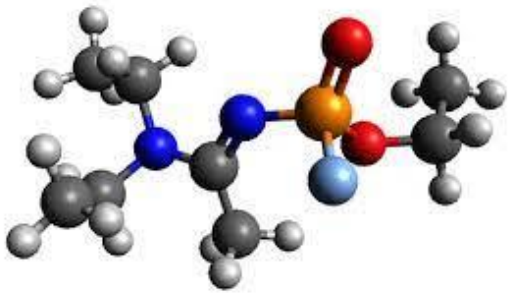

Application of PBK Modeling and (Q)IVIVE for Prioritization of Chemicals for Toxicity Testing

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SimulationPlus MIDD+ 2023 Conference

February 16, 2023

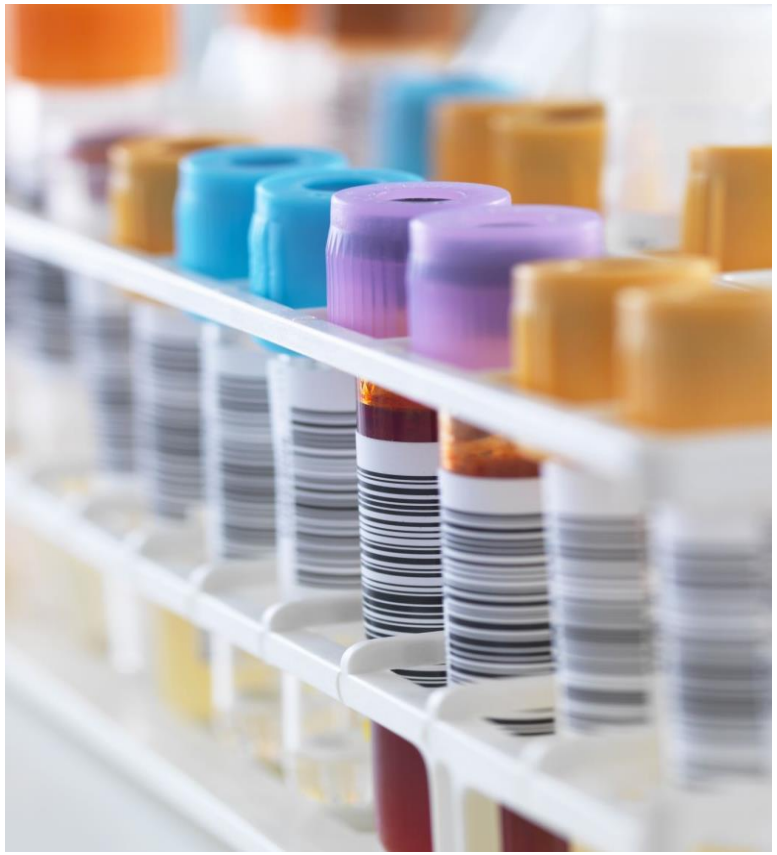
Disclaimer

I declare that I have no conflict of interest. The views expressed in this presentation are those of the author and do not necessarily represent the views or policies of Inotiv or NICEATM.

Outline

- Current trend of chemical safety evaluations
  - Replacement of in vivo studies with in vitro assays
- NICEATM
  - To promote the application of new approach methodologies
- PB(P)K model and (Q)IVIVE
  - To put in vitro data into in vivo context
  - Key components
  - Case studies

Promote Use of NAMs for Chemical Safety Evaluation



- New Approach Methodologies (NAMs)
  - *In vitro* tests, *in chemico* assays and *in silico* models
  - Promote the "3Rs" (reduce, refine, replace)
  - Can provide mechanistic-based, more human-relevant information
  - Can be high throughput
- Goals/Challenges
  - To relate in vitro data to in vivo outcomes
  - Acceptance into regulatory decision making
- Possible approaches
  - (Q)IVIVE: in vitro to in vivo extrapolation

NICEATM

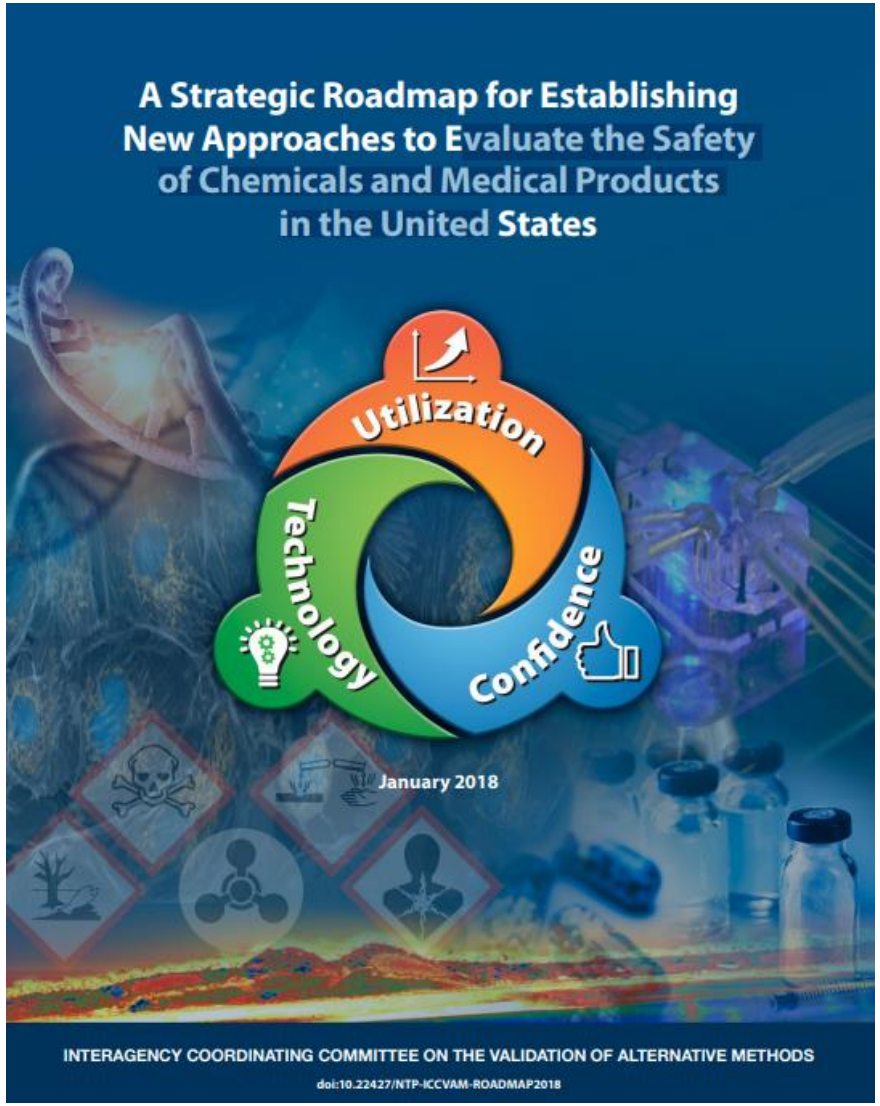
https://ntp.niehs.nih.gov/whatwestudy/niceatm/index.html

NICEATM: the **N**ational Toxicology Program (NTP) **I**nteragency **C**enter for the **E**valuation of **A**lternative **T**oxicological **M**ethods

Focuses on developing and evaluating data from alternative test methods

Provides operational and scientific support to **I**nteragency **C**oordinating **C**ommittee on the **V**alidation of **A**lternative **M**ethods (ICCVAM)

Provides information through its website

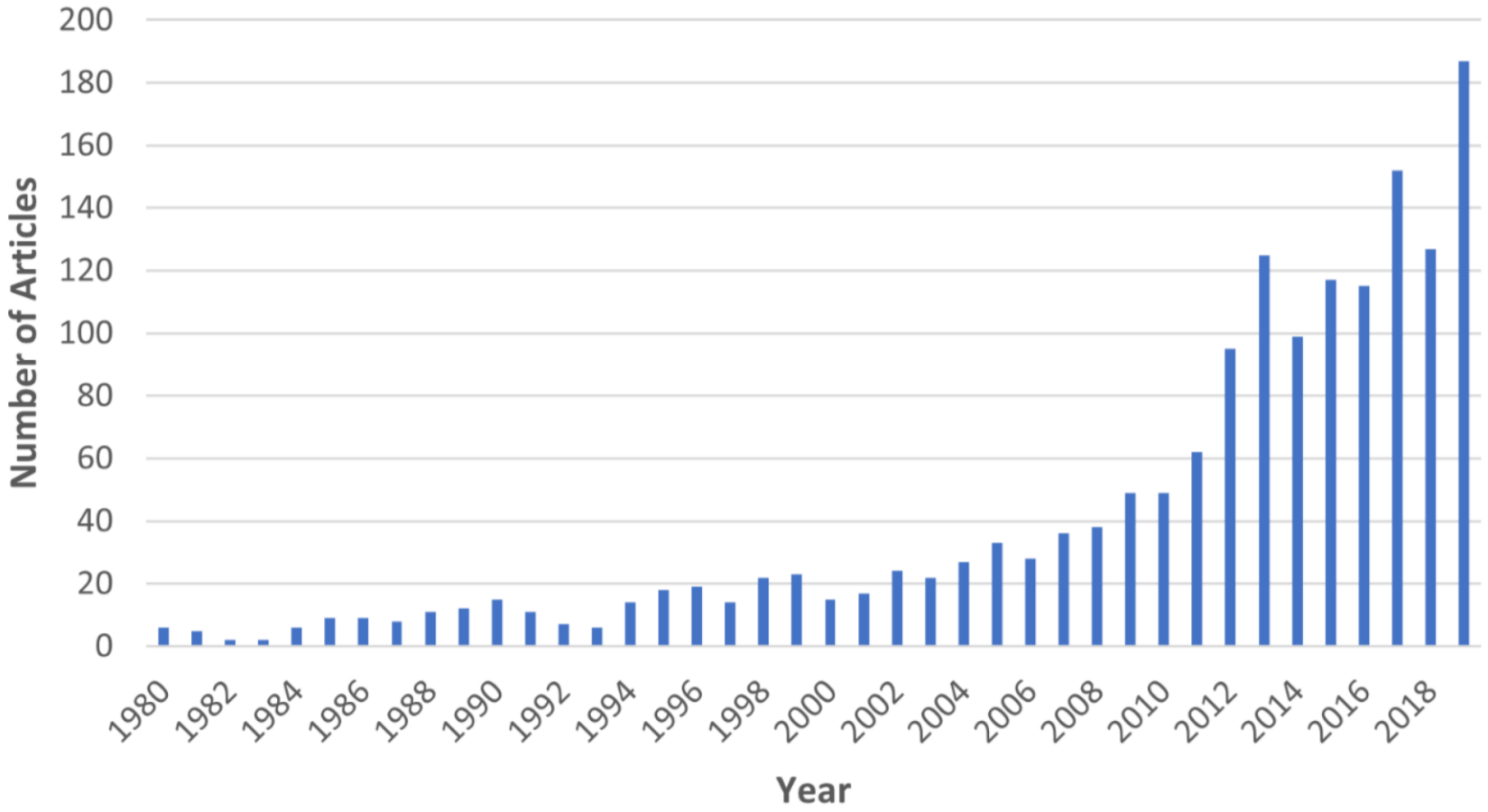


ICCVAM Strategic Roadmap for Establishing New Approaches

- To connect end users (e.g., regulators, industry) with the developer of NAMs
  - End-users help guide the development of the alternative methods
- To establish confidence in new methods
  - Use efficient and flexible approaches
- To ensure adoption of new methods by both federal agencies and industry

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). https://dx.doi.org/10.22427/NTP-ICCVAM-ROADMAP2018

In Vitro to In Vivo Extrapolation (IVIVE) Relevant Publications



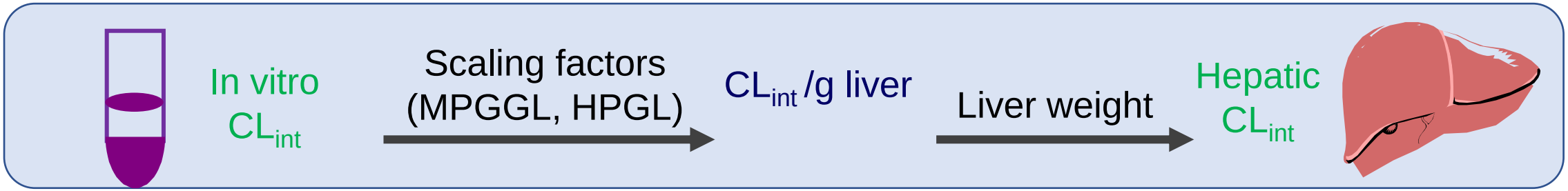
Chang X, et al. IVIVE: Facilitating the Use of In Vitro Toxicity Data in Risk Assessment and Decision Making. Toxics 2022, 10(5), 232

Clarification of the "IVIVE" Terminology

Broadly defined: an approach utilizing **in vitro experimental data** to predict **in vivo phenomena or outcomes** (e.g., exposure, effects)

IVIVE of ADME parameters

E.g., scaling from in vitro metabolic clearance to hepatic clearance

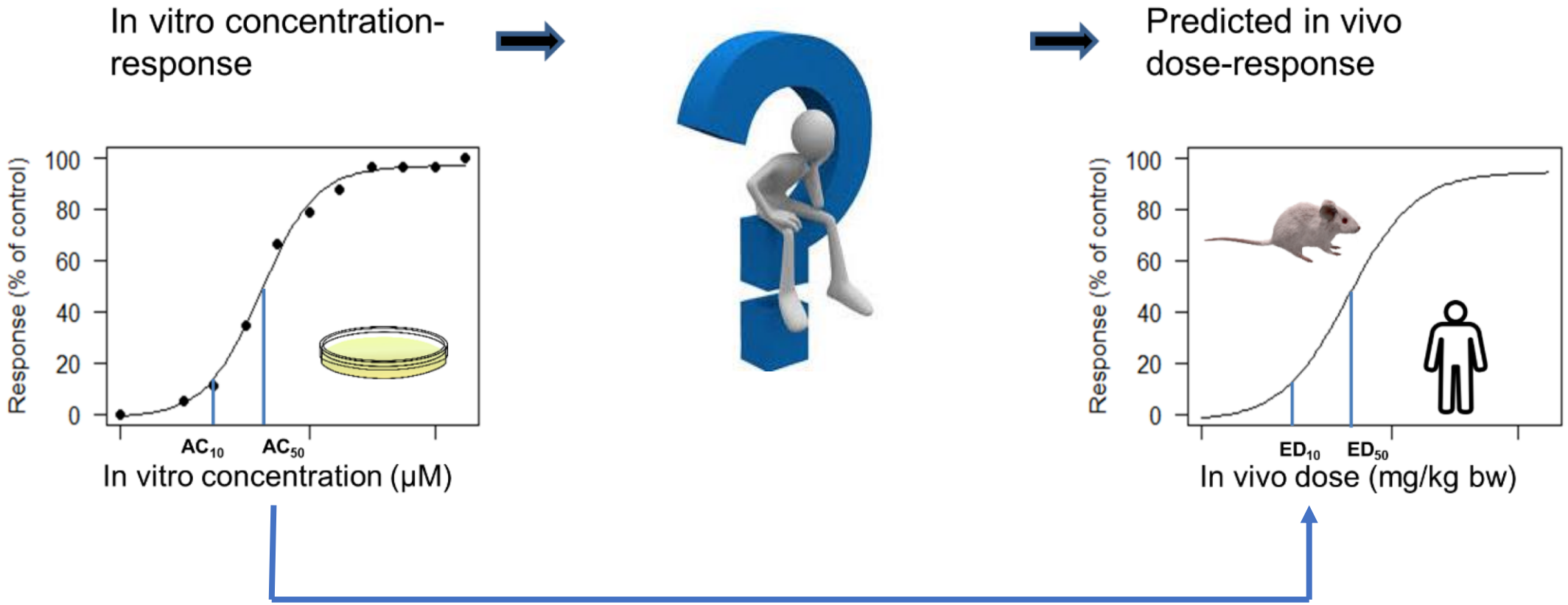


ADME: absorption, distribution, metabolism, and exposure
MPPGL: microsomal protein per gram of liver
HPGL: hepatocellularity per gram of liver

Chang X, et al. IVIVE: Facilitating the Use of In Vitro Toxicity Data in Risk Assessment and Decision Making. Toxics 2022, 10(5), 232

IVIVE of Dosimetry: Makes In Vitro Assay Results Human Relevant

To apply PK/TK models to translate the in vitro activity concentration (AC_x) to an in vivo exposure that would lead to an internal (blood or tissue) concentration equal to the AC_x



Chang X, et al. IVIVE: Facilitating the Use of In Vitro Toxicity Data in Risk Assessment and Decision Making. Toxics 2022, 10(5), 232

Forward versus Reverse Dosimetry

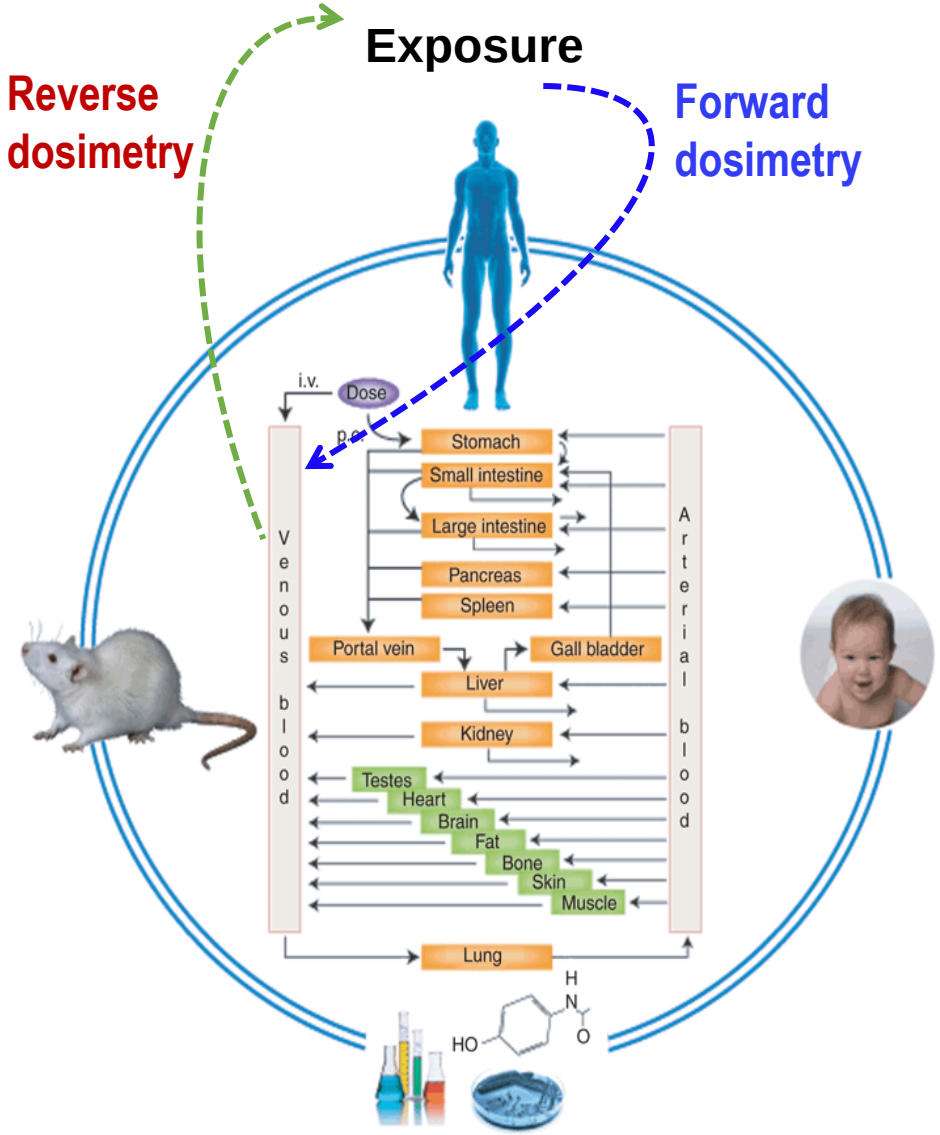
- **Forward dosimetry**

  External exposure → Internal concentration

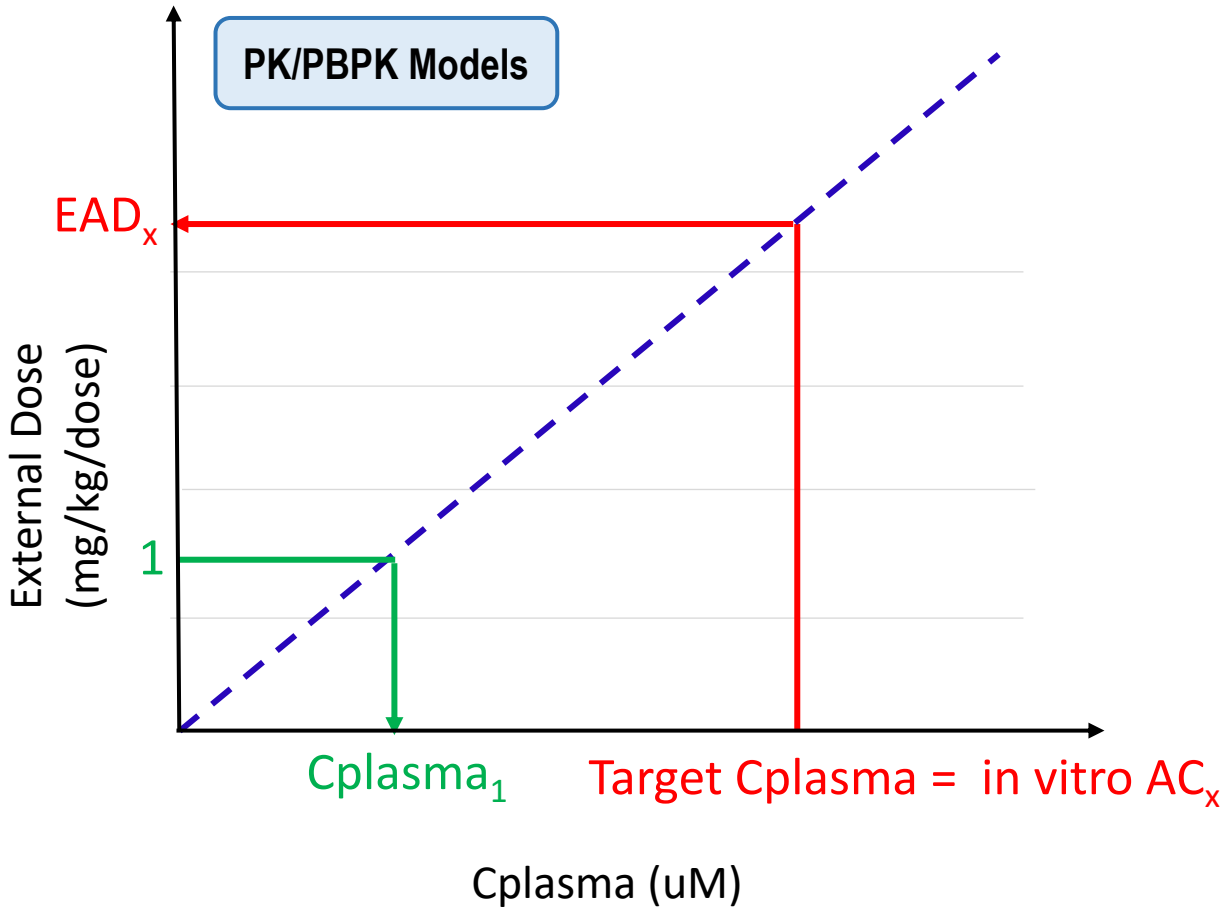
- **Reverse dosimetry**

  Biological monitoring data (e.g., blood, urine chemical levels) → External exposure

- **(Q)IVIVE**
  - In vitro activity concentration (e.g., AC50) + reverse dosimetry
  - To estimate external exposures that could lead to internal concentration equal to AC50 values



Reverse Dosimetry for (Q)IVIVE



Linear Extrapolation Assumption

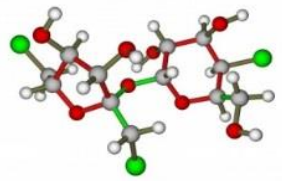
$$EAD_X = In\ Vitro\ AC_X * \frac{1\ mg/kg\ /dose}{\boldsymbol{Cplasma_1}}$$

EAD: Equivalent administrated dose
ACx: activity concentration at x% of maximum response
Cplasma: plasma concentration, Css or Cmax

Wetmore et al, Toxicol Sci. 2012. 125(1):157-74

How is (Q)IVIVE Carried Out?

Experimental measurement



QSAR/QPPR Prediction

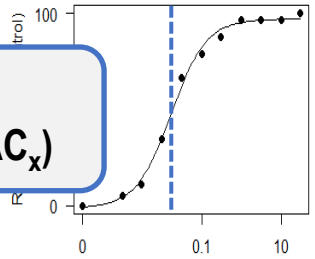
Physiochemical and PK parameters: hepatic clearance, fu, Kp, gut absorption, etc

Physiological parameters: BW, tissue weight, tissue blood flow

Kinetics: Simple PK or PBPK models

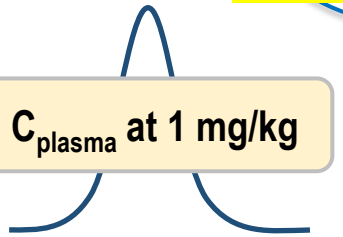
In Vitro Assays

Activity Concentration (AC_x)



Target plasma / tissue Concentration

Reverse dosimetry



C_{plasma} at 1 mg/kg

$$AC_x * \left(\frac{1\ mg/kg}{C_{plasma}\ at\ 1\ mg/kg}\right)$$

AOP

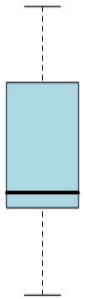
In Vivo responses

In Vivo LELs

RfD

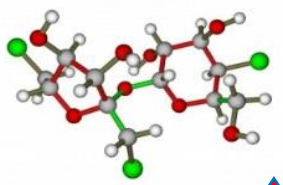
MoE

Equivalent administered doses (EADs) (mg/kg/day)



Sources of Variability

Experimental measurement



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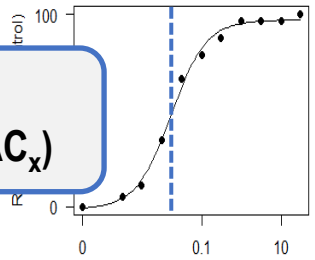

Target plasma / tissue Concentration

Reverse dosimetry

C_{plasma} at 1 mg/kg

$$AC_x * \left(\frac{1\ mg/kg}{C_{plasma}\ at\ 1\ mg/kg}\right)$$

AOP

In Vivo responses

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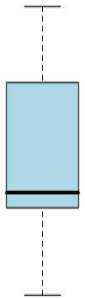
Equivalent administered doses (EADs) (mg/kg/day)



Case study 1: Using VPA and its analogues to demonstrate how (Q)IVIVE can be applied to predict in vivo toxicity exposure

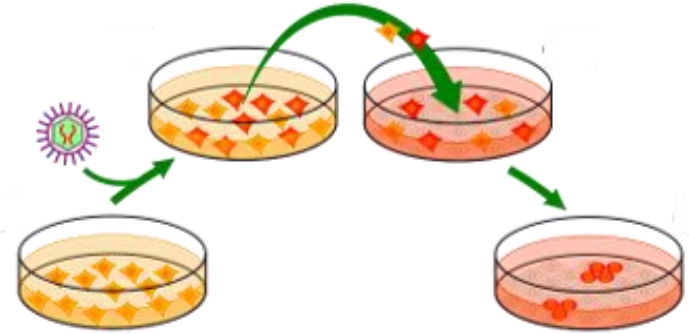
Birth Defects

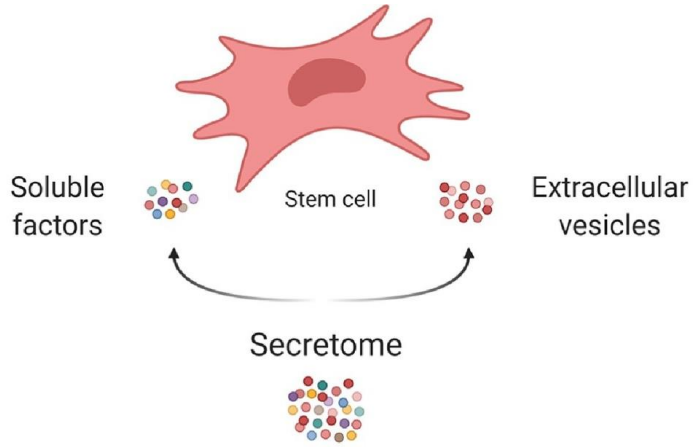
- Data & Statistics on birth defects
  - Affect approximately 3% of all babies born in the United States each year (CDC, 2020)
  - Many of birth defects are caused by in utero exposure to various pharmaceutical and environmental chemicals (Weinhold B, 2009)
  - Faster and cheaper methods are required for large-scale screening
- Example Chemicals: valproic acid (VPA) and its 9 analogues
  - VPA is anti-convulsant and anti-epileptic drug
  - VPA is well known to be teratogenic in humans and animals (Ornoy A, 2009).
  - Short-chain aliphatic acids

CDC, 2020. https://www.cdc.gov/ncbddd/birthdefects/data.html;
Weinhold B, et al., 2009. Environ Health Perspect 117(10): A440-A447; Ornoy A. 2009. Reprodctive Toxicology 28(1): 1-10

Stemina devTOX quickPredict (devTOX^{qP}) Assay

- A biomarker-based human pluripotent stem cell assay for developmental toxicity screening (Palmer JA et al. 2017)
- Measured changes in secreted and consumed metabolites in spent medium after chemical exposure
- Ornithine (o) & Cystine (c)
  - Involved in metabolic pathways critical for cell proliferation and differentiation during embryonic and fetal development
  - o/c Ratio as predictive of developmental toxicity potential

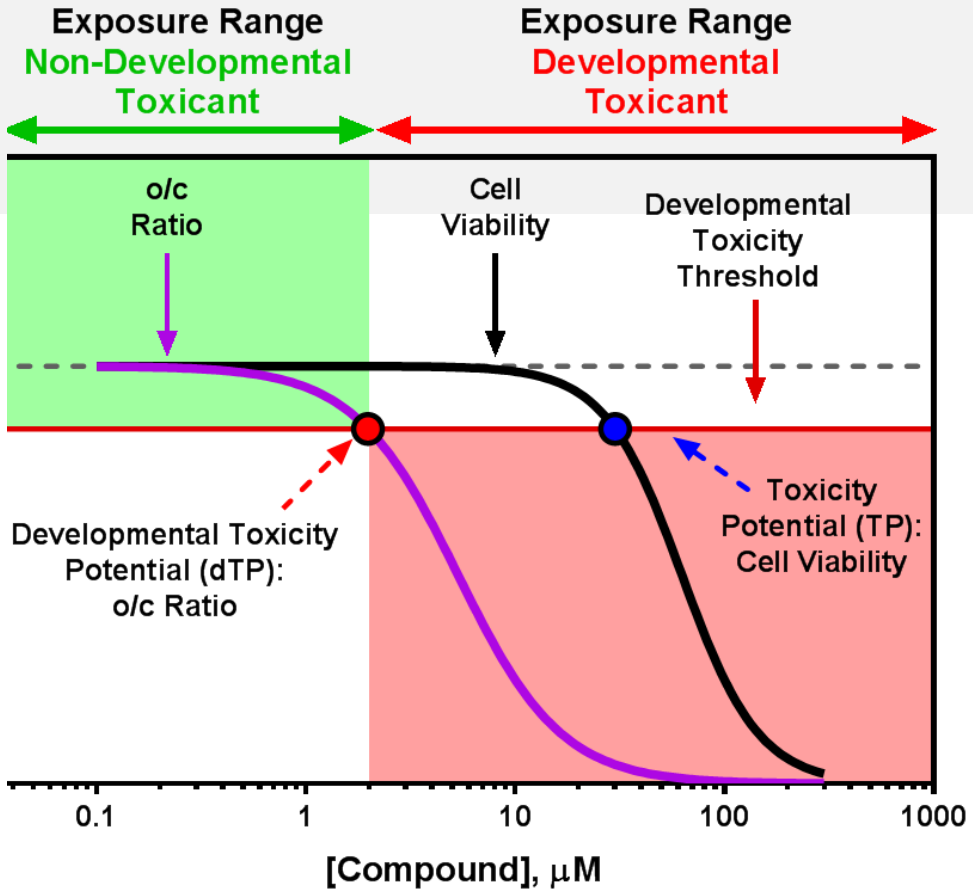




Palmer JA et al., 2017. Reprod Toxicol 73:350-361. Daneshmandi L, et al., 2020. Trends in Biotechnology, 38(12)

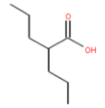
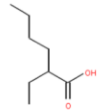
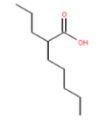
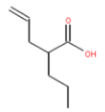
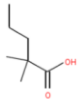
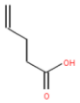
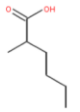
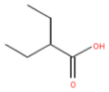
Interpreting devTOX^{qP} Assay Dose Response Data

- Viability and o/c ratio are function of chemical exposure
- Identify exposure level that alters metabolism
  - Developmental Toxicity Threshold (dTT)
  - **D**evelopmental Toxicity Potential (dTP) concentration
  - **T**oxicity **P**otential (**TP**) concentration (Cytotoxic)



Correlation between In Vitro and In Vivo Assays

- The devTOX^{qP} Assay Results are concordant with available in vivo mammalian potency data

Analogue (Preferred Name)	CAS	Structure	Molecular Weight	devTOX^{qP} dTP (µM)	$dTP_{Analogue}$ / dTP_{VPA}	*In Vivo* Potency[a]
Valproic acid (VPA)	99-66-1		144.21	**236**	**1.0**	+++[1,2,3]
2-Ethylhexanoic acid (2EHA)	149-57-5		144.21	**399**	**1.7**	+[4]
2-Propylheptanoic acid (2PHA)	31080-39-4		172.26	**546**	**2.3**	+++[1]
2-Propyl-4-pentenoic acid (4-ene-VPA)	1575-72-0		142.20	**695**	**2.9**	++[1,2,3]
2,2-Dimethylvaleric acid (2,2DVA)	1185-39-3		130.18	**784**	**3.3**	-[2,3]
4-Pentenoic acid (4PA)	591-80-0		100.12	**913**	**3.9**	-[2,3]
2-Methylhexanoic acid (2MHA)	4536-23-6		130.18	**976**	**4.1**	-
2-Ethylbutyric acid (2EBA)	88-09-5		116.16	**1,071**	**4.5**	-[2,3]

[a]Potency relative to VPA based on results in the NMRI exencephaly-mouse model using decision criteria in Eikel et al.[1]
ND: Not Determined

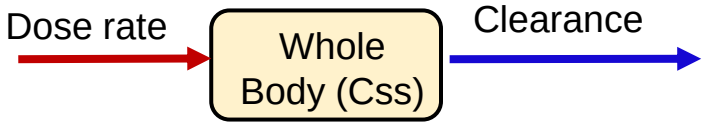
Table is provided by Jessica Palmer

Selected PK/PBPK Modeling Tools

Types	Examples	Pros	Cons
Commercial PBPK building software	GastroPlus / SimCyp / PKSim	Ready to use, allowing for more complex modeling, e.g., non-linear kinetics	Costly, not transparent, not designed for reverse dosimetry
Commercial modeling software	Matlab / Berkeley Madonna / acslX	Flexibility, better transparent	Costly, steep learning curve
Open-source modeling software	R language	Open source, transparent, flexibility	Learning curve
Open-source modeling package	High throughput toxicokinetic (HTTK) R package https://cran.r-project.org/package=httk	Open source, transparent, environmental chemicals	Learning curve
Read to use, open-access web tool	Integrated Chemical Environment (ICE) https://ice.ntp.niehs.nih.gov/	Open source, transparent, user-friendly interface	Limited flexibility

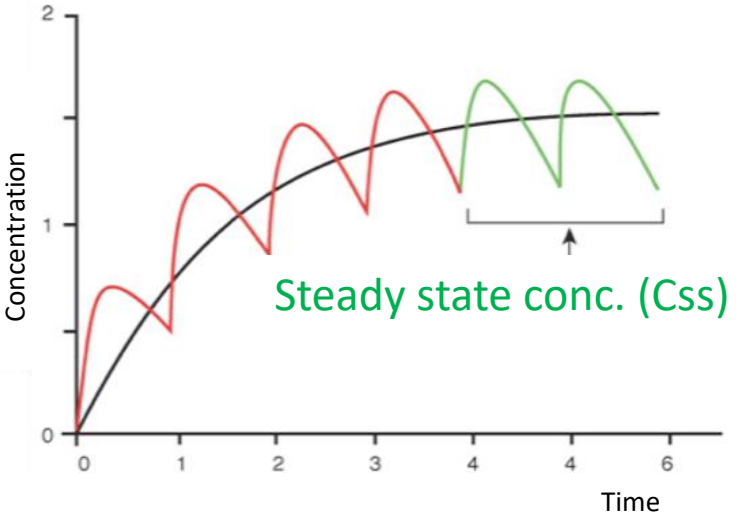
PK/PBPK Models for (Q)IVIVE

A PPK model

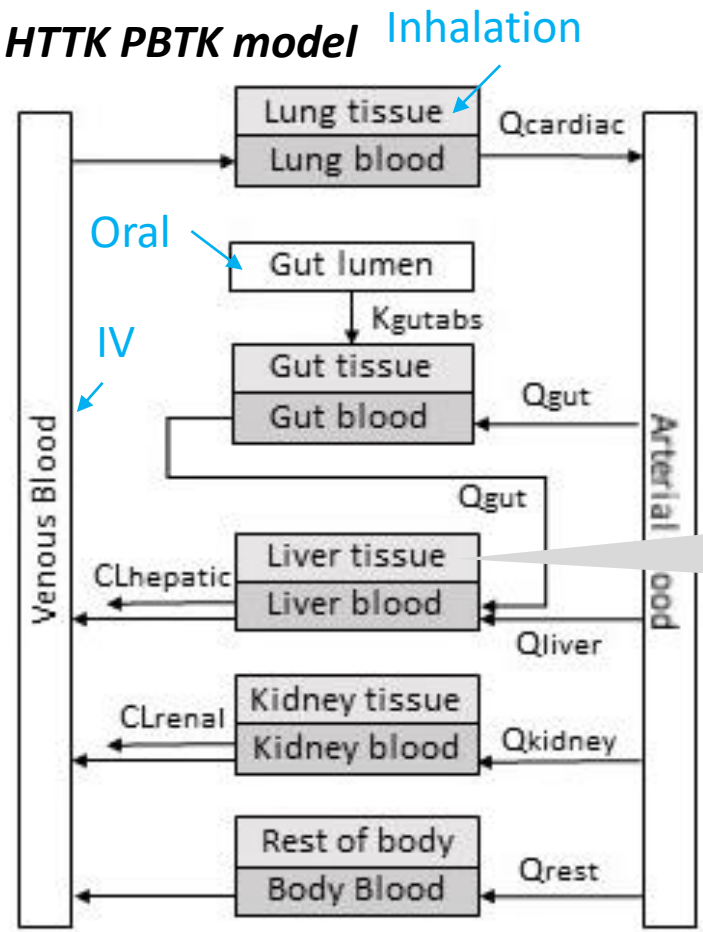


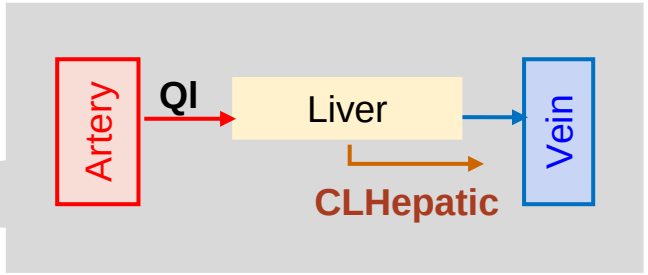
$$C_{ss} = \frac{\text{Dose rate}}{GFR * fu + Q_{liver} * \frac{fu * Clint}{Q_{liver} + fu * Clint}}$$

Clint: Intrinsic clearance;
Qliver: Blood flow to liver;
GFR: Glomerular filtration rate;
fu: fraction unbound to plasma protein



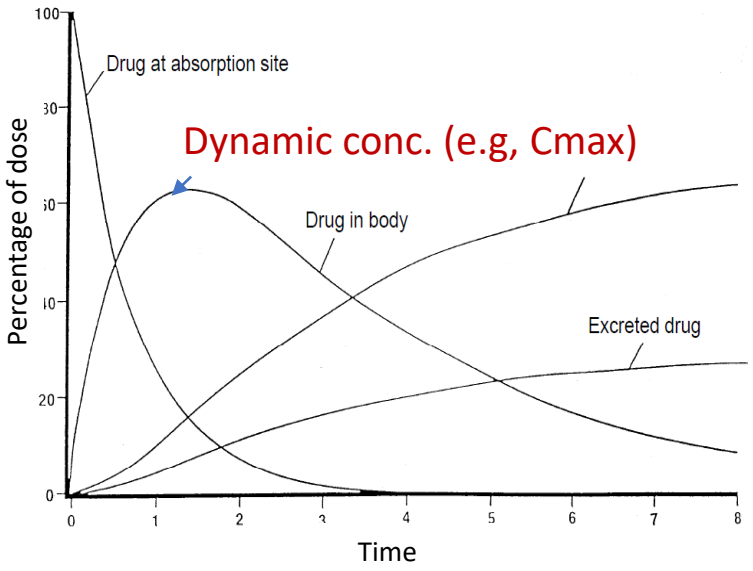
B HTTK PBTK model





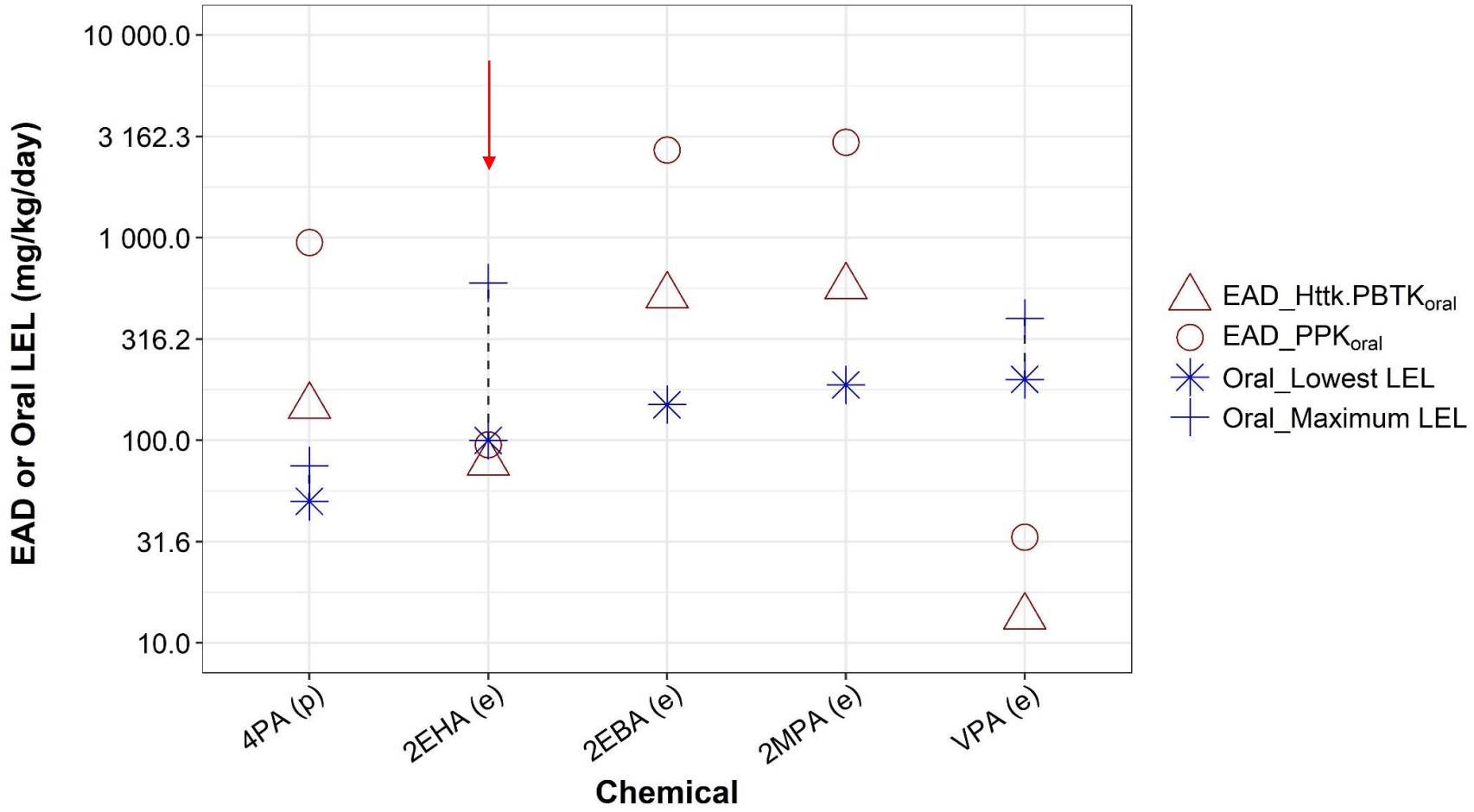
$$\frac{dA_i}{dt} = Q_i \left(C_{arterial} - \frac{A_i}{P_i * Vi} \right) - Ci * fu * CLi$$

A_i: amount in tissue i ; Q_i: blood flow to tissue I;
V_i: volume of tissue i; CL_i: metabolic clearance;
P_i: the tissue to plasma partition coefficient



Rowland and Tozer, 1995

Impact of Different PK Models on IVIVE: Compared to Rat LELs



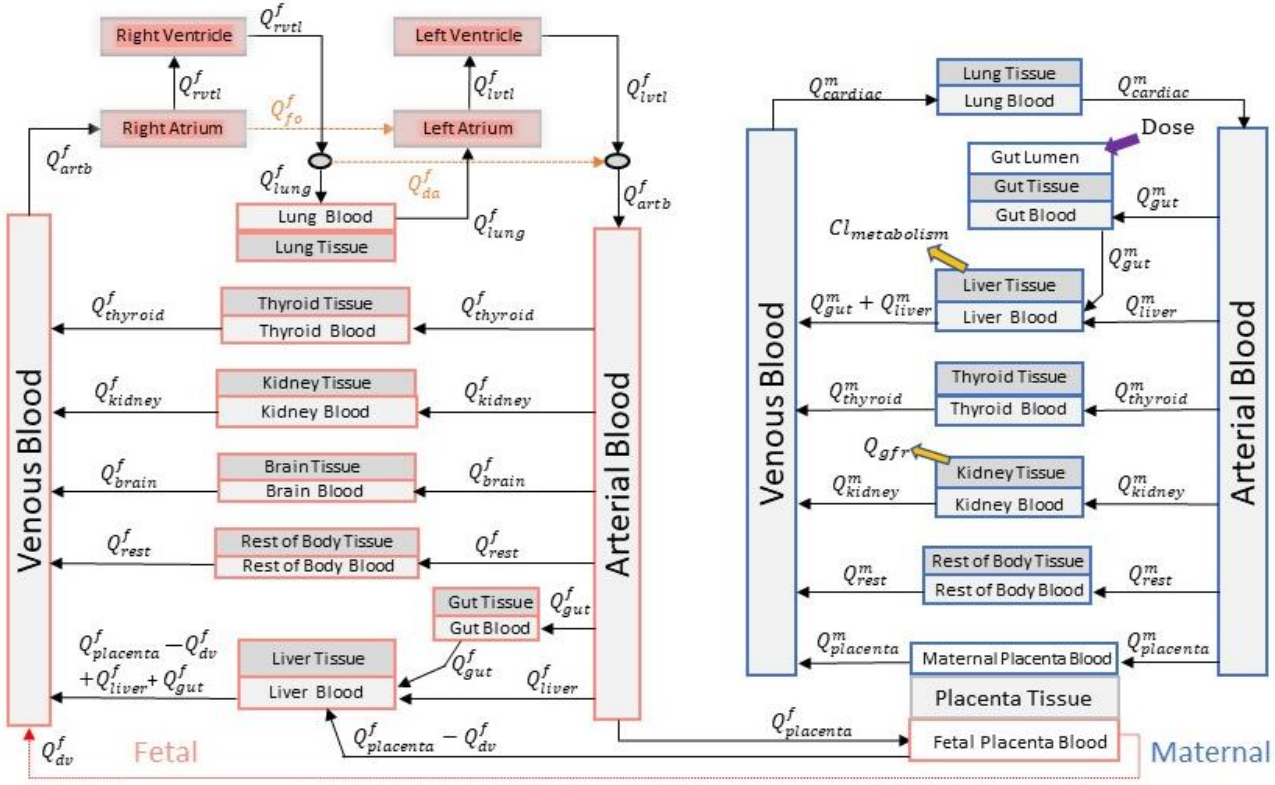
(e): using experimental Clint values
(p): using predicted Clint values from QSAR model

Httk.PBTK: PBTK model in httk R package (v1.8)
PPK: population-based PK model
LEL: lowest effect level from in vivo rat developmental toxicity study

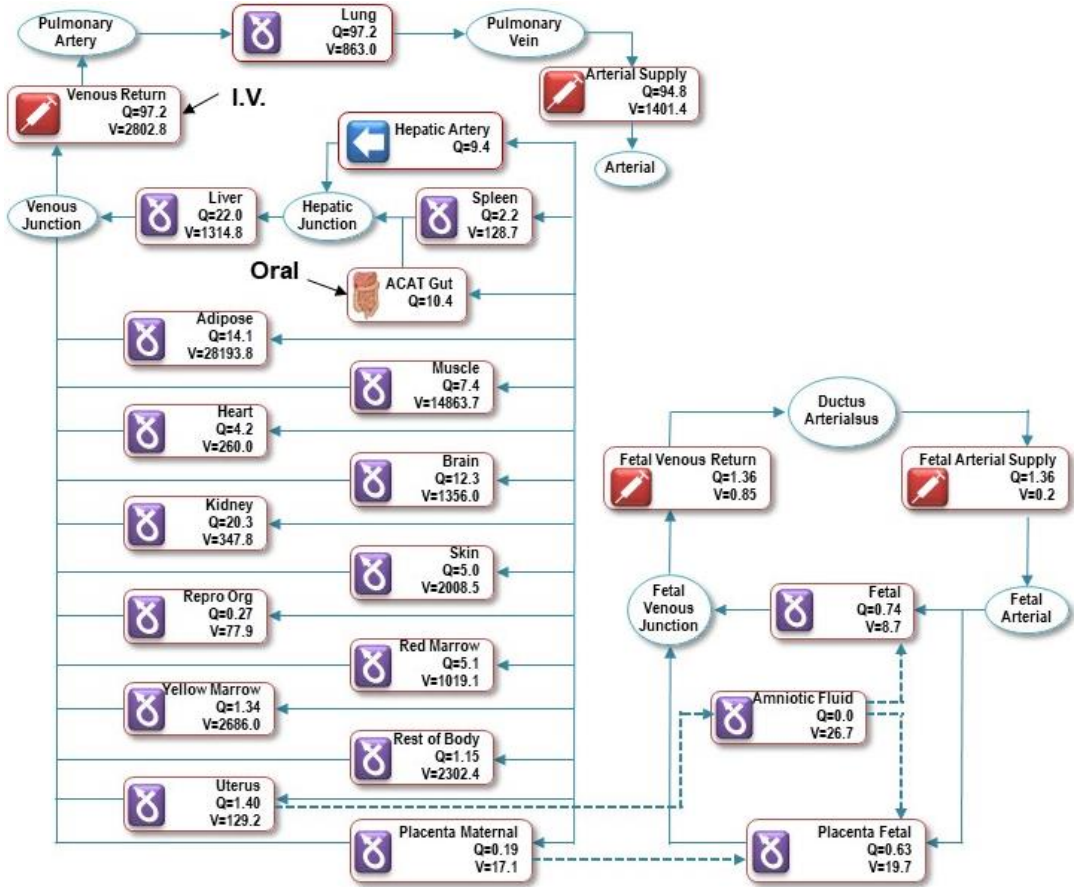
Pregnancy-specific PBPK Models for IVIVE

C HTTK human gestational dose model (HTTK.fPBTK)

(Kapraun et al., https://doi.org/10.1016/j.reprotox.2022.09.004)

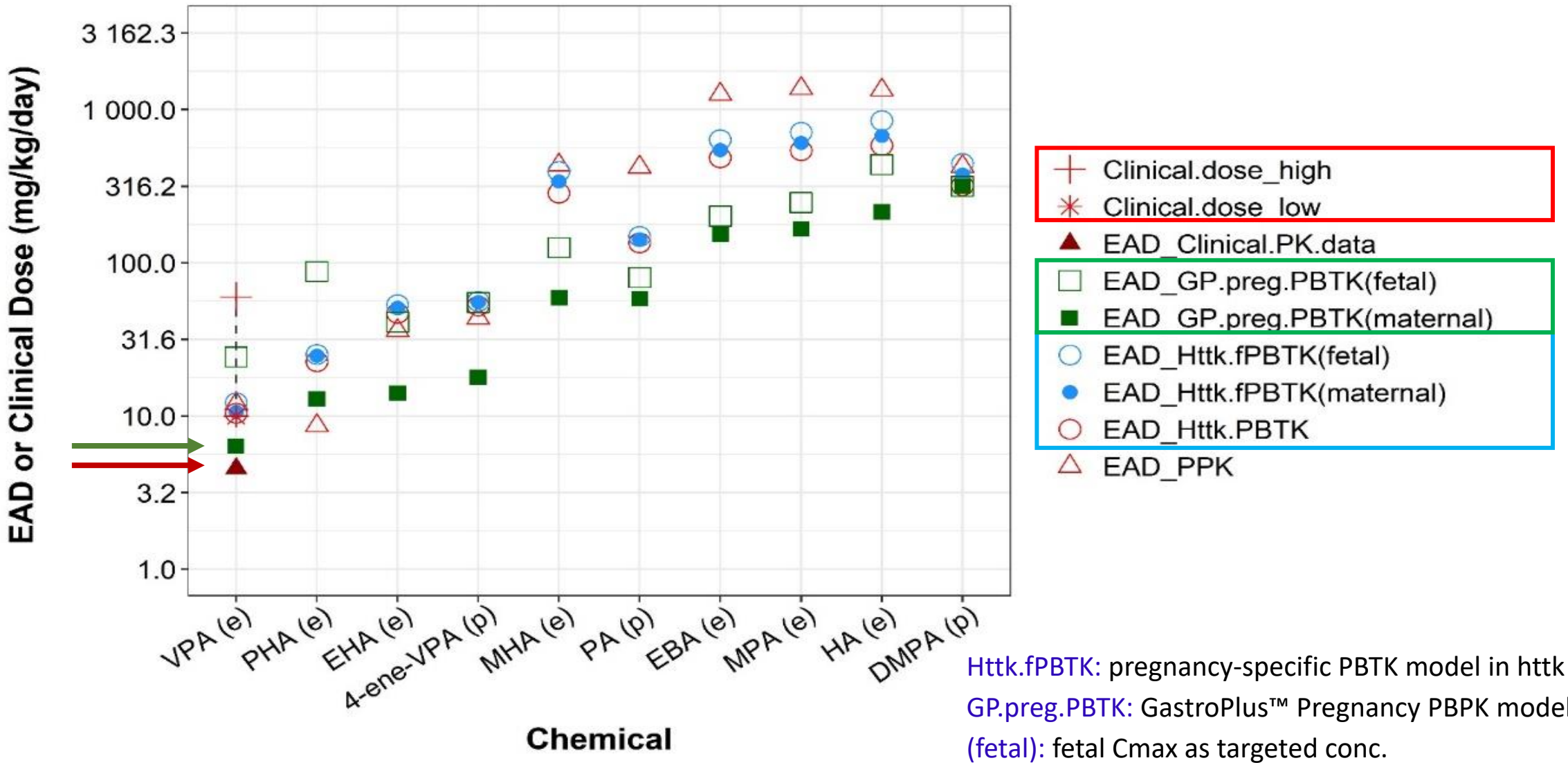


D GastroPlus™ Pregnancy PBPK model



BW, body weight; CL, clearance; CLint, intrinsic clearance; GFR, glomerular filtration rate; I.V., intravenous injection; Q, blood flow rate; ACAT, advanced compartmental absorption and transit model; V, volume

Impact of Different PK/PBPK Models on IVIVE: Human Data



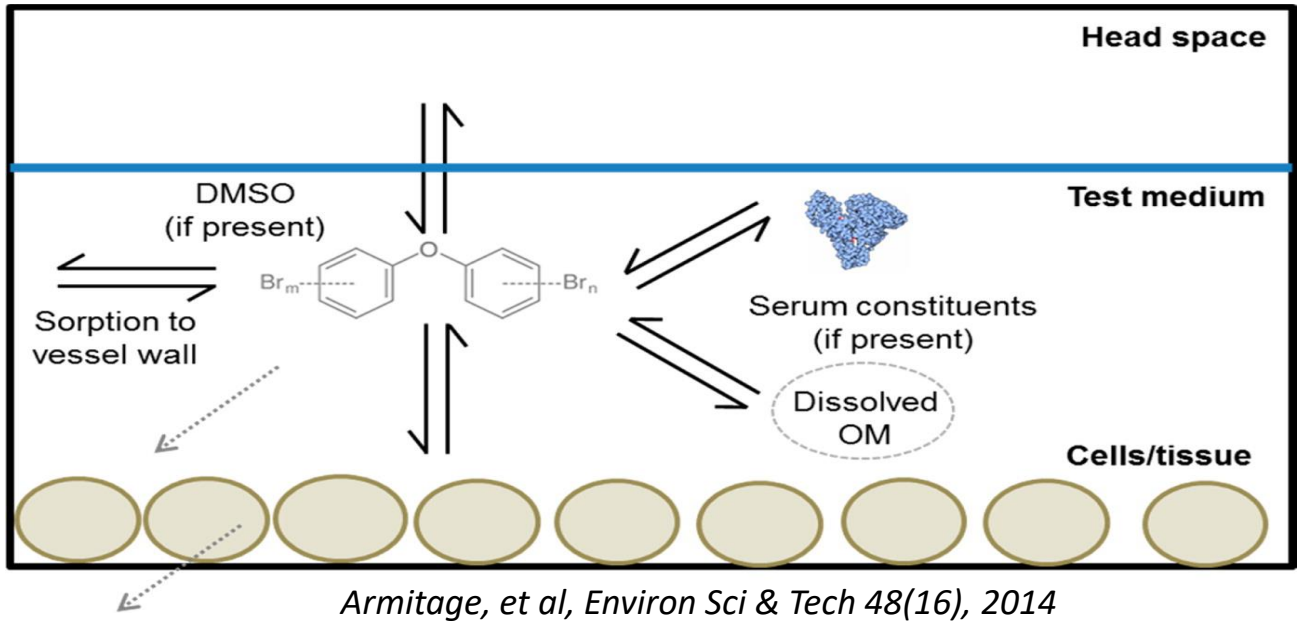
Httk.fPBTK: pregnancy-specific PBTK model in httk

GP.preg.PBTK: GastroPlus™ Pregnancy PBPK model

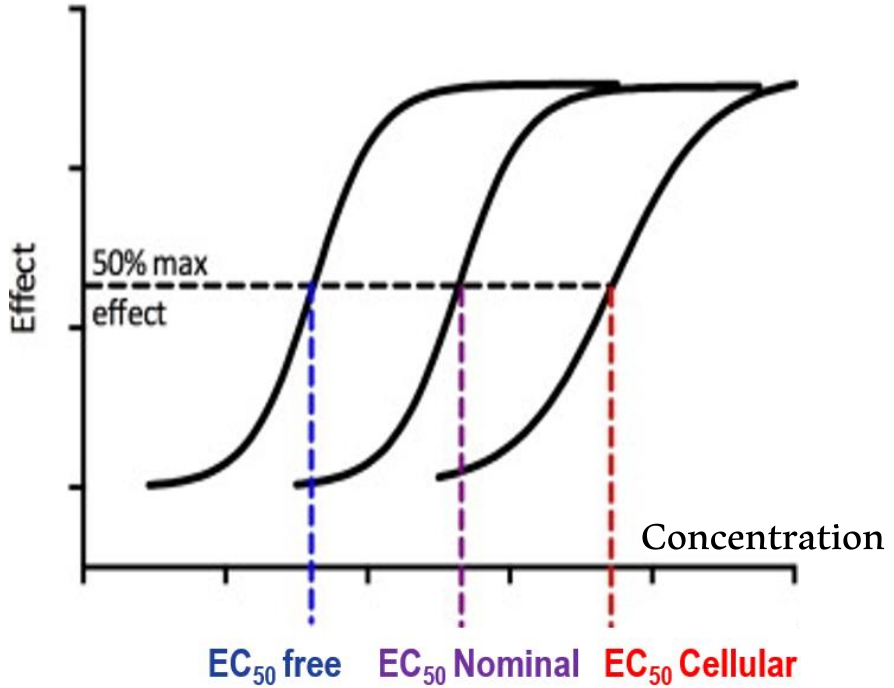
(fetal): fetal Cmax as targeted conc.

(maternal): maternal Cmax as targeted conc.

Mass Balance Model for Predicting Chemical Distribution in In Vitro Assay System



Armitage, et al, Environ Sci & Tech 48(16), 2014



Adapted from Groothuis et al 2015

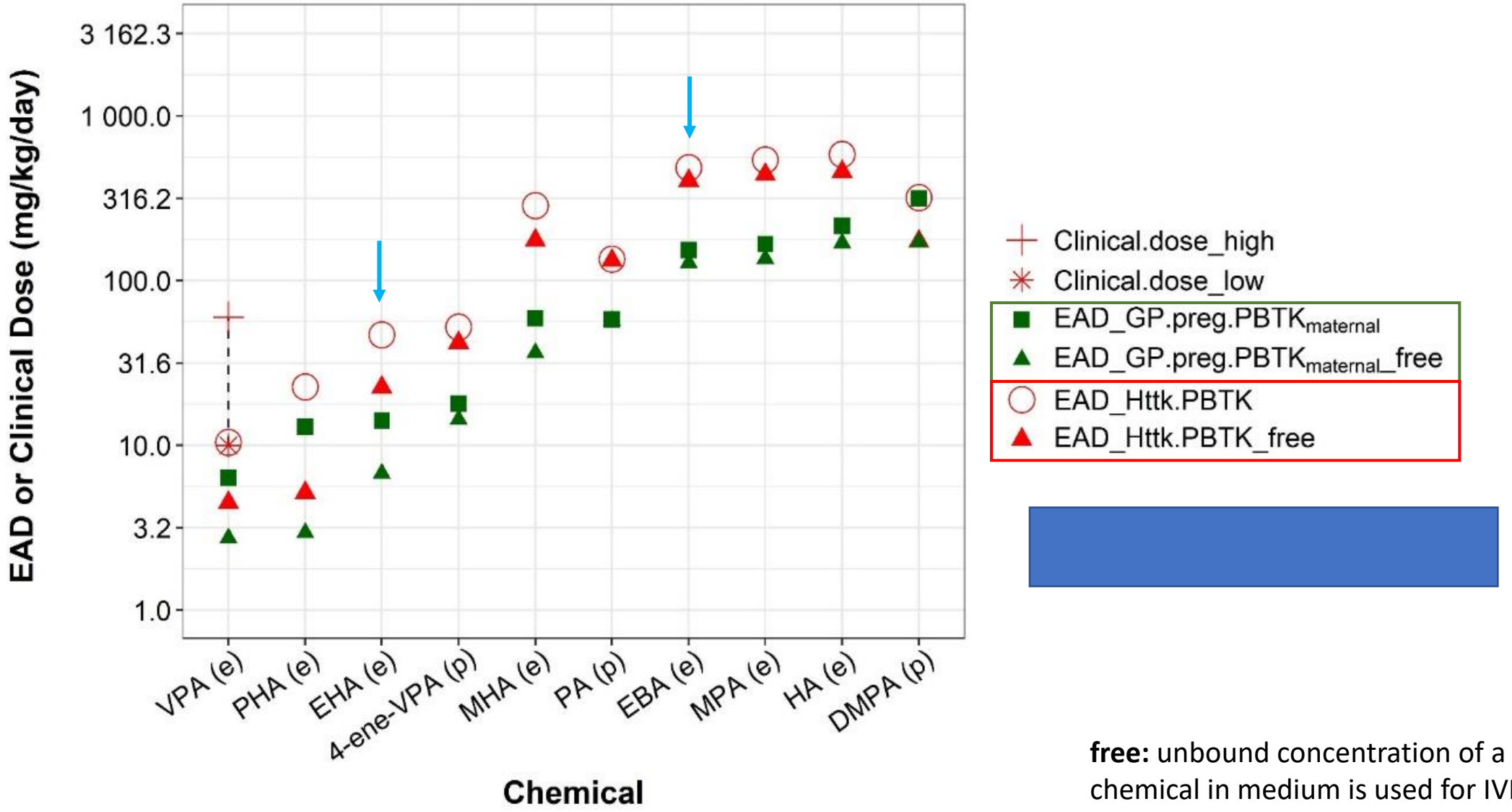
Factors influencing the fraction of test chemical in cells:

- **In vitro assay specific parameters**
  - Cell number
  - Incubation temperature
  - Percentage fetal bovine serum (% FBS)
  - Well-volume
  - Head space
- **Chemical specific parameters**
  - Octanol-water partition coefficient (K_{OW})
  - Air-water partition coefficient (K_{AW})

Free Medium Concentration Predicted from Mass Balance Model

Chemical	Nominal Conc. (uM)	Free Conc.	Ratio: Free vs Nominal Conc.
2-Ethylhexanoic acid	100	47.8	0.48
Valproic acid	100	43.0	0.43
2,2-Dimethylvaleric acid	100	54.2	0.54
Hexanoic acid	100	78.6	0.79
2-propylpent-4-enoic acid	100	80.1	0.80
2-Propylheptanoic acid	100	22.7	0.23
2-Methylhexanoic acid	100	61.6	0.62
4-Pentenoic acid	100	98.3	0.98
2-Ethylbutyric acid	100	83.5	0.83
2-Methylpentanoic acid	100	81.4	0.81

IVIVE Adjustment Using In Vitro Kinetic Model: Human Data



free: unbound concentration of a chemical in medium is used for IVIVE

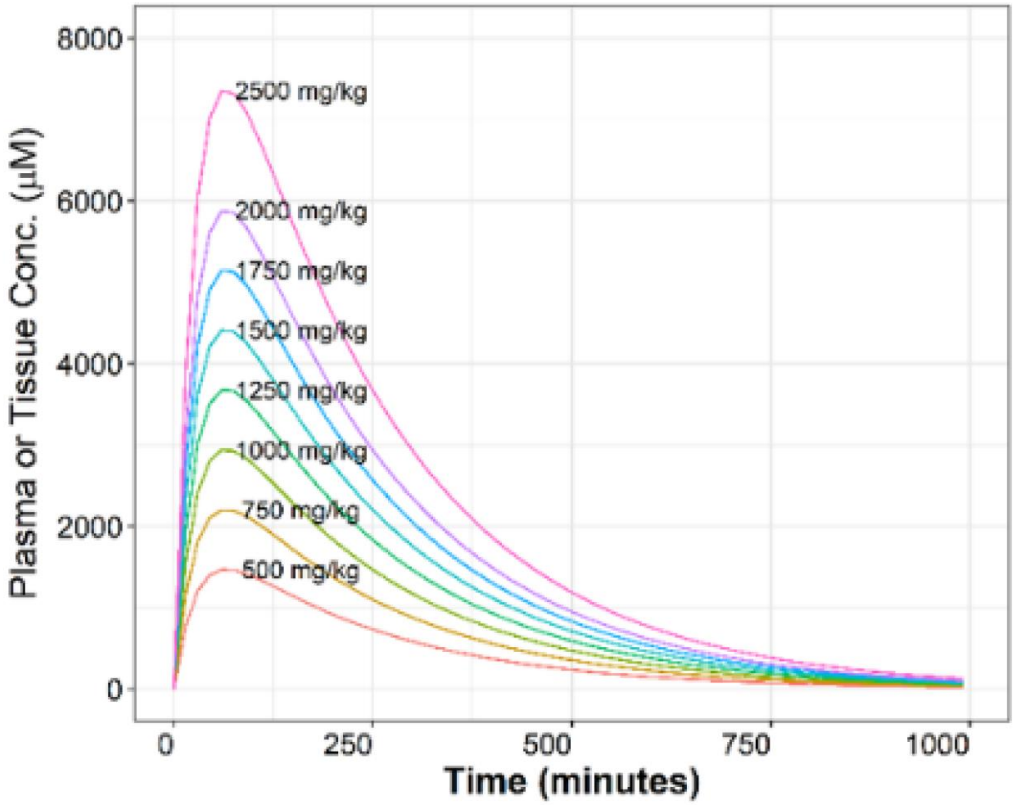
Summary of VPA IVIVE Study

- The EAD estimates for the VPA analogues based on different PK/PBPK models were quantitatively similar to in vivo data for both rats and humans
- The variations on EADs using different types of PK/PBPK models for IVIVE are within expected ranges
  - For rat, the Httk.PBTK model provided the most accurate overall predictions for the rat developmental toxicity LELs
  - For human, GastroPlus pregnancy model with maternal plasma Cmax as target internal concentration provides the most conservative estimation
- Impact of in vitro kinetics on EAD estimates is chemical-dependent

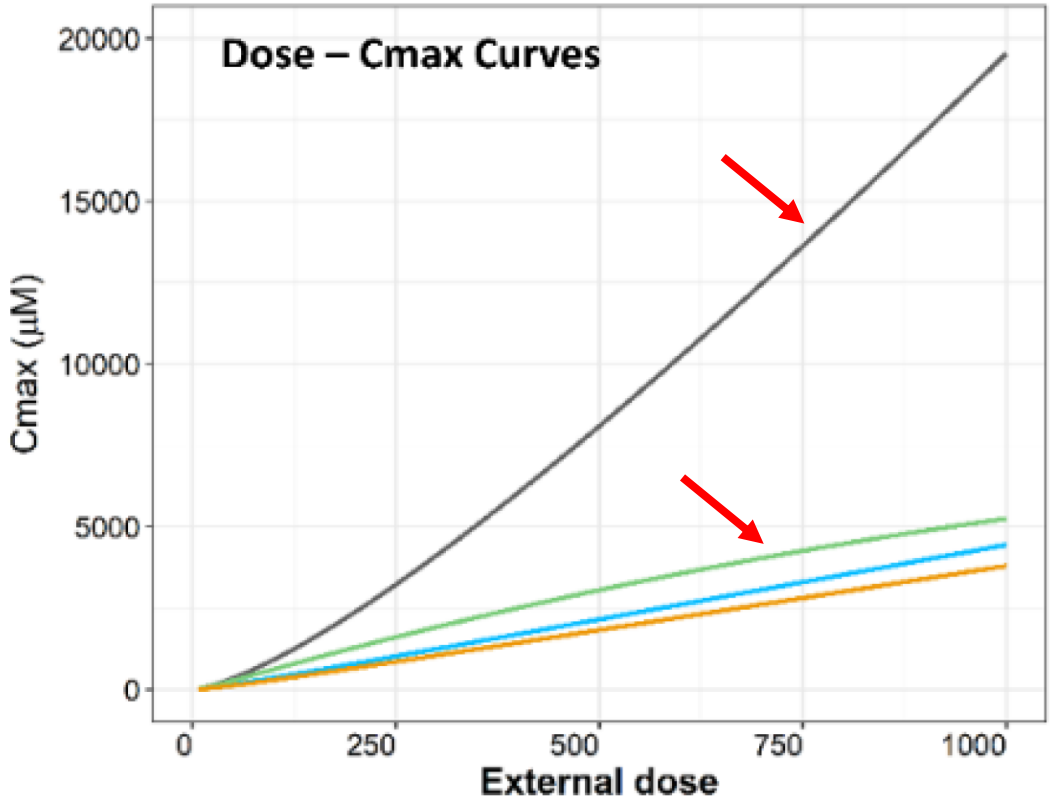
Case study 2: QIVIVE to Facilitate Evaluation of Herbicides for Genotoxic Activity Using In Vitro Assays

Stephanie SR, et al. Evaluation of the Herbicide Glyphosate, (Aminomethyl)phosphonic Acid, and Glyphosate-Based Formulations for Genotoxic Activity Using In Vitro Assays. Environmental and Molecular Mutagenesis (Accepted)

Reverse Dosimetry for (Q)IVIVE



Non-linear Extrapolation

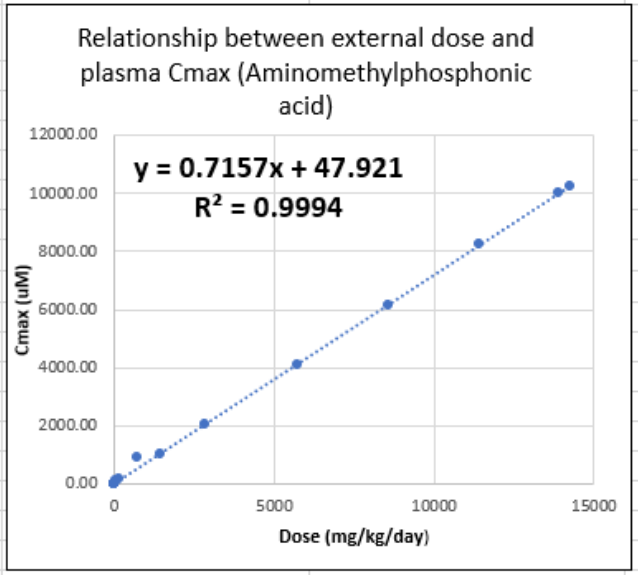


Chang X, et al. IVIVE: Facilitating the Use of In Vitro Toxicity Data in Risk Assessment and Decision Making. Toxics 2022, 10(5), 232

Predicted Cmax at Multiple External Doses Using GastroPlus Model

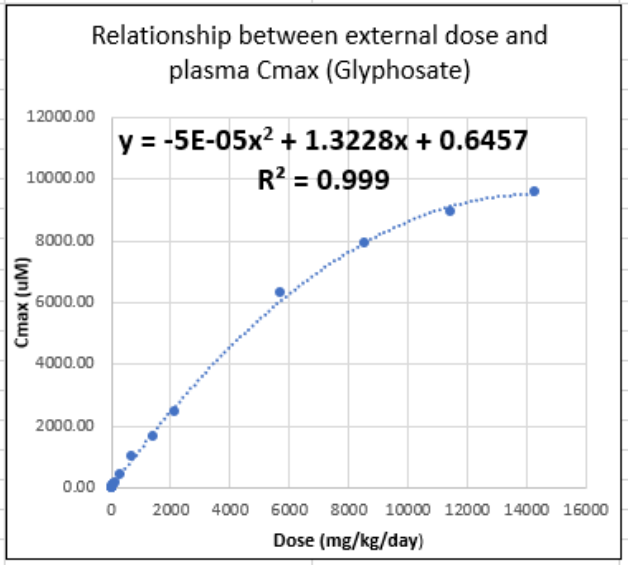
Aminomethylphosphonic acid

Dose (mg/kg/day)	Cmax (uM)
1	1.25
2	2.50
4	4.99
8	9.98
16	19.97
32	39.93
71	89.14
143	178.28
714	891.40
1429	1029.11
2857	2058.30
5714	4116.52
8571	6174.73
11429	8232.77
13896	10000.18
14286	10270.36



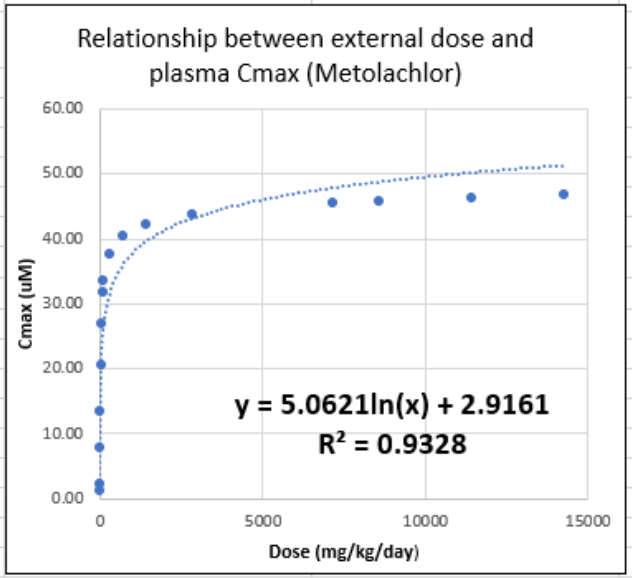
Glyphosate

Dose (mg/kg/day)	Cmax (uM)
1	1.44
3	4.12
7	10.30
14	20.59
29	41.19
57	82.37
86	123.56
114	164.74
286	411.85
714	1029.64
1429	1636.69
2143	2455.03
5714	6324.26
8571	7944.38
11429	8914.20
14286	9597.63



Metolachlor

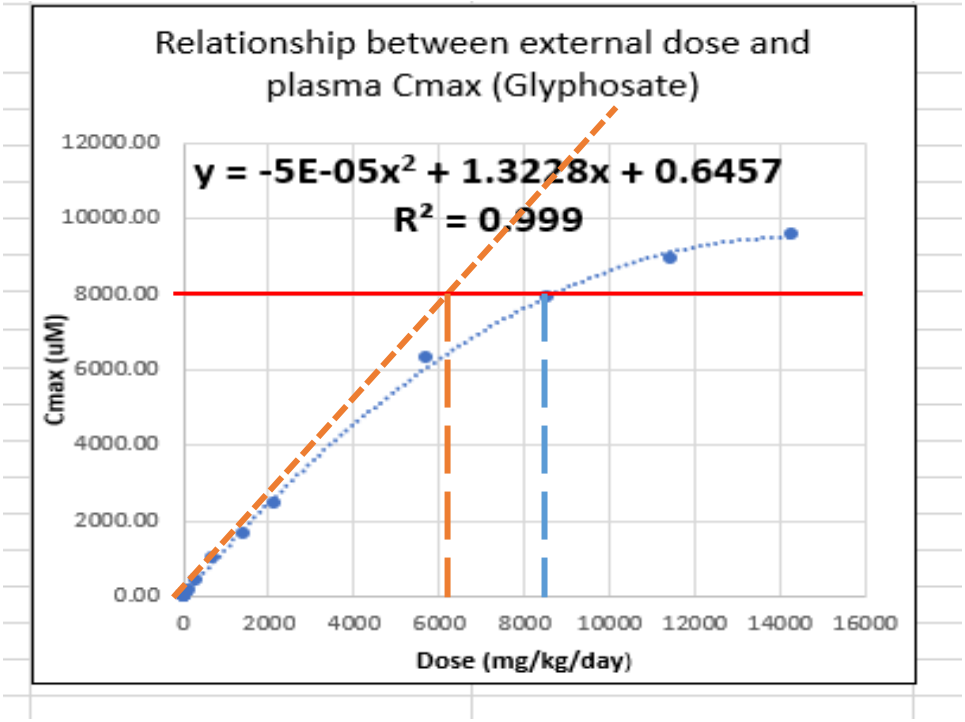
Dose (mg/kg/day)	Cmax (uM)
1	1.16
2	2.25
8	7.78
16	13.36
32	20.41
57	27.02
86	31.62
114	33.43
286	37.52
714	40.34
1429	42.04
2857	43.57
7143	45.42
8571	45.76
11429	46.30
14286	46.71



EAD Comparison Between Using GastroPlus and HTTK PBK Models

Chemicals	ACC or Top Testing Conc. (uM)	GastroPlus EAD (mg/kg/day)	Httk EAD (mg/kg/day)
Aminomethylphosphonic acid	10000	13895.7	871.8
Glyphosate	10000	15968.0	1357.0
Metolachlor	159	1.08E+13	121.7

Glyphosate



Stephanie SR, et al. Evaluation of the Herbicide Glyphosate, (Aminomethyl)phosphonic Acid, and Glyphosate-Based Formulations for Genotoxic Activity Using In Vitro Assays. Environmental and Molecular Mutagenesis (Accepted)

Take Home Message

- (Q)IVIVE
  - Putting in vitro toxicity data into in vivo setting
  - Promotes use and acceptance of NAMs in risk assessment
  - Promising results with using in vitro assay that are mechanistic relevant to in vivo outcomes
- Extrapolation with linear assumption between external exposure and plasma concentration
  - Provides a more conservative estimate for human risk assessment
- Challenges
  - Variability and uncertainty
- Future opportunities

Acknowledgments

Integrated Chemical Environment

https://ice.ntp.niehs.nih.gov/

https://github.com/kmansouri/OPERA

Thank You

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