Model conception, parameterization using *in silico* methods, and computational implementation

Michael Lawless, Ph.D.
Simulations Plus, Inc.
Lancaster CA
Phone: 661-723-7723
Email: michael.lawless@simulations-plus.com

Conflict of Interest Statement

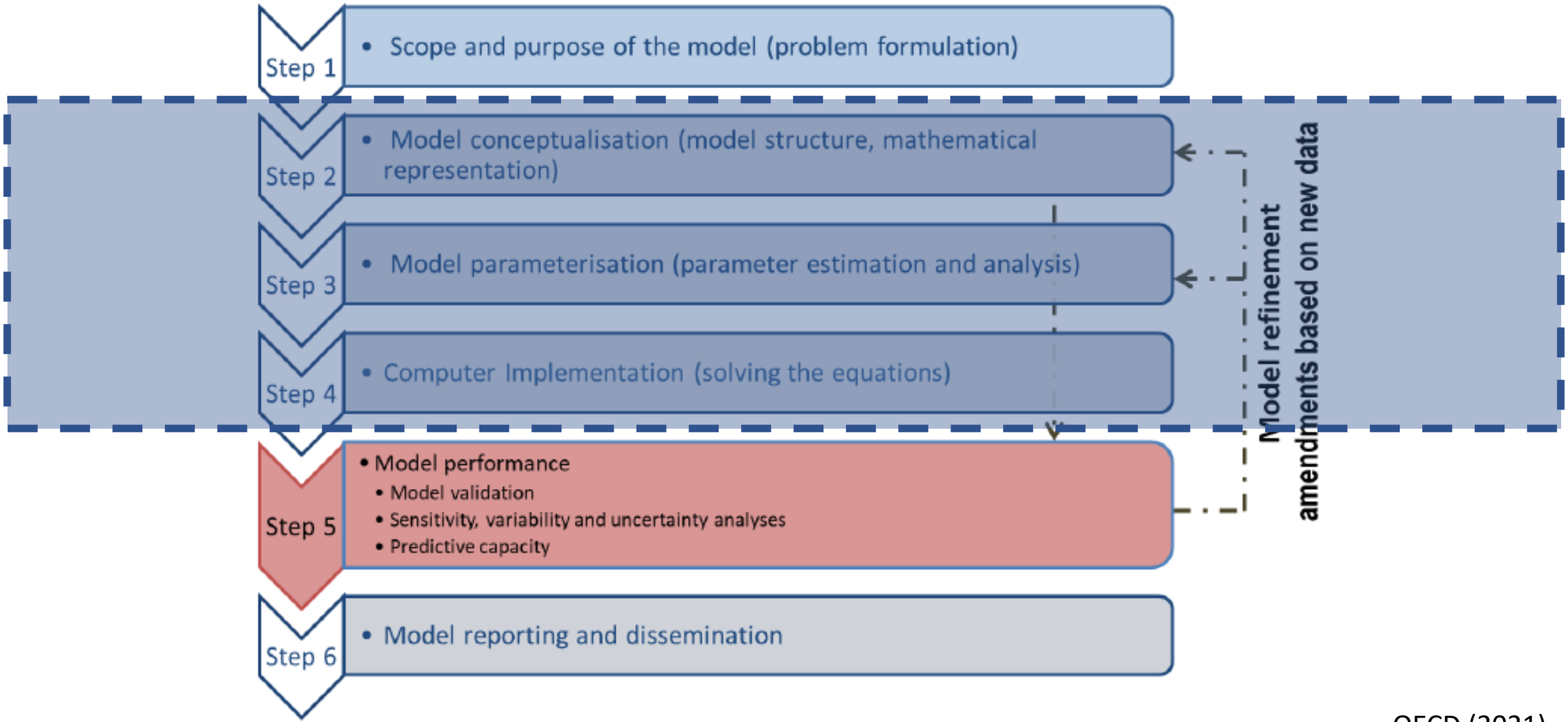
The author declares no conflict of interest.

Abbreviations

- **%F**: oral bioavailability
- **%Fa**: fraction absorbed
- **%FDp**: fraction of dose in portal vein
- **ACAT**: advanced compartmental absorption and transit
- **AD**: applicability domain
- **ADME**: absorption, distribution, metabolism and excretion
- **BCRP**: breast cancer resistant protein
- **GI**: gastrointestinal
- **OATP1B1**: organic anion transporting polypeptide 1B1
- **ODE**: ordinary differential equation
- **RMSE**: root mean square error
- **QSAR**: quantitative structure activity relationship
- **QSPR**: quantitative structure property relationship
- **PBK**: physiologically based kinetic
- **PepT1**: peptide transporter 1
- **Pgp**: P-glycoprotein

Outline

- PBK model conception
  - Model structure and mathematical representation
- PBK model parameterization
- PBK computational implementation
- Integrating machine learning into PBK simulations
- Machine learning model background
- Case study - PBK simulations to predict herbicide absorption and bioavailability

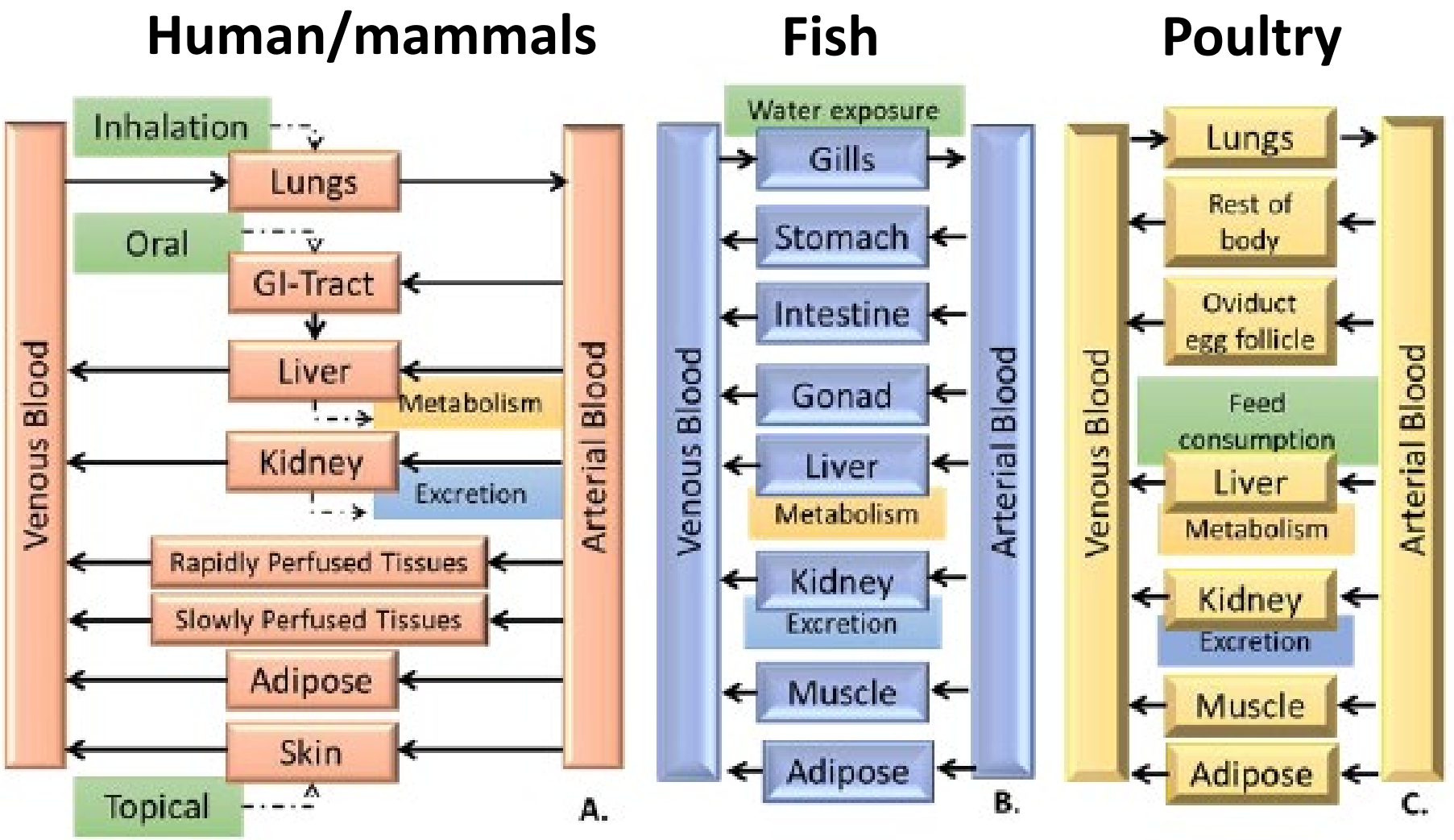

Step 1
• Scope and purpose of the model (problem formulation)
Step 2
• Model conceptualisation (model structure, mathematical representation)
Step 3
• Model parameterisation (parameter estimation and analysis)
Step 4
• Computer Implementation (solving the equations)
Step 5
• Model performance
• Model validation
• Sensitivity, variability and uncertainty analyses
• Predictive capacity
Step 6
• Model reporting and dissemination
Model refinement
amendments based on new data

OECD (2021)

Step 2: Model Conceptualization (Model structure and mathematical representation)

- The structure of the PBK model is informed by the problem formulation, knowledge of the biokinetic mechanism, and availability of suitable data
- The model should only be as complex as necessary to address the problem, e.g., a one-component model might be sufficient
- Chemical partitioning into compartments assumed to be instantaneous
  - Tissue and organ masses should be within the body mass
  - Total blood flow equals sum of the flows to the compartments
- Chemical distribution into compartments is perfusion or permeability limited uptake

PBK Model Conceptualization



Chemical administration routes are in green box

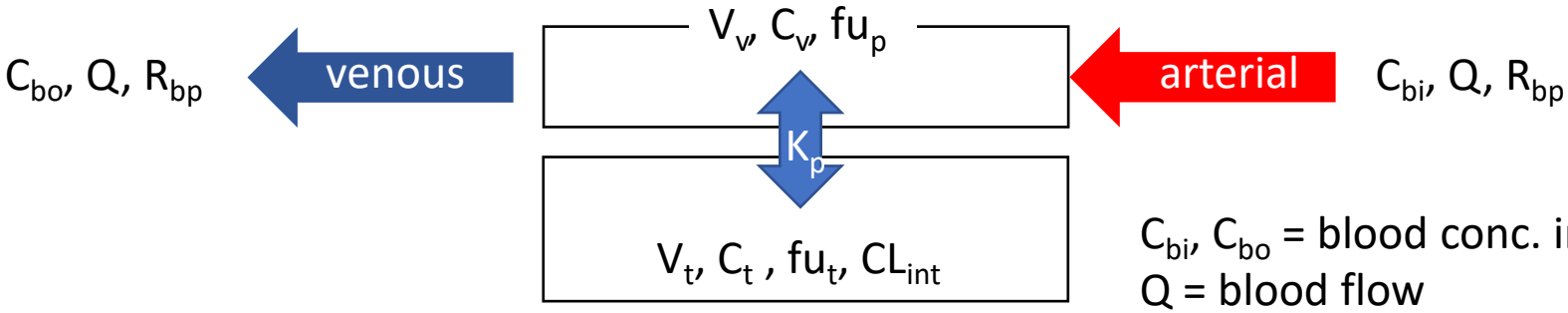
Tissues and organs are compartments that have:

- A specific volume
- Blood perfusion rate
- Volume fractions of lipids and proteins
- Tissue/plasma partition coefficient (K_p)
- Enzyme/transporter expression levels

OECD (2021)

PBK Model Conceptualization

PBK models solve a series of differential equations that describe a chemical's time dependent absorption, distribution, metabolism and excretion (ADME).



C_{bi}, C_{bo} = blood conc. in and out
Q = blood flow
R_{bp} = blood to plasma ratio
V_v, V_t, = plasma and tissue volume
fu_t, fu_p = fraction unbound tissue and plasma
CL_{int} = tissue intrinsic clearance
K_p = tissue/plasma coefficient
C_v, C_t = plasma and tissue concentration

$$V_t \frac{dC_t}{dt} = Q\left(C_{bi} - \frac{C_t \times R_{bp}}{K_p}\right)$$

Non-eliminating, perfusion-limited tissue

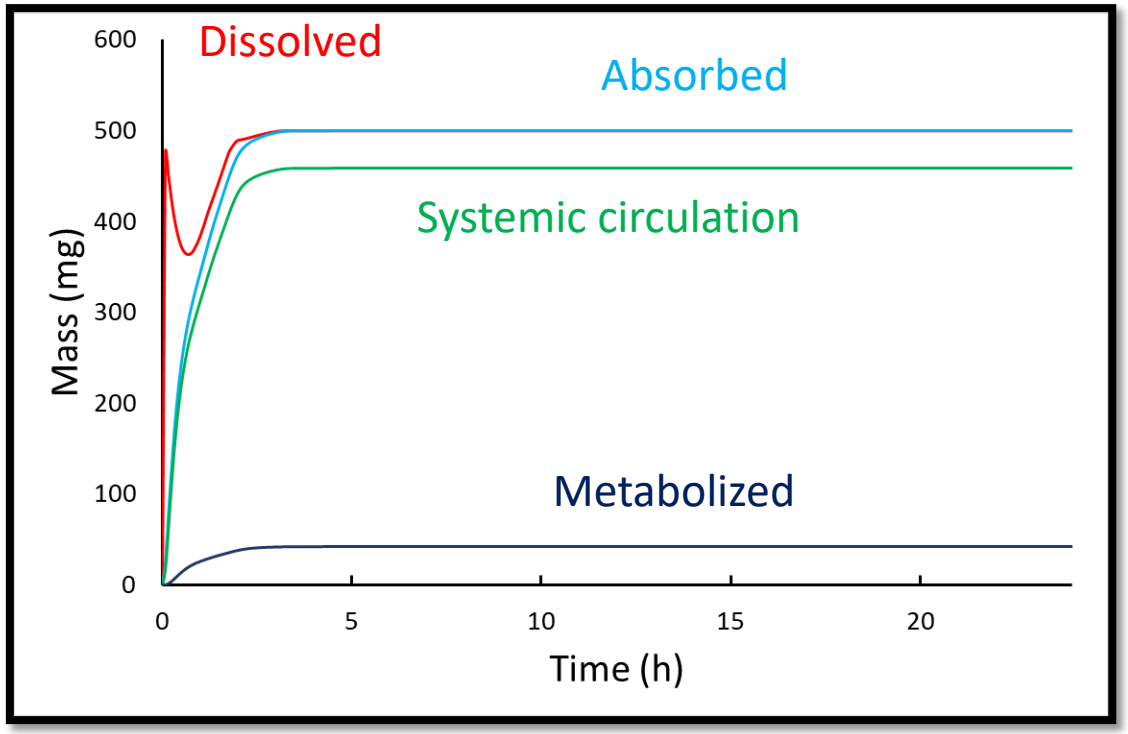
$$V_t \frac{dC_t}{dt} = \left(Q \times C_{bi} - \frac{Q \times C_t \times R_{bp}}{K_p} - CL_{int}\left(\frac{C_t \times fu_p}{K_p}\right)\right)$$

Last term represents clearance in the tissue

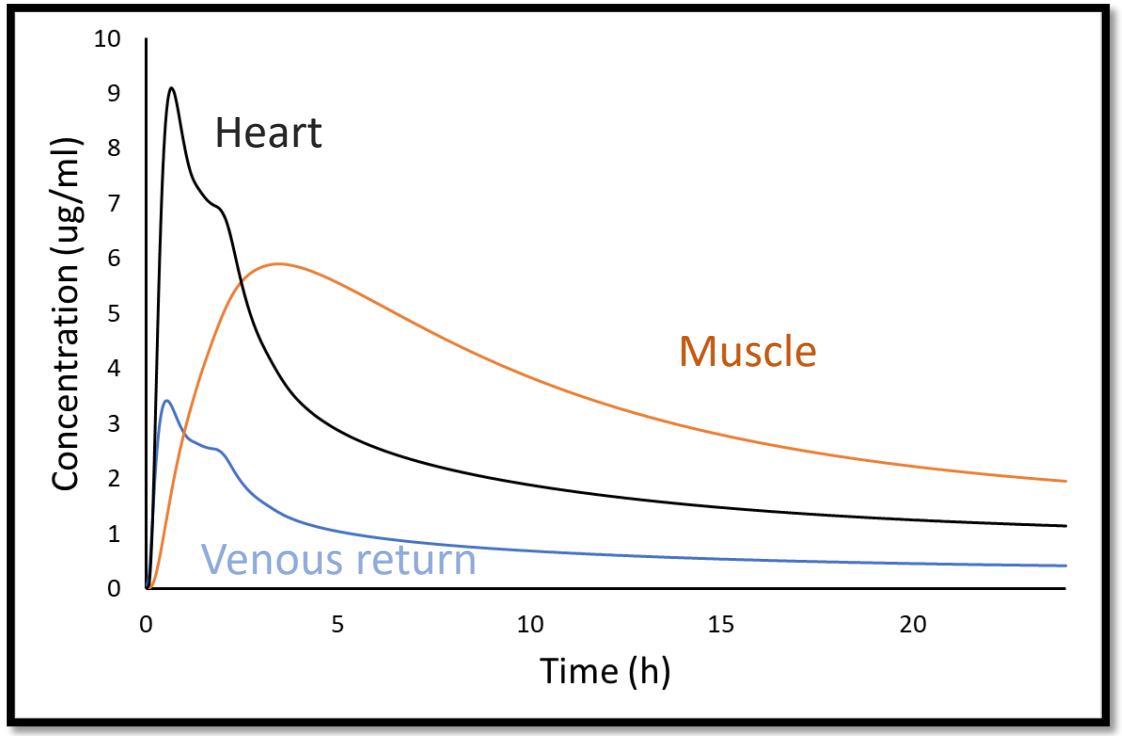
(Jeong Y et al. 2017 and Espie P 2009)

PBK Model Conceptualization

500 mg dose



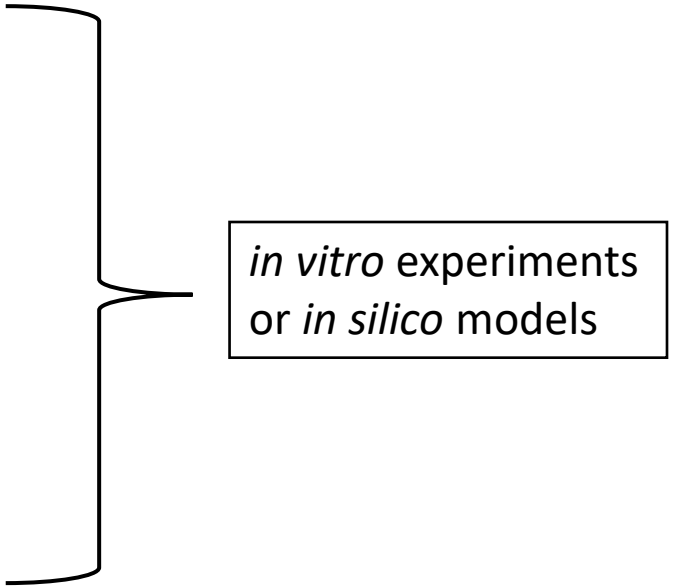
Amount of compound dissolved and absorbed in gastrointestinal tract, in systemic circulation, and metabolized. The amount metabolized equals the difference between the amount absorbed and in systemic circulation.



Compound concentration versus time in heart, muscle, and venous return.

Step 3: PBK Model Parameterization

- Physiological parameters
  - Volume, blood flow, pH (GI tract compartments), etc.
  - Literature values (Brown et. al., 1997, Davis and Morris 1993, https://www.interspeciesinfo.com)
- Chemical specific ADME parameters
  - Rate of absorption (Ka)
  - pKa
  - Octanol-water distribution coefficient (logD)
  - Solubility
  - Intrinsic clearance (CLint)
  - Km and Vmax
  - Fraction unbound to plasma
  - Blood to plasma ratio



Step 3: PBK Model Parameterization

- Absorption across external barriers (passive, uptake, efflux)
  - Oral, intravenous, dermal, inhalation
- Partitioning
  - Organism and environment
  - Organ/tissue and plasma
- Active transport into or out of cells
  - Efflux transporters, e.g., Pgp and BCRP, on the basolateral membrane of the cell transport chemicals out of the cell
  - Influx transporters, e.g., PepT1, OATP1B1, on the apical membrane of the cell transport chemicals into the cell

Step 3: Model Parameterization

- Systemic clearance
  - Removal of the compound from systemic circulation
  - Exhalation for volatile chemicals
  - Renal clearance
  - Metabolism
  - Biliary clearance (excretion from hepatocyte into bile)

Step 4: Computer Implementation

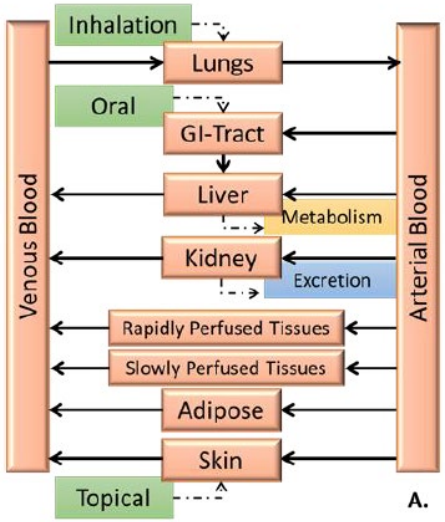
- Software packages that solve ordinary differential equations (ODE)
- Methods are well established and not a significant source of uncertainty
- Examples of PBK modeling software (Annex 1, Table 1C)
  - Cloe® (Cyprotex)
  - High throughput toxicokinetics – Httk (EPA)
  - GastroPlus® (Simulations Plus)
  - Simcyp™ Simulator (Certara)
  - PK-Sim® (Bayer) open source
  - PLETHEM (ScitoVation)
  - Berkley Madonna (https://berkeley-madonna.myshopify.com/)

In Silico Property Estimation and PBK

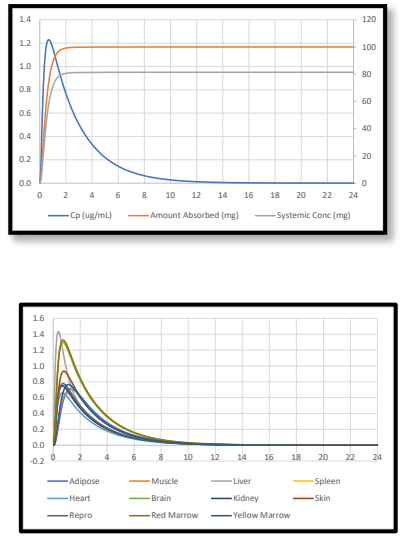
metribuzin

Permeability, solubility, pKa, logD Fup, blood:plasma ratio, CLint

Machine learning models



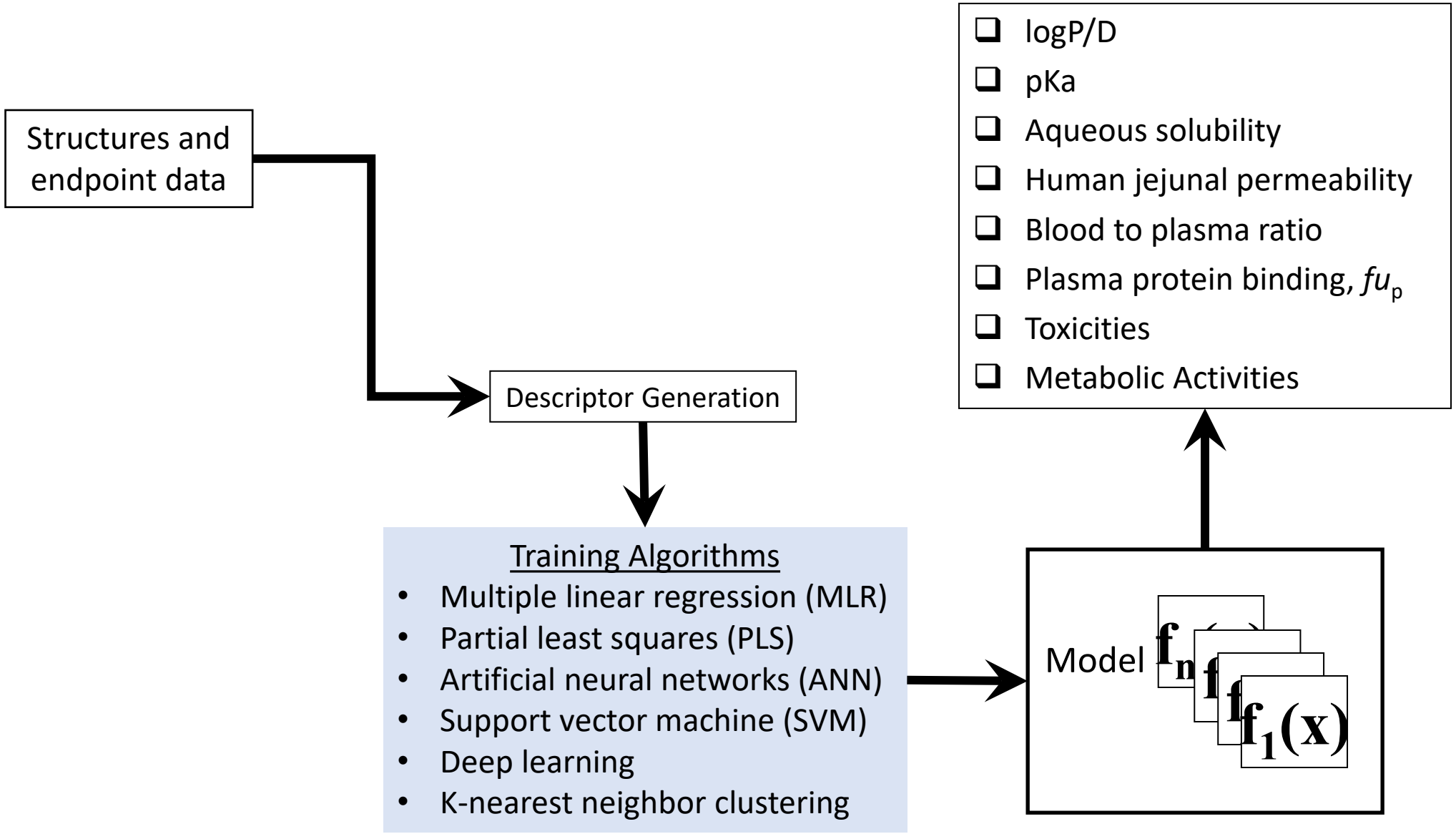
PBK



<u>Internal exposure</u>
Plasma conc.
Tissue conc.

Goal: provide parameters for PBK simulations based on 2D structure of the molecule

Machine Learning Technology



OECD Principles for validating QSAR models (OECD 2007)

- A defined endpoint
- An unambiguous algorithm
- A defined domain of applicability
- Appropriate measures of goodness-of-fit, robustness, and predictivity
  - R^2, root mean square error
- A mechanistic interpretation, if possible

Goodness-of-fit, Robustness, and Predictivity of Regression Models

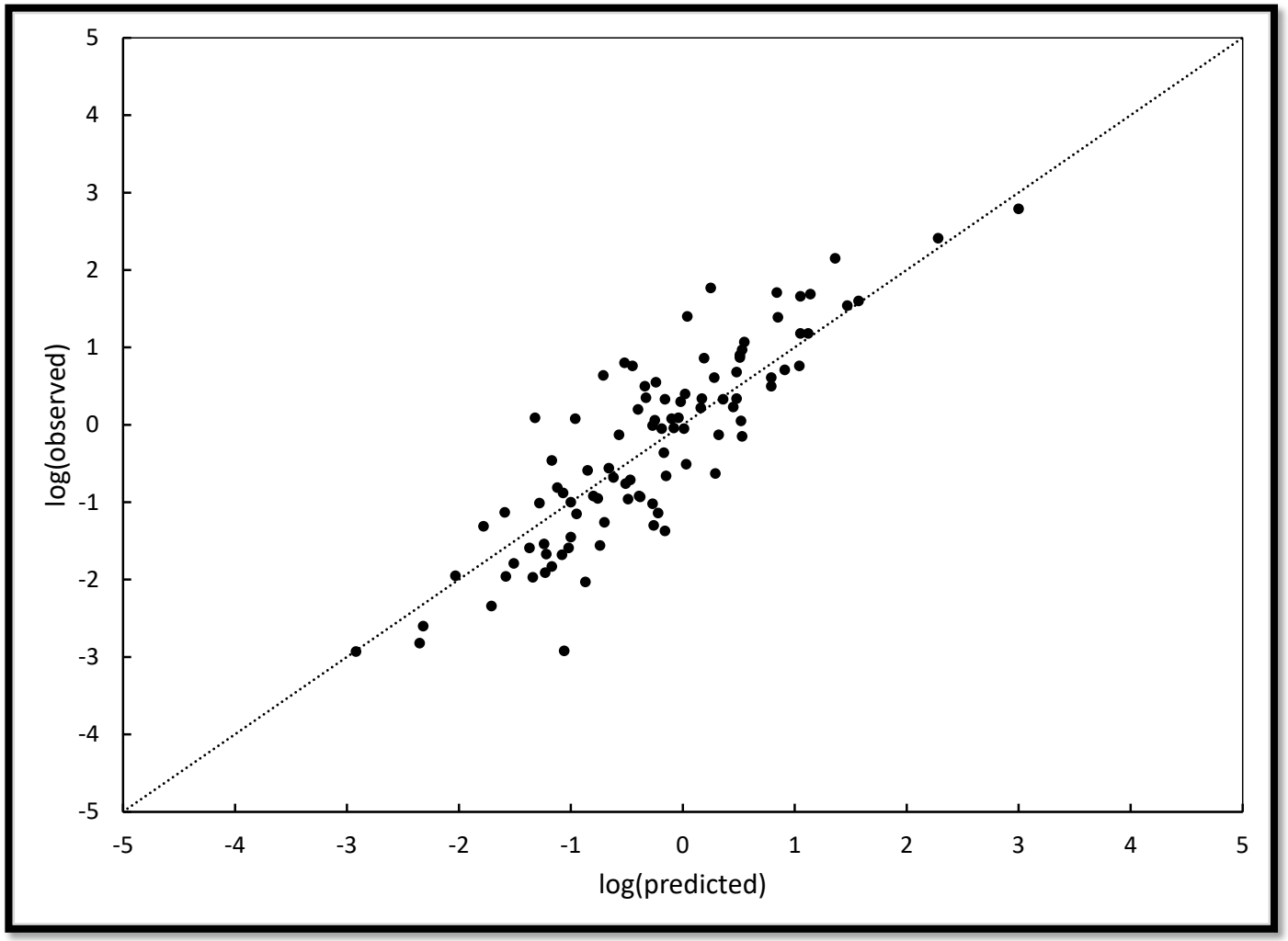
Alexander, et al 2015 suggests reporting the coefficient of determination (R^2) and RMSE on a **test** set.

$$R^2 = 1 - \frac{\sum(y - \hat{y})^2}{\sum(y - \bar{y})^2}$$

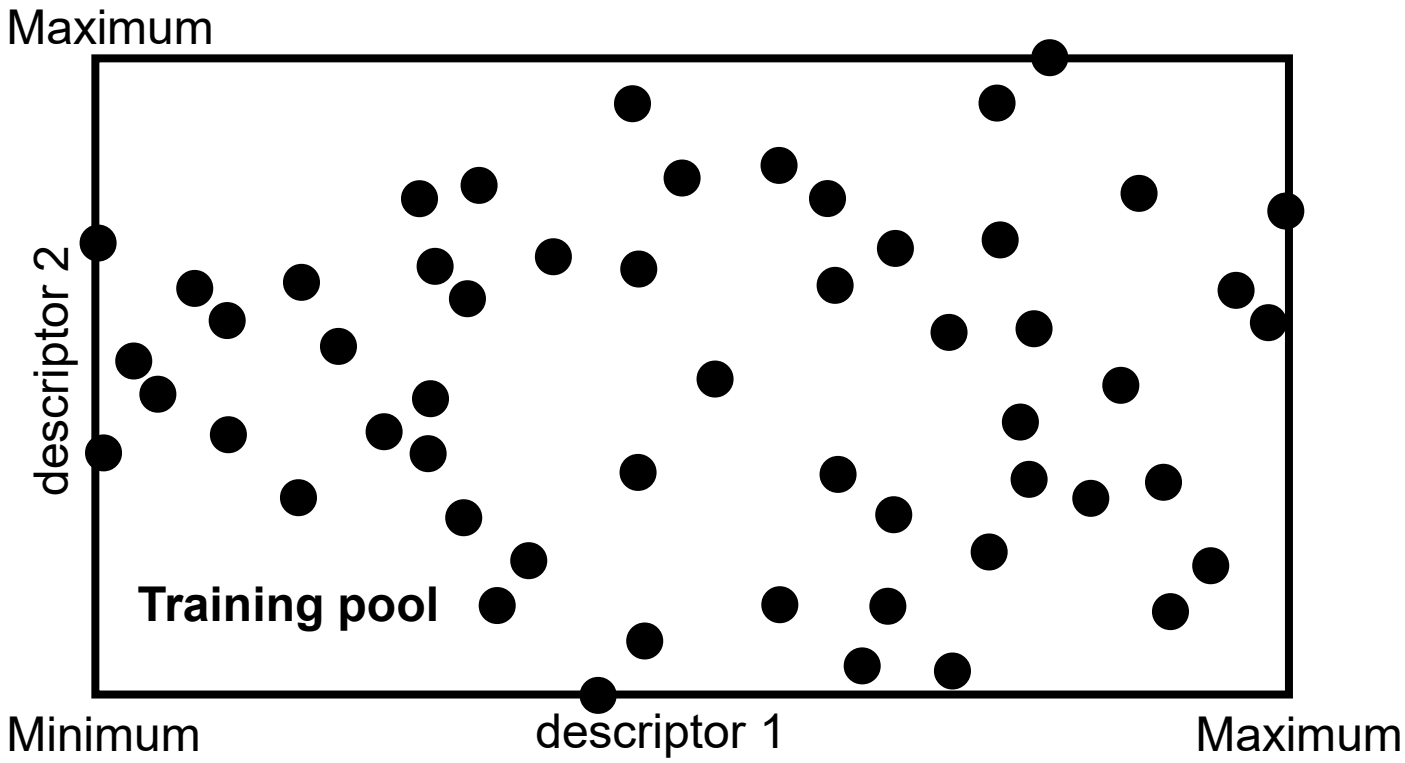
y is the observed value
$\hat{y}$ is the predicted value
$\bar{y}$ is the mean

$$\text{RMSE} = \sqrt{\frac{\sum(y - \hat{y})^2}{n}}$$

RMSE is the root mean square error
n is the number of observations



Simple Applicability Domain Method



OECD (2007)

Predictions for molecules whose descriptors are outside of the applicability domain of the model should be subjected to parameter sensitivity analysis.

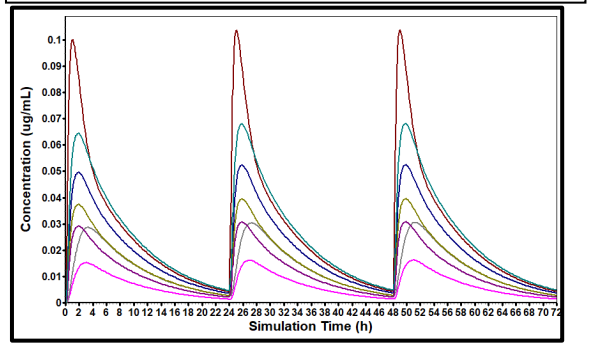
In Silico Tools*

Resource	Available from
ACD/Percepta	ACD Labs
ADMET Predictor®	Simulations Plus, Inc.
ALOGPS 2.1	http://www.vcclab.org/lab/alogps/
ChemAxon	ChemAxon
Computational Toxicology Dashboard	EPA, includes OPERA predictions (Mansouri 2018)
EPIK, QikProp	Schrodinger
Episuite	US-EPA
MOE (Molecular Modelling Environment)	Chemical Computing Group
MoKa, VolSurf	Molecular Discovery
OECD QSAR toolbox	https://www.qsartoolbox.org/
SwissADME	http://www.swissadme.ch/

* OECD (2021) Annex 1, Table 1A

What's happening *in vivo* for an orally administered compound

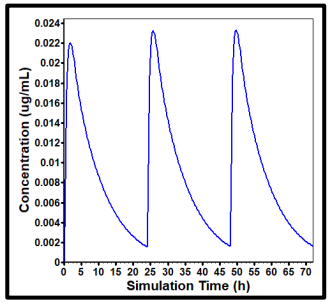
Adipose Liver Brain
Spleen Muscle Heart
Kidney



Tissue distribution
Plasma protein binding
Blood:plasma concentration ratio
Systemic clearance

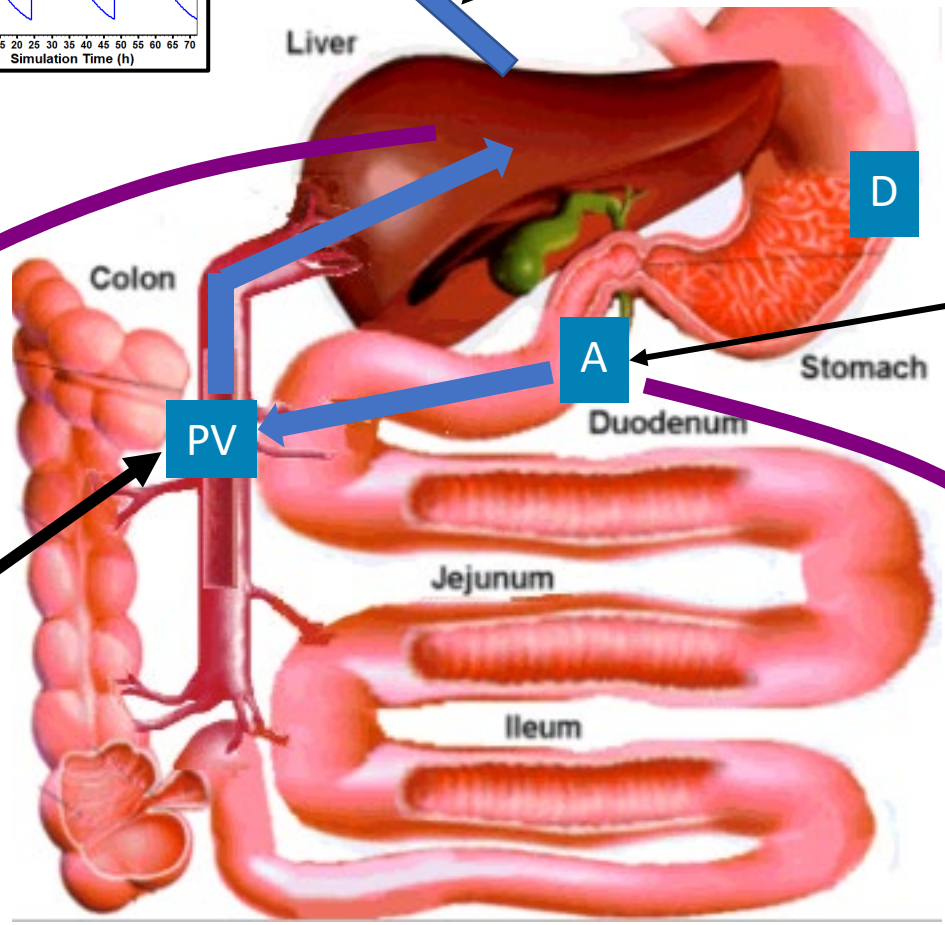
Metabolism

Liver metabolism
Non-linear clearance
Hepatic uptake
Biliary secretion



F%
(not Fa%)

SC



pK_a
Solubility vs. pH
Biorelevant solubility
Precipitation kinetics

Fa%

Transcellular permeability
Paracellular permeability
logD vs. pH
Gut extraction
Carrier-mediated transport

FDp%

Metabolism

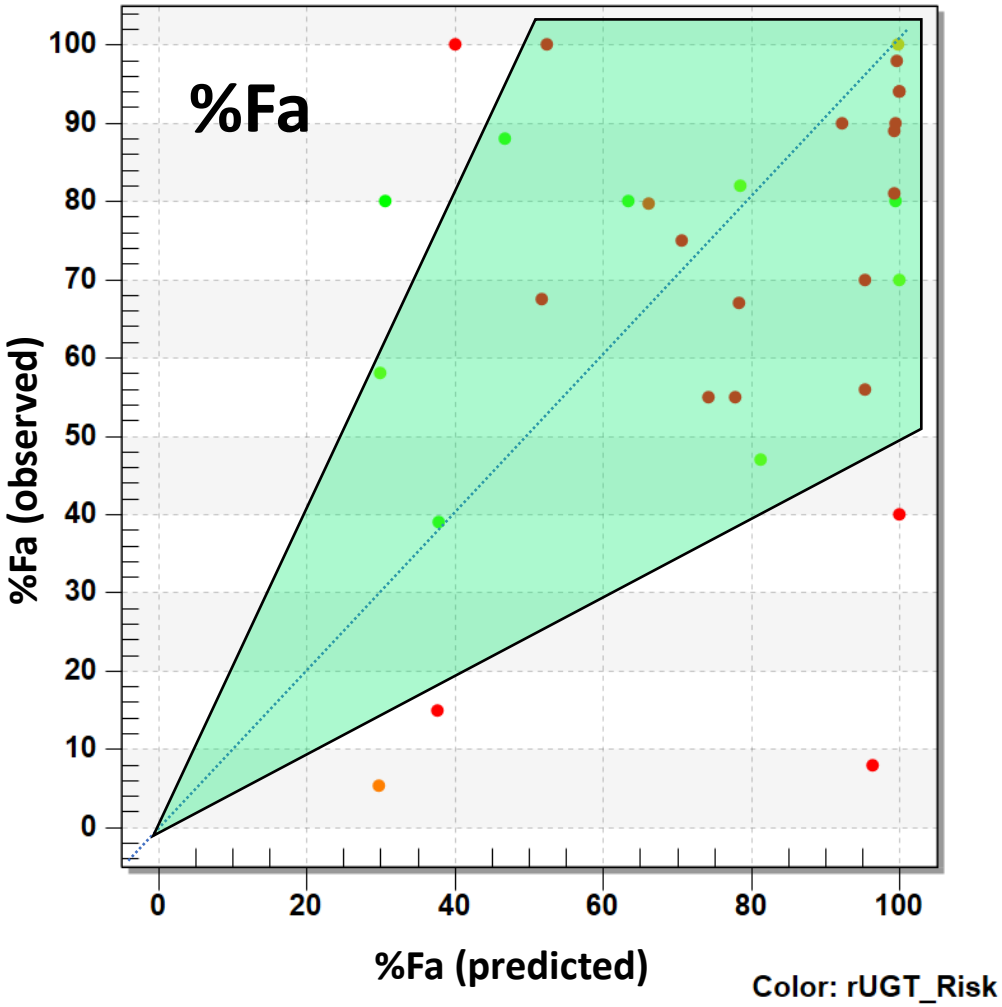
Capsugel (1998)

Herbicide absorption and bioavailability prediction workflow

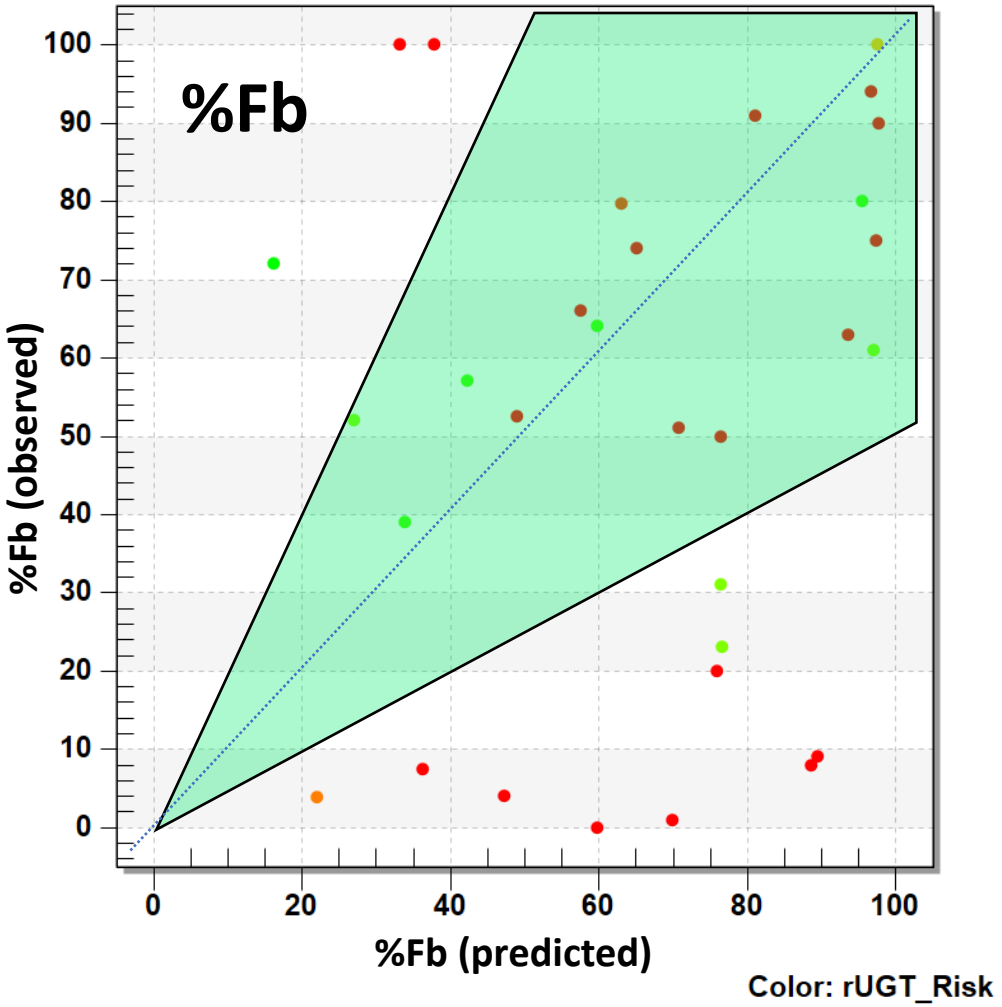
- Searches of EPA, EFSA and WHO risk assessments and databases provided observed %Fa and %Fb estimates for 31 of 37 representative herbicides cited by Zhang 2018
- Fasted rat physiology details (gastrointestinal compartment sizes and transit times, liver volumes, etc.) were taken from the ACAT™ model in GastroPlus® 9.0.
- Rat HTPK simulations were run for each herbicide in ADMET Predictor® 9.0 using predicted logP, pKa, aqueous solubility, effective jejunal permeability, fraction unbound in microsomes (fumic), fraction unbound in rat plasma (fup), and the ratio of blood to plasma for rat
- Hepatic clearance was based on the predicted total cytochrome P450 (CYP) clearance in rat liver microsomes (RLM)
- Renal clearance was taken as glomerular filtration rate (GFR) * fup
- Calculated fractions ionized and molecular size parameters were used to account for paracellular permeability

Herbicide absorption and bioavailability prediction workflow

- The predicted %Fa was compared graphically to the percentage of radiolabel not recovered in the feces
- The predicted %Fb was compared with the amount of parent recovered from the urine
- Note that both “observed” values are lower bounds for the values that would be obtained by sampling the blood
- Points in the predicted vs. observed plots were color-coded by a qualitative measure of their predicted susceptibility to glucuronidation in rat liver and by whether or not they were predicted to be substrates for P-glycoprotein (P-gp)
  - This was done to flag cases where the simplifying assumptions made may break down



81% predicted within 2-fold of the reported value



59% predicted within 2-fold of the reported value, with only 10% underestimated by more than 2-fold.

Summary and Conclusion

- The PKB model structure depends on the problem, knowledge of the biokinetic mechanism, and availability of suitable data
- Model parameterization includes physiological and ADME parameters
  - *In vitro* experiments and/or machine learning models can be used as model parameters
- PKB models are implemented by software packages which have well established methods and are not a significant source of uncertainty
- OECD guidance 69 lists several principles for validating QSAR machine learning models
- 81% of %Fa and 59% of %Fb predictions were within 2-fold of the observed value for 37 herbicides

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