rat experimental data

PBPK Case Study: *In Vivo* Data for Model Validation

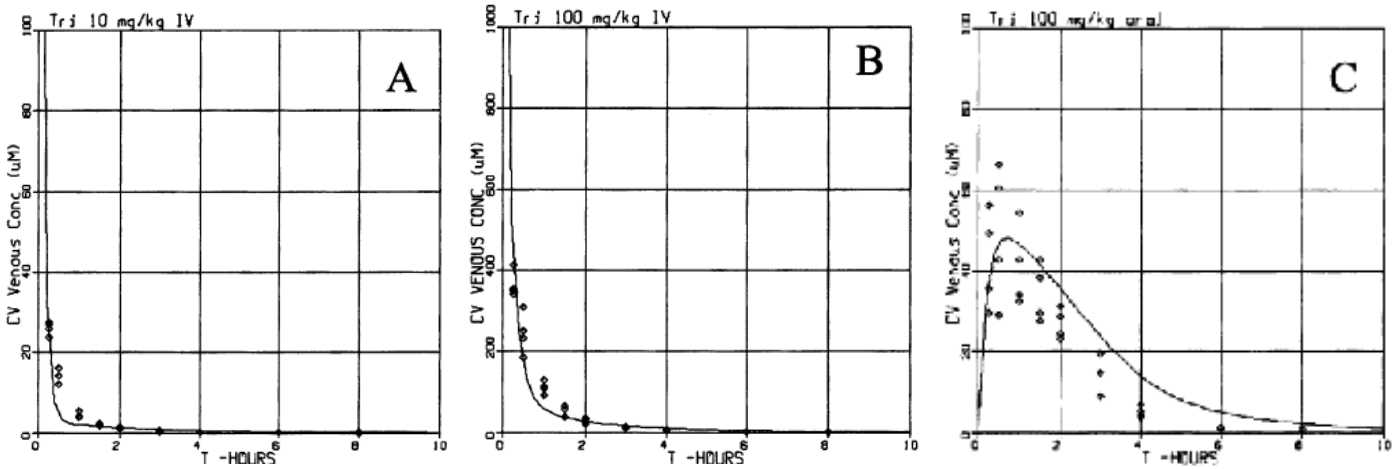
- **Rat kinetic data**
  - **Oral** and **intravenous** dosing of male Wistar rats with radiolabelled TCE
    - IV doses:10, 75 mg/kg
    - Oral doses: 100, 1000 mg/kg
  - **Excretion:**
    - Radioactivity measured in **exhaled air** at multiple hours (0.5-12 h)
    - Urinary and fecal excretion measured at multiple time points (6-168 h)
  - **Blood concentration-time profile**
    - Measured at multiple time points (0.25-72 h) using gas chromatography)
  - **Tissue concentration** measured for various tissues at 168 h

- **Model Validation**
  - The rat PBPK model was validated against **blood kinetics data, exhaled fraction, and urinary excretion**
  - Using the *in vitro* determined parameters **Km** and **Vmax**, rat PBPK model
    - Overestimated experimental blood TCE levels
    - Underestimated percentages of metabolized TCE
  - These results indicate that **oxidative metabolism was underestimated**
  - **A scaling factor (~7× applied to Vmax value) was required to** reconcile difference in CYP2E1 activity between the *in vitro* and *in vivo* situations.

TCE: trichloroethylene

PBPK Case Study:
Simulated vs. Observed Blood Concentration

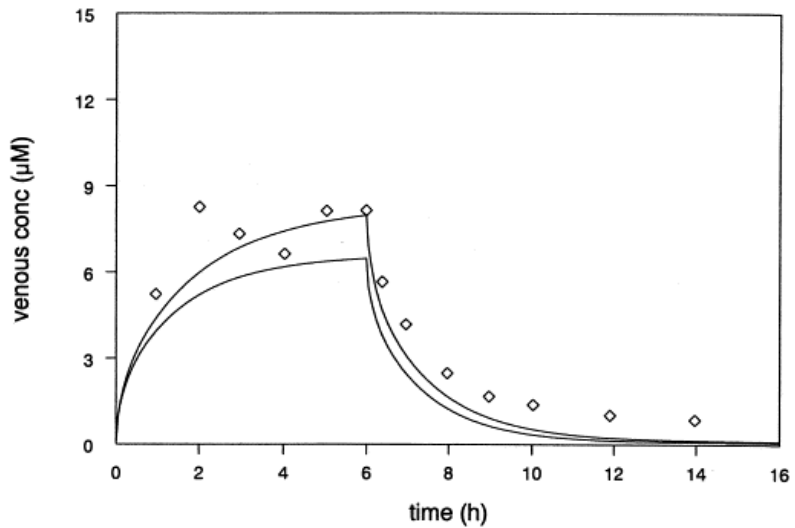
Rat, oral and IV dosing



Results: With the scaled Vmax value for **CYP2E1**, the rat PBPK model adequately predicted the TCE blood levels.

Human volunteers, Inhalation exposure (100 ppm TCE for 6 h)

The upper and lower curves show the results predicted using the lowest and highest Vmax values from 14 human liver microsomal samples



Results: Using the same scaling factor derived from rat model validation, the human PBPK model adequately predicted human TCE blood levels.

PBPK Case Study: Main Results

Comparison of rat and human PBPK model predictions: Fate of TCE at 24 h after an 8 h inhalation exposure to 35 ppm

	Rat	Human
C_{max} in blood	5.53 uM	2.15–2.48 uM
% Metabolized of total	78	62–66
% Metabolized by P450 (of total)	99.999	≥99.9999
% Metabolized by GST (of total metabolized)	0.001	μ neg: 0.00003–0.00004 μ pos: 0.00005–0.00008
% Exhaled	22	25–28
% In fat	0.007	8–10

Interindividual differences:
- Vmax of CYP2E1
- between for μ-positive and μ-negative individuals

- **Main results:**
  - CYP2E1 dominates TCE metabolism.
  - GST-mediated metabolism (nephrotoxicity-relevant pathway) is much higher in rats than in humans.
  - Humans exhibit greater retention of parent TCE in fat.
- **Risk assessment implications:**
  - Assuming GST-derived metabolites drive toxicity, humans are less sensitive to TCE nephrotoxicity than rats, despite similar external exposures.

PBPK Case Study

Strengths

- Mechanistically grounded PBPK model
- Use enzyme-specific *in vitro* data rather than empirical fitting alone
- Direct comparison of species differences and interindividual variability in metabolism
- High relevance to TCE risk assessment

Weaknesses

- Empirical scaling factor required for CYP2E1
- High-dose oral rat data not well simulated due to saturation effects
- Toxicodynamics (e.g., DNA damage, repair, tumor formation) not modeled
- Reliance on the assumption that GST pathway drives nephrotoxicity

PBPK Case Study: Summary

The study demonstrated that PBPK modeling informed by *in vitro* enzyme kinetics can quantitatively explain species and human variability in TCE metabolism.

By identifying pathway-specific **internal dose metrics** rather than relying on external exposure, the model showed that humans are likely **less susceptible** than rats to GST-mediated TCE nephrotoxicity.

The paper exemplifies how PBPK modeling provides mechanistic insights and risk-relevant conclusions, which cannot be obtained using default uncertainty factor approaches.

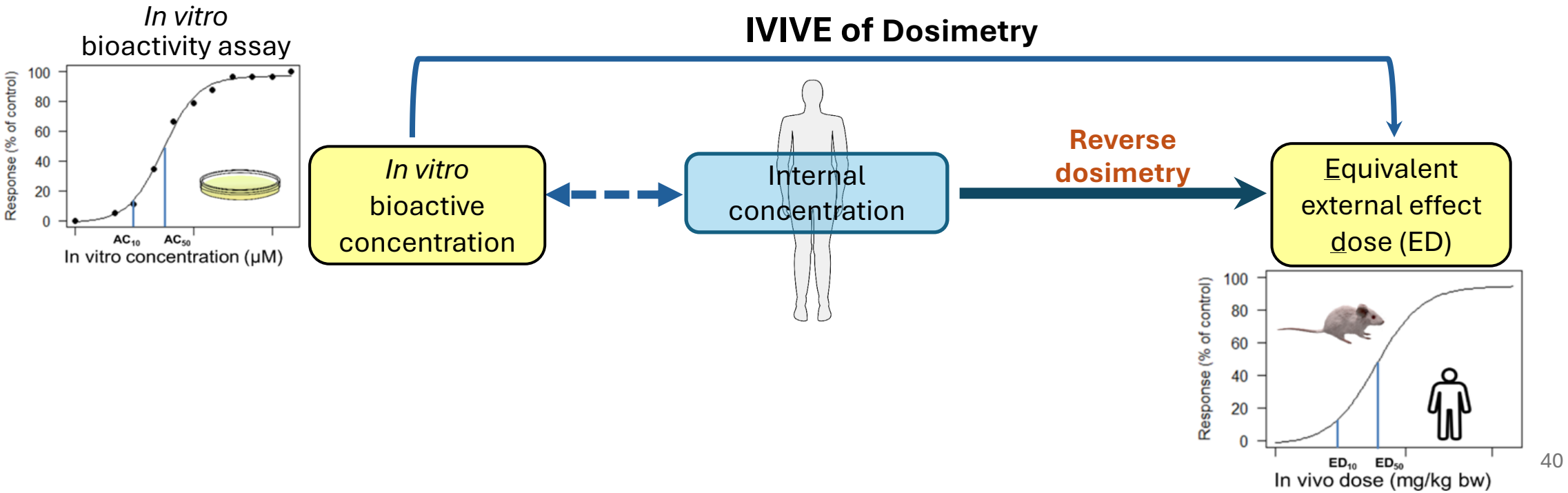
Based on the case study, is GST-mediated metabolism higher in rats or human?

IVIVE

IVIVE

- What *in vivo* dose corresponds to an active *in vitro* concentration?
- Can cell-based assay results predict human health effects?
- What is the human equivalent dose for observed *in vitro* effects?

Purpose: ***In vitro* to *in vivo* extrapolation** (IVIVE) translates *in vitro* assay results (cell-based, biochemical) into predictions relevant to *in vivo* dose or toxicity in whole organisms.



IVIVE Case Study

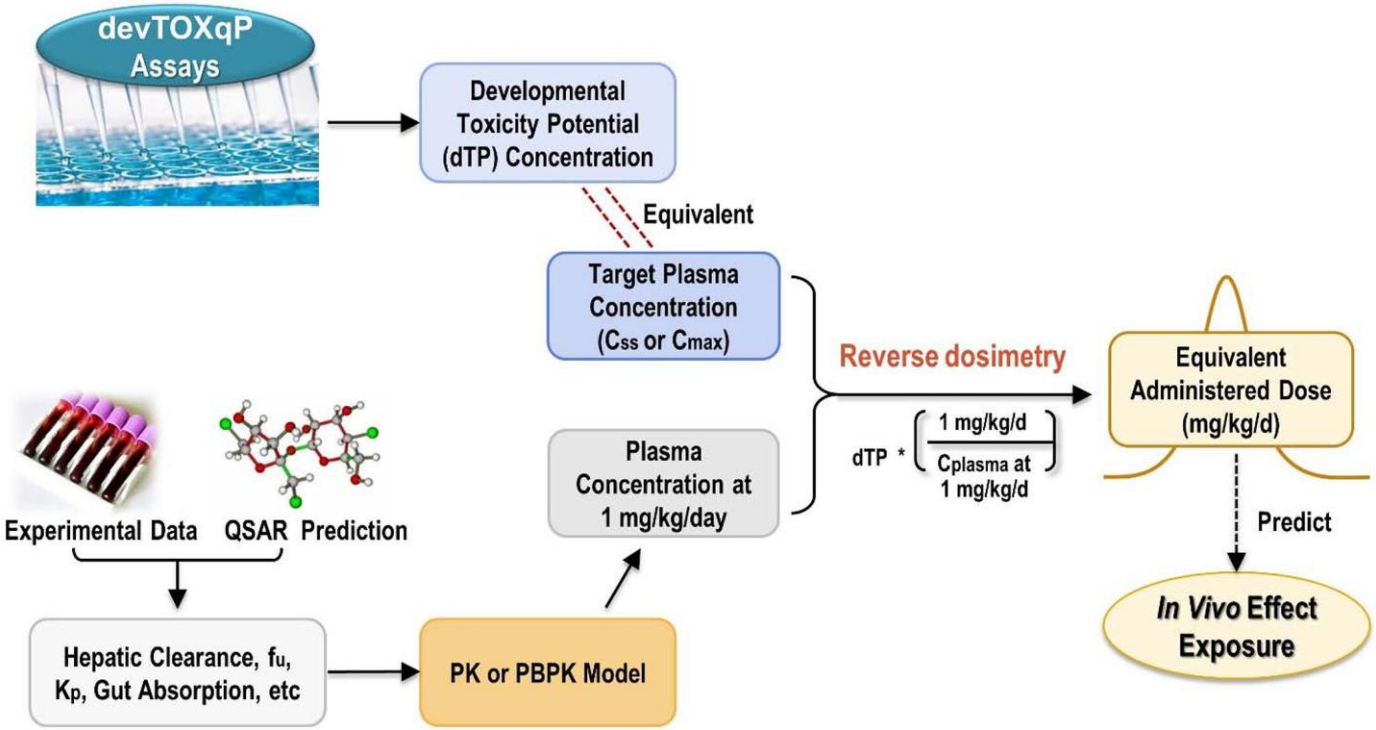
Quantitative *in vitro* to *in vivo* extrapolation for developmental toxicity potency of valproic acid analogues

Chang et al., 2022

Birth Defect Research

doi: 10.1002/bdr2.2019

Can ***in vitro*** **activity concentrations** from a human stem cell-based developmental toxicity assay be used to quantitatively predict ***in vivo*** **doses relevant for developmental toxicity** for valproic acid (VPA) and its analogues?



IVIVE: Predicting equivalent administered doses (EADs) from in vitro bioactivity concentrations in the devTOXqP assay

IVIVE Case Study: *In Vitro* Data

Chemicals:

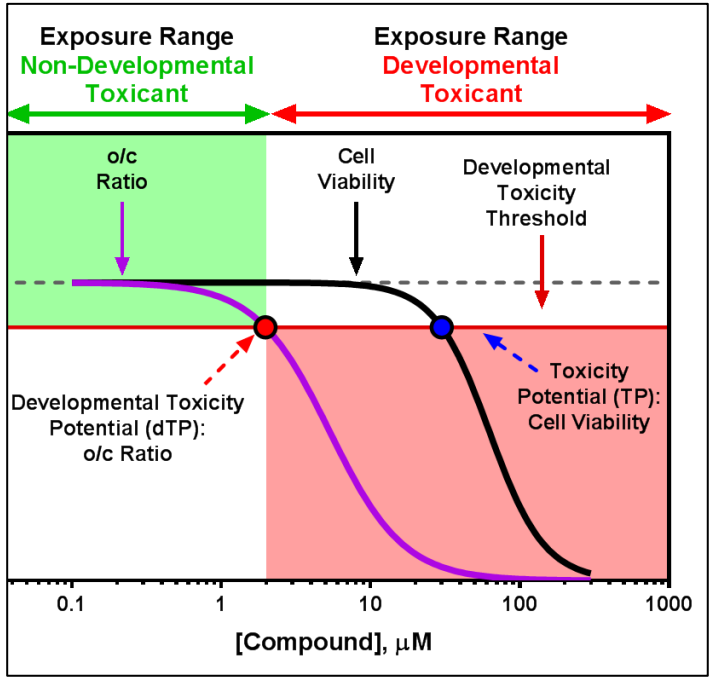
- Valproic acid (VPA) and its nine analogues
- Short-chain aliphatic acids
- VPA is an anticonvulsant and antiepileptic drug
- VPA is well known to cause birth defects in humans and animals (Ornoy et al., 2009)

***In vitro* assay data:**

- Developmental toxicity potential (dTP) concentration from the devTOX^{qP} assay

- The devTOX^{qP} assay:
  - **A biomarker-based human pluripotent stem cell assay for developmental toxicity screening** (Palmer et al., 2017)
  - Detects changes in secreted and consumed ornithine (o) and cystine (c) in spent medium
  - The **o/c ratio** is predictive of developmental toxicity potential

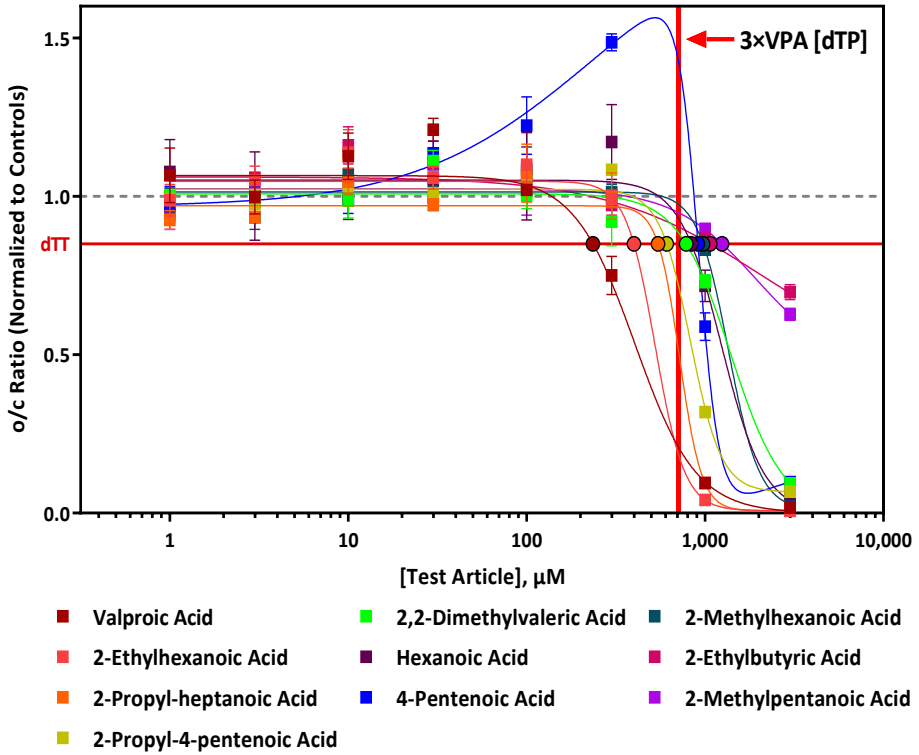
Stemina devTOX quickPredict assay



Stemina Biomarker Discovery, Inc.

IVIVE Case Study: *In Vitro* Data

***In vitro* assay data:** Developmental toxicity potential (dTP) concentration from devTOX^{qP} assay



Dose response curves of VPA analogues in devTOX^{qP} assays. The x-axis represents the concentration of the test article, and the y-axis represents the normalized o/c ratio.

Chemical Name†	dTP (µM)	$dTP_{analogue}$ / dTP_{VPA}	dTP-free medium (µM)	*In vivo* potency‡
Valproic acid	236	1.0	101.5	+++
2-Ethylhexanoic acid	399	1.7	191.5	+
2-Propylheptanoic acid	546	2.3	125.6	+++
2-Propyl-4-pentenoic acid	611	2.6	488.8	++
2,2-Dimethylpentanoic acid	784	3.3	423.4	-
Hexanoic acid	838	3.6	662.0	ND
4-Pentenoic acid	913	3.9	894.7	-
2-Methylhexanoic acid	976	4.1	605.1	-
2-Ethylbutyric acid	1071	4.5	888.9	-
2-Methylpentanoic acid	1248	5.3	1010.9	ND

†, chemicals are sorted from the lowest to highest dTP values; ‡, Potency relative to VPA based on results in the NMRI exencephaly-mouse model using decision criteria in Eikel et al. (2006).

IVIVE Case Study: PK/PBPK Models

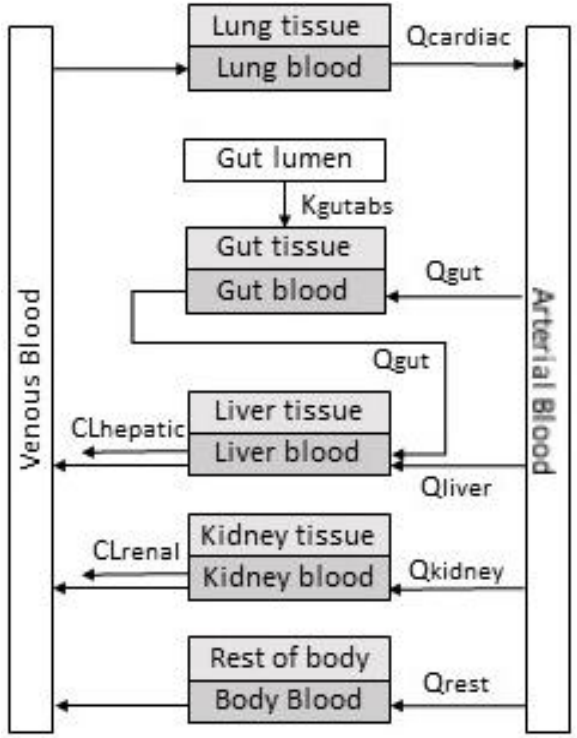
Structures of PK/PBPK models used for IVIVE

A population-based PK (PPK) model

$$C_{ss} = \frac{\text{Standard oral dose rate (1 mg/kg/day)}}{(\text{GFR} * f_u) + \left(\text{Q}_{\text{liver}} * f_u * \frac{CL_{\text{int}}}{\text{Q}_{\text{liver}} + f_u * CL_{\text{int}}} \right)}$$

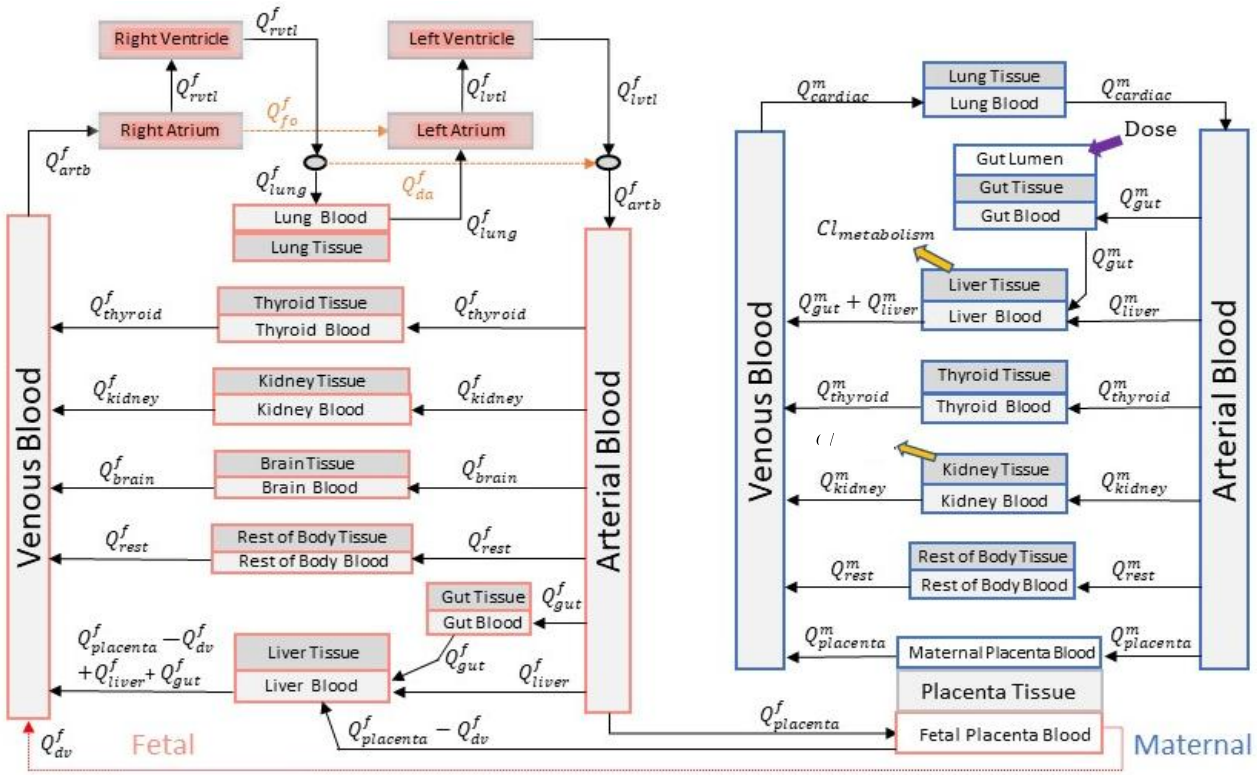
CLHepatic, hepatic clearance (L/h); CLint, intrinsic hepatic clearance (L/h); CLRenal, renal clearance (L/h); fu, fraction of chemical unbound to plasma protein; GFR, glomerular filtration rate (L/h); Q, blood flow rate

B HTTK PBTK model

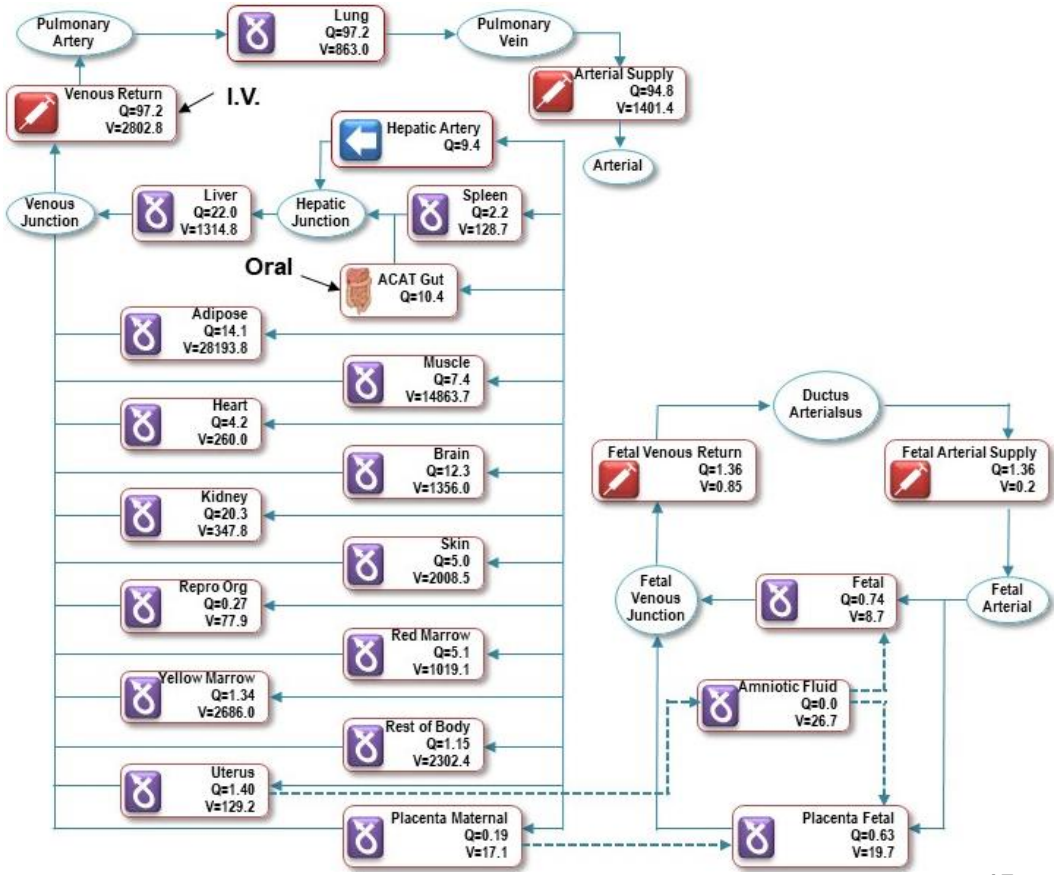


IVIVE Case Study: PK/PBPK Models

C HTTK pregnancy-specific PBTK model (HTTK.fPBTK)



D GastroPlus™ Pregnancy PBPK model (Proprietary)



ACAT, advanced compartmental absorption and transit model; BW, body weight; Cl, clearance; CLint, intrinsic clearance; Clrenal, renal clearance; I.V., intravenous injection; Q, blood flow rate; Q^f, fetal tissue blood flow; Q^m, maternal tissue blood flow; V, volume

IVIVE Case Study: Rat Data for Validation

Rat lowest effect levels (LELs) derived from *in vivo* toxicity studies

Chemical	Dose range (mg/kg/day)	Strains	Route	Gestational dosing period	LEL: maternal toxicity (mg/kg/day)	LEL: fetal toxicity (mg/kg/day)	Reference
VPA	0-800	Sprague-Dawley	NA	GD 8-17	NA	200	Table 2 (Binkerd et al. 1988)
	0-400	Sprague-Dawley	Corn oil gavage	GD 6-15	400	200	Tables 5 and 6 (Narotsky, Francis, and Kavlock 1994)
EHA	0-600	Wistar	Drinking water	GD 6-19	600	100	(Pennanen et al. 1992)
	0-1000	Fischer 344	NA	GD 6-15	500	250	(Hendrickx et al. 1993)
PA	0-100	Sprague-Dawley	Corn oil gavage	GD 6-15	75	50	Tables 1 and 8 (Narotsky, Francis, and Kavlock 1994)
EBA	0-200	Sprague-Dawley	Corn oil gavage	GD 6-15	150	NA	Table 1 (Narotsky, Francis, and Kavlock 1994)
MPA	0-250	Sprague-Dawley	Corn oil gavage	GD 6-15	188	NA	Table 1 (Narotsky, Francis, and Kavlock 1994)

NA, not available; GD, gestation day

IVIVE Case Study: Results

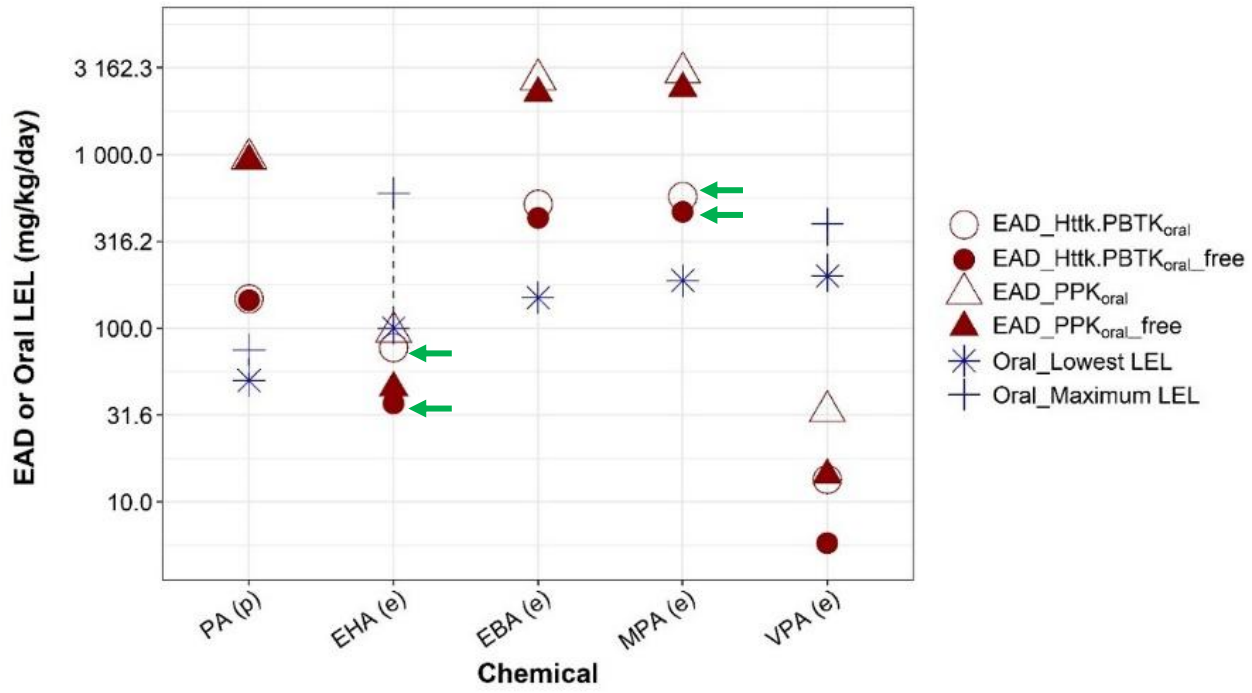


Fig 1. Equivalent administered doses (EADs) compared to oral rat lowest effect levels (LELs)

Main results:

- The EADs can adequately predict rat developmental toxicity LELs
- The **Httk.PBTK model** provided the **most accurate overall predictions** for the rat developmental toxicity LELs
- For 4 of 5 VPA analogues, EADs estimated using the Httk.PBTK model are within **3.5-fold** of the lowest or highest rat LELs.
- The difference in EAD estimates between the PPK and Httk.PBTK models ranges from 1.9- to 6.5-fold across chemicals.
- EAD estimates based on free media concentrations are lower than nominal concentrations for all chemicals.

(e): using experimental Clint values
(p): using predicted Clint values from QSAR model

Httk.PBTK: PBTK model in httk R package
PPK: population-based PK model
free: unbound concentration in medium used for IVIVE
LEL: lowest effect level from rat developmental toxicity study

IVIVE Case Study: Results

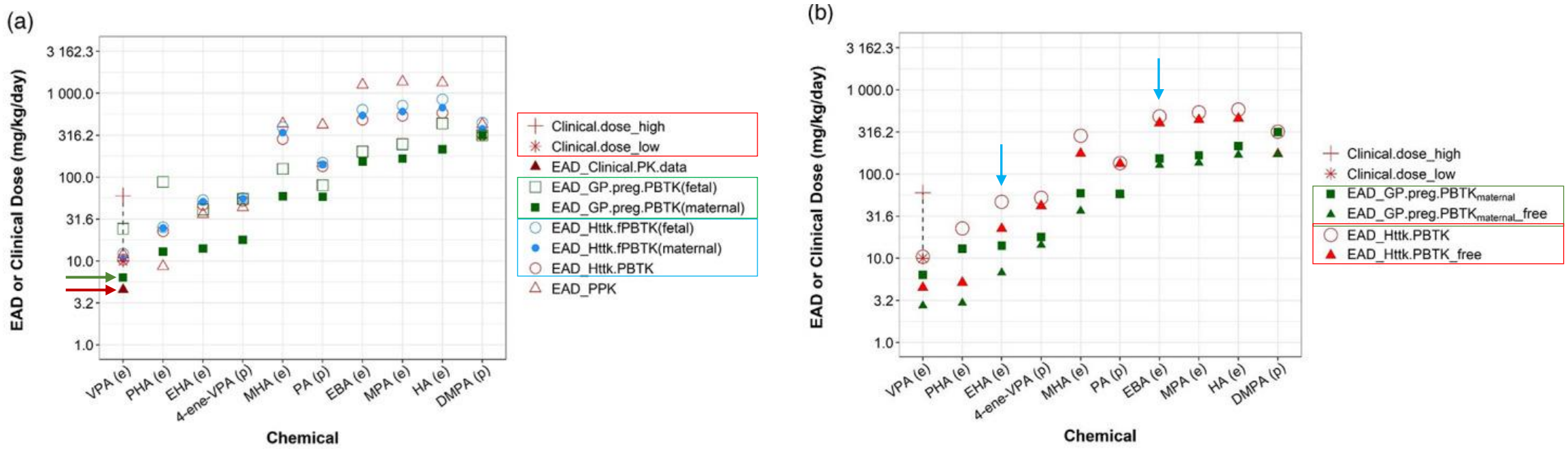


Fig 2. Human EADs compared to human clinic data

Httk.PBTK: PBTK model in httk R package
Httk.fPBTK: pregnancy-specific PBTK model in httk
GP.preg.PBTK: GastroPlus™ Pregnancy PBPK model
free: unbound concentration in medium used for IVIVE
(fetal): fetal Cmax as targeted concentration
(maternal): maternal Cmax as targeted concentration

Main results:

- All human PK models produce EADs that approximate the minimum clinical dose of VPA.
- The GastroPlus pregnancy model, using maternal plasma Cmax as the target internal concentration, provides the most conservative estimate.
- The impact of in vitro kinetics on EAD estimates is chemical-dependent.

IVIVE Case Study

Strengths

- Use human-relevant, mechanistically based in vitro bioactivity data
- Cross-platform comparison of multiple PK/PBPK modeling approaches, including pregnancy-specific models, enabling evaluation of model-dependent variability
- The majority of modeling platforms are open-source
- Assessment of the impact of in vitro kinetics on EAD estimates

Weaknesses

- A limited number of chemicals were used in the study
- Use of linear kinetic assumptions in most PK/PBPK models
- Lack of toxicodynamic modeling

IVIVE Case Study: Summary

In vitro assay-derived EADs aligned well with in vivo rat LELs and human clinical doses, especially when PBPK models were used.

Adjusting for free in vitro concentrations yielded more conservative EADs

This study provides a promising example of using in vitro mechanistic relevant assay and IVIVE to predict in vivo toxicity exposure.

What is the main purpose of IVIVE in this study?

In this IVIVE study, which dose metric was used to link in vitro activity concentrations to in vivo exposure?

Questions?

Visit https://www.thepsci.eu/in-silico-tools-webinars/ for webinar materials

- *In Silico* Methods Quick Reference Sheet
- Glossary
- List of Additional Resources

Aswani.Unnikrishnan@inotiv.com

Xiaoqing.Chang@inotiv.com