High-throughput PBPK and Microdosimetry: Cell-level Exposures in a Virtual Tissue Context

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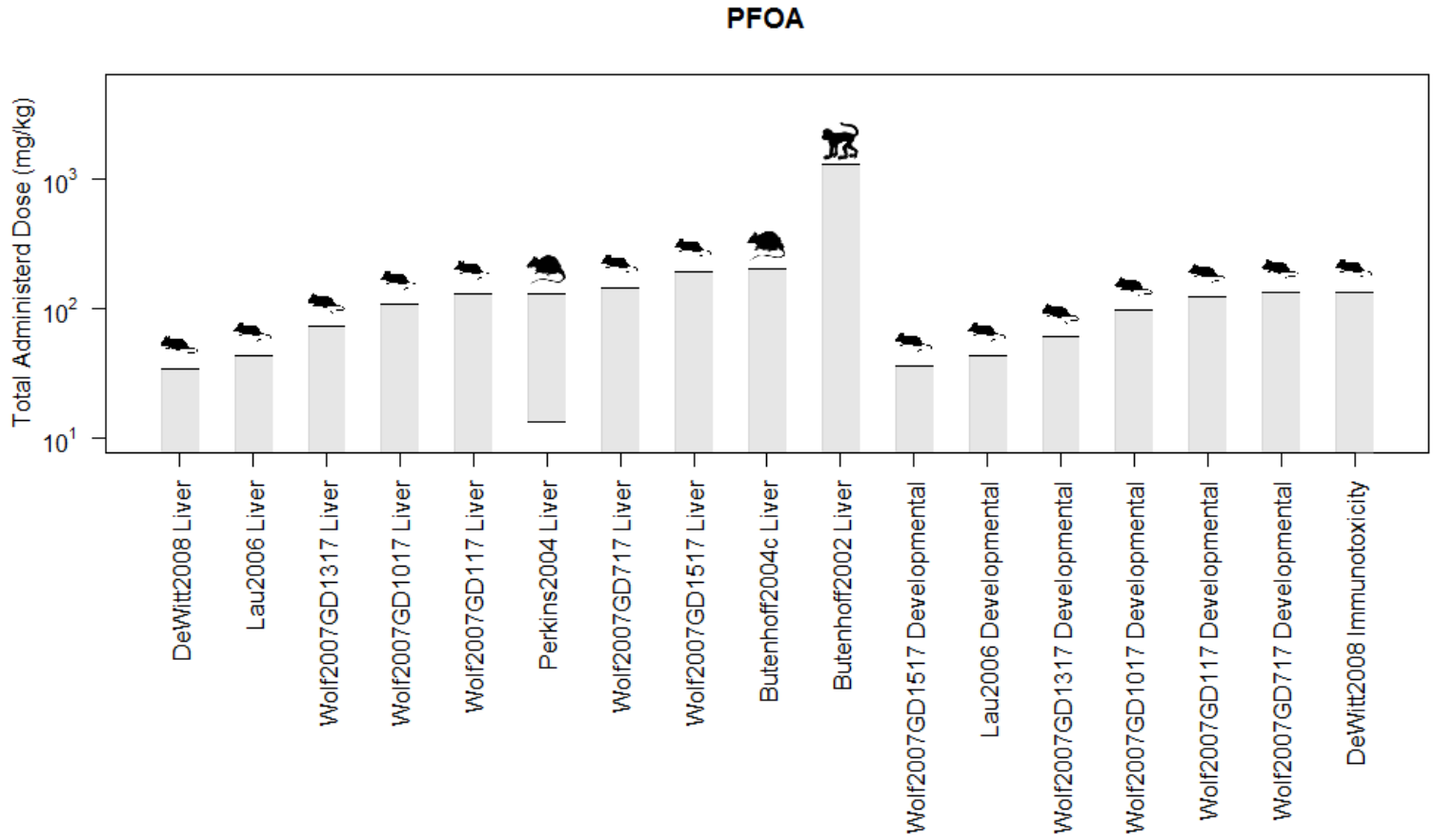
Introduction

- Toxicokinetic (TK) models can determine whether chemical exposures produce potentially hazardous tissue concentrations
- Tissue microdosimetry TK models relate whole-body chemical exposures to cell-scale concentrations.
- Successful methods have been developed for pharmaceutical compounds to determine high throughput TK (HTTK) from limited *in vitro* measurements and chemical structure-derived property predictions
- vLiverPBPK is an R package that contains data tables and tools for making predictions of tissue concentrations in various species; allows simulation of dose metrics from *in vivo* toxicity studies and determination of bioactive doses from *in vitro* hazard profiling assays data
- End goal is **quantitative *in vitro* – *in vivo* extrapolation for chronic dose-response**

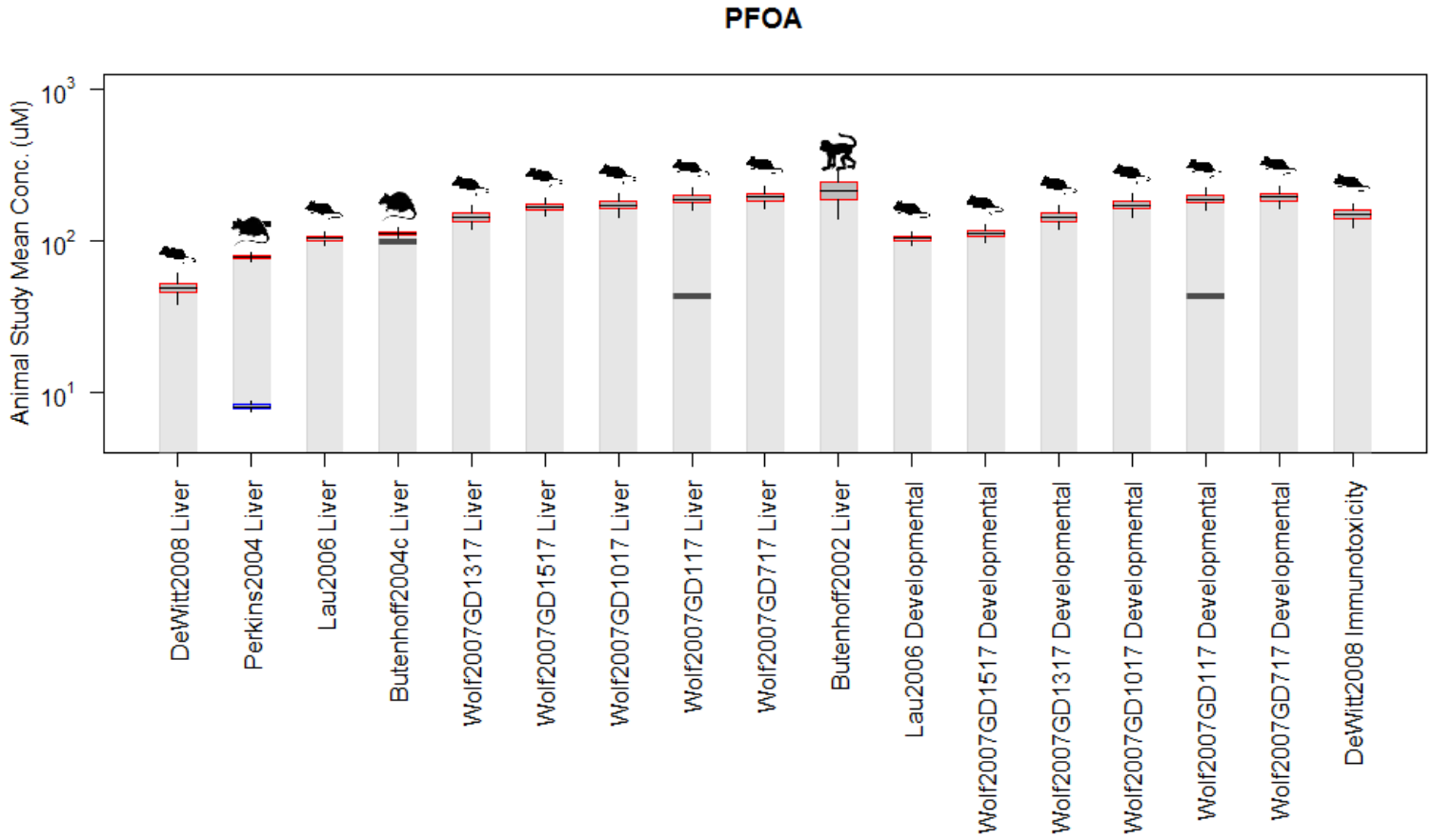
Pharmacokinetics Matters



Differences in species and dosing regimen can create apparent differences in doses needed to produce adverse effects.

Wambaugh *et al.* Tox. Sci. (2013)

Pharmacokinetics Matters



PK Modeling of tissue concentrations can reconcile these differences.

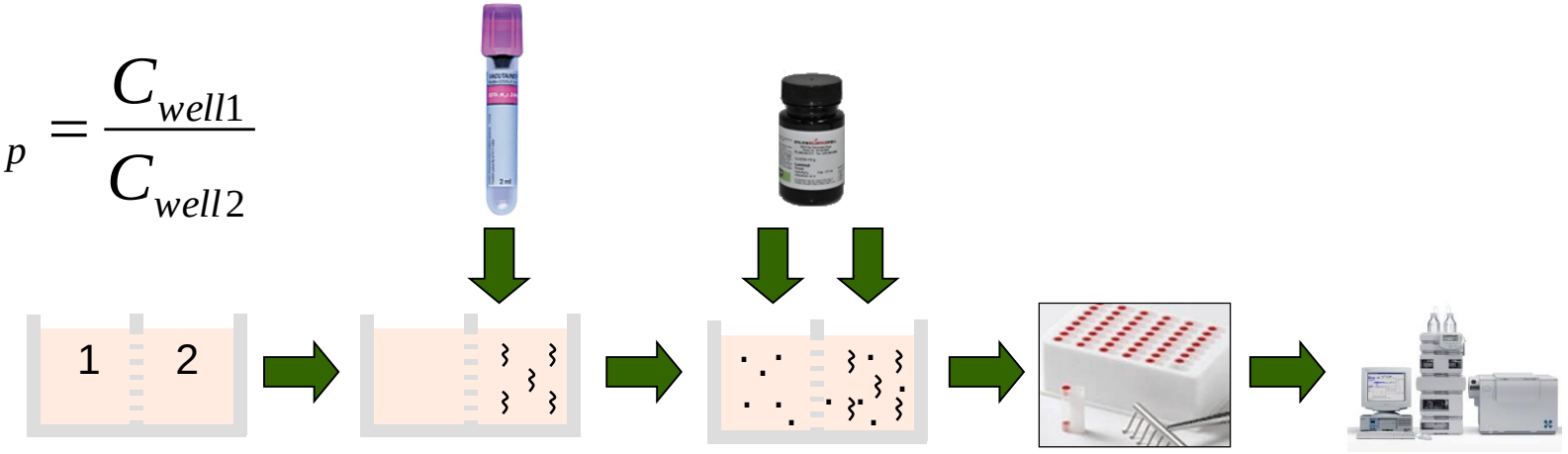
Wambaugh *et al*. Tox. Sci. (2013)

High Throughput Physiologically-based Toxicokinetic Models (HTPBPK)

- Out of 239 ToxCast chemicals examined by Wetmore et al. (2012), only 11 had some sort of human-relevant TK data or model
- HTTK predictions of steady-state behaviors were generated in Wetmore et al. (2012) using *in vitro* TK methods
- Can build generic, high throughput PBPK (HTPBPK) models parameterized with
  - the same *in vitro* HTTK data used for steady-state work, **plus**
  - QSARs for tissue-specific properties
  - Assumptions about unknown dynamic processes, such as absorption
- These HTPBPK models can provide a simulated in vivo context for tissue simulations

Plasma Protein Binding (Fraction Unbound in Plasma)

$$F_{ub,p} = \frac{C_{well1}}{C_{well2}}$$



Double-wells connected by semi-permeable membrane on a Rapid Equilibrium Dialysis (RED) Plate

Add plasma (6 donor pool for human) to one well

Add chemical

Incubate plates to allow wells with and without protein to come to equilibrium

Determine concentration in both wells (analytical chemistry)

RED Method:
Waters *et al.* (2008)

- Data on ToxCast chemicals initially collected at Hamner Institutes
- Published:
  - Rotroff et al. (2010) - Pilot study using 38 Phase I ToxCast Chemicals
  - Wetmore et al. (2012) - Remainder of easily analyzed Phase I chemicals
  - Wetmore et al. (2013) Rat PK for 50 ToxCast/ToxRefDB compounds

Intrinsic Hepatic Clearance

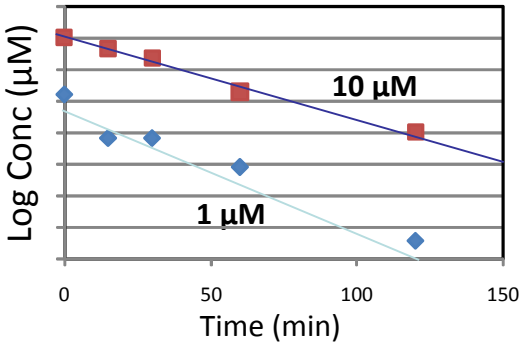
Cryopreserved Hepatocytes (10 donor pool for human)

Add Chemical (1 and 10 µM)

Remove Aliquots at 15, 30, 60, 120 min

Analytical Chemistry

The rate of disappearance of parent compound (slope of line) is the **hepatic clearance** (µL/min/10^6 hepatocytes)

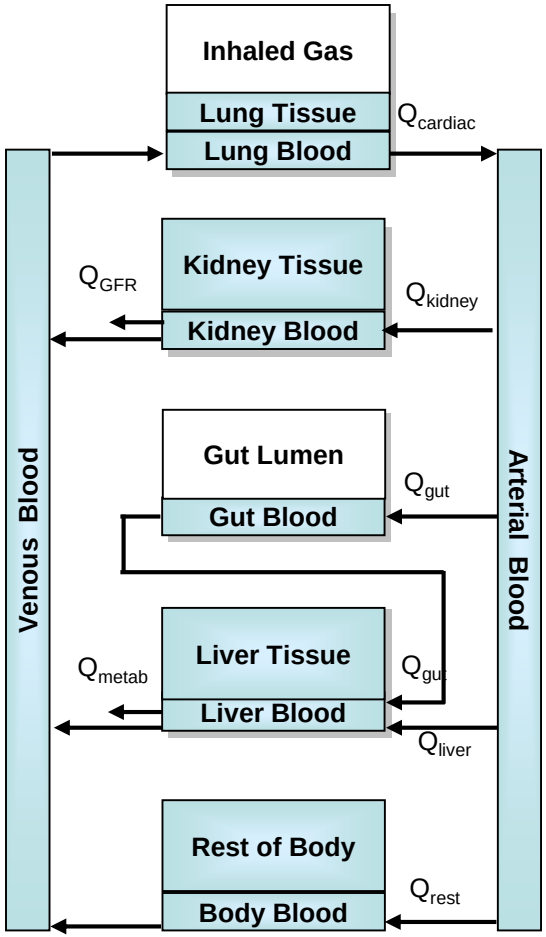


We perform the assay at 1 and 10 µM to check for saturation of metabolizing enzymes.

Cryopreserved hepatocyte Method: Shibata *et al.* (2002)

- Data on ToxCast chemicals initially collected at Hamner Institutes

Physiologically-based Toxicokinetic (PBPK) Model



- Some tissues (*e.g.*, arterial blood) are simple compartments, while others (*e.g.*, kidney) are compound compartments consisting of separate blood and tissue sections.
- Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (*e.g.*, fat, brain, bones) are lumped into the "Rest of Body" compartment.
- Chemical enters the body primarily through oral absorption, but we don't know absorption rate and bioavailability (assume "fast", *i.e.* 1/h and 100%)
- The only ways chemicals "leaves" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

Physiological Data

	Volume (L/kg)					Blood Flow (ml/min/kg)				
Tissue	**Mouse**	**Rat**	**Dog**	**Human**	**Rabbit**	**Mouse**	**Rat**	**Dog**	**Human**	**Rabbit**
Adipose	0.07	0.07	0.05	0.21	0.05	10.80	1.60	3.50	3.71	12.80
Bone	0.05	0.04	0.04	0.07	0.04	23.31	36.11	1.30	3.36	36.11
Brain	0.02	0.01	0.01	0.02	0.01	13.20	5.20	4.50	10.00	5.20
Gut	0.04	0.03	0.04	0.02	0.05	72.50	39.20	23.00	16.43	44.40
Heart	0.00	0.00	0.01	0.00	0.00	14.00	15.60	5.40	3.43	6.40
Kidneys	0.02	0.01	0.01	0.00	0.01	65.00	36.80	21.60	17.71	32.00
Liver	0.05	0.03	0.03	0.02	0.04	90.00	47.20	30.90	20.71	70.80
Lung	0.01	0.00	0.01	0.01	0.01	2.00	6.22	10.56	2.00	6.22
Muscle	0.37	0.39	0.44	0.38	0.54	45.50	30.00	25.00	10.71	62.00
Skin	0.15	0.17	0.17	0.03	0.04	20.50	23.20	10.00	4.29	23.20
Spleen	0.00	0.00	0.00	0.00	0.00	5.50	4.07	1.65	1.10	3.60
Rest	0.03	0.05	0.00	0.05	0.03	110.19	90.00	5.59	2.97	90.00

Volumes and flows from Schmitt (2008) + Rabbit (Sipes)

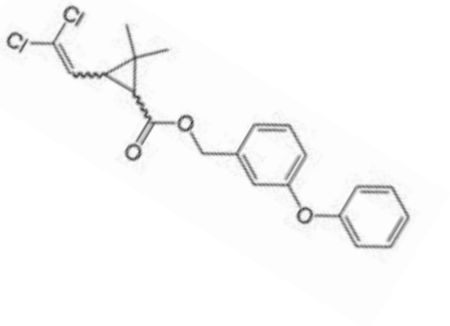
Other parameters from Davies and Morris (1993) + Rabbit (Sipes)

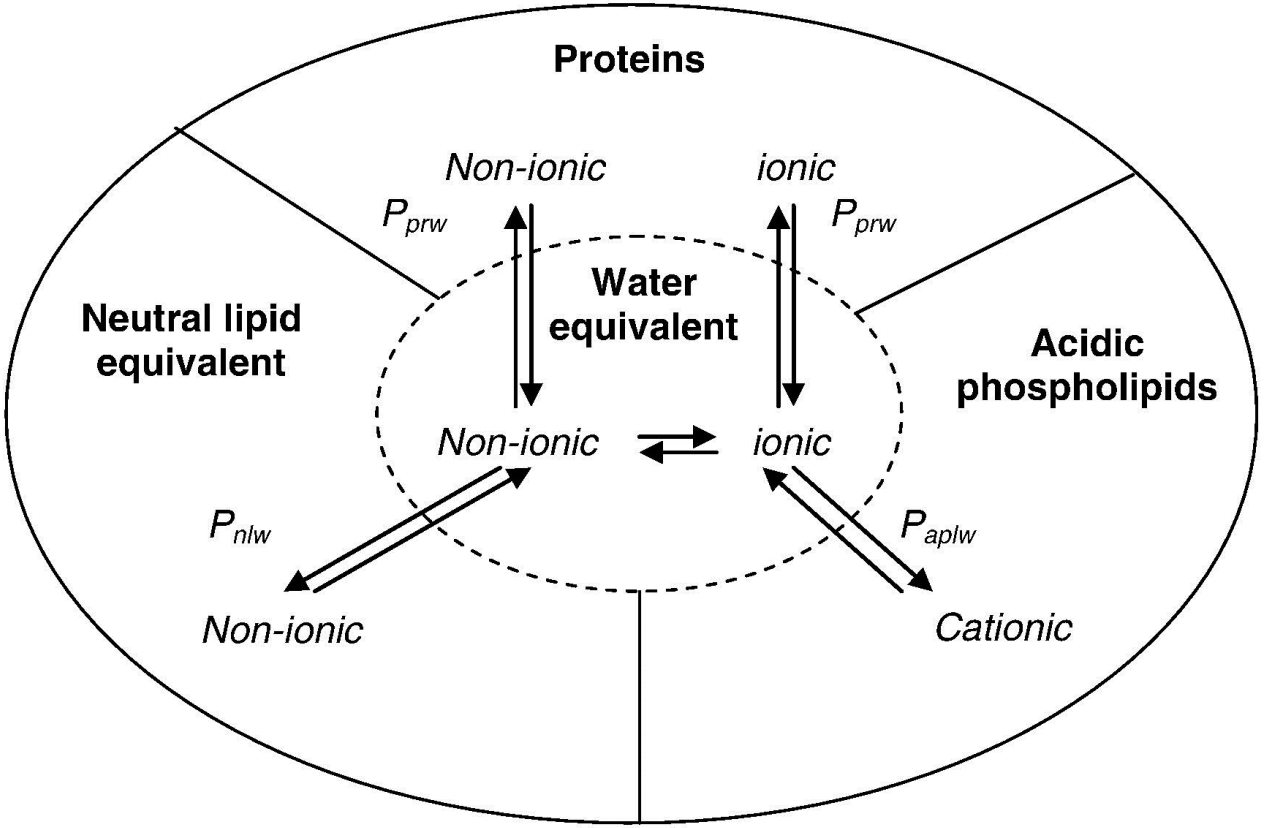
	Units	**Mouse**	**Rat**	**Dog**	**Human**	**Rabbit**
Total Body Water	ml/kg	725.00	668.00	603.60	600.00	716
Plasma Volume	ml/kg	50.00	31.20	51.50	42.86	44
Cardiac Output	ml/min/kg	400.00	296.00	120.00	80.00	212
Average BW	kg	0.02	0.25	10.00	70.00	2.5
Total Plasma Protein	g/ml	0.06	0.07	0.09	0.07	0.057
Plasma albumin	g/ml	0.03	0.03	0.03	0.04	0.0387
Plasma a-1-AGP	g/ml	0.01	0.02	0.00	0.00	0.0013
Hematocrit	fraction	0.45	0.46	0.42	0.44	0.36
Urine	ml/min/kg	0.035	0.139	0.021	0.014	0.0417
Bile	ml/min/kg	0.069	0.063	0.008	0.003	0.0833
GFR	ml/min/kg	14.0	5.2	6.1	1.8	3.12

Predicted Partition Coefficients

Tissue-specific partitioning estimated (Schmitt 2008) using:

- Physicochemical properties (logP, pKa) predicted from structure (EpiSuite, QikProp)
- Measured free fraction in plasma





Partitioning figure is from Peyret (2010), which described a new method that is being implemented for vLiverPBPK by Cory Strope

Schmitt (2008) Tissue Composition Data

	Fraction of total volume[a]		Fraction of cell volume[b]			Fraction of total lipid			
Tissue	Cells	Interstitium	Water	Lipid	Protein	Neutral Lipid[c]	Neutral Phospholipid[c]	Acidic Phospholipid[c]	pH[d]
Adipose	0.86	0.14	0.03	0.92	0.06	1	0.0022	0.0006	7.10
Bone	0.9	0.1	0.26	0.02	0.21	0.85	0.11	0.04	7.00
Brain	1	0.004	0.79	0.11	0.08	0.39	0.48	0.13	7.10
Gut	0.9	0.096	0.78	0.07	0.15	0.69	0.26	0.05	7.00
Heart	0.86	0.14	0.7	0.11	0.19	0.48	0.43	0.09	7.10
Kidneys	0.78	0.22	0.73	0.06	0.21	0.26	0.61	0.13	7.22
Liver	0.82	0.18	0.68	0.08	0.21	0.29	0.59	0.11	7.23
Lung	0.5	0.5	0.74	0.04	0.11	0.51	0.38	0.11	6.60
Muscle	0.88	0.12	0.76	0.01	0.19	0.49	0.42	0.09	6.81
Skin	0.69	0.31	0.47	0.14	0.41	0.9	0.08	0.02	7.00
Spleen	0.79	0.21	0.75	0.02	0.23	0.3	0.54	0.15	7.00
Red blood cells	1	–	0.63	0.01	0.33	0.3	0.59	0.1	7.20

a Values taken from (Kawai et al., 1994). Original values given as fraction of total organ volume were rescaled to tissue volume by subtracting vascular volume

b Values taken from (ICRP, 1975). Original values given as fraction of total tissue mass were rescaled to cellular volume as follows: Water fraction of total tissue reduced by interstitial volume and subsequently all values normalized by cellular fraction.

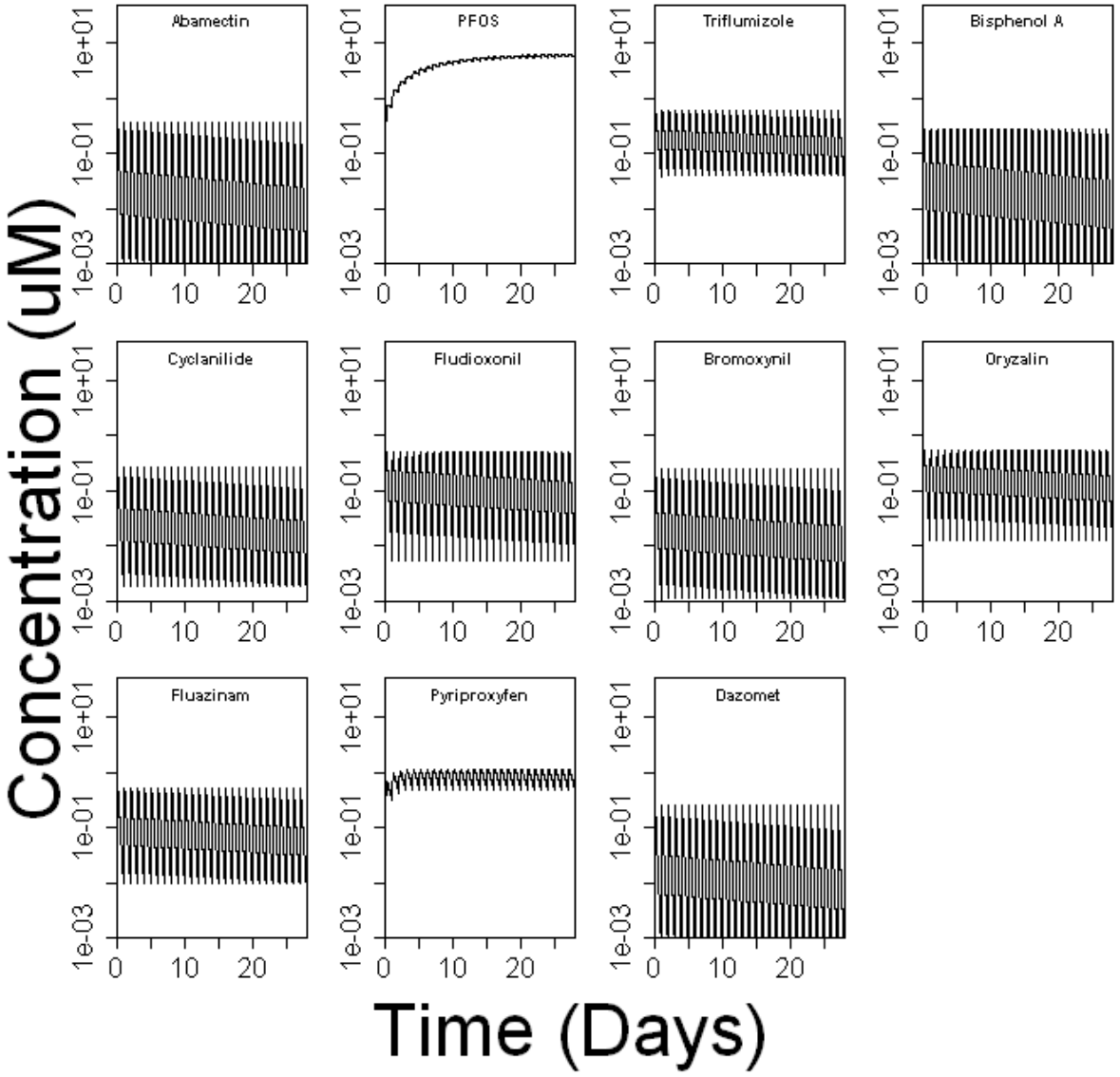
c Data taken from (Rodgers et al., 2005a).

d Values taken from ([Waddell and Bates, 1969], [Malan et al., 1985], [Wood and Schaefer, 1978], [Schanker and Less, 1977], [Harrison and Walker, 1979] and [Civelek et al., 1996]). Mean values were calculated when more than one value was found for the same tissue.

e Data taken from (Gomez et al., 2002).

Predicted PK Metrics

- Human hepatic concentration of various chemicals as a function of 28 daily doses (10 mg/kg/day)
- Can predict mean and peak concentration and time integrated area under the curve (AUC) for various tissues

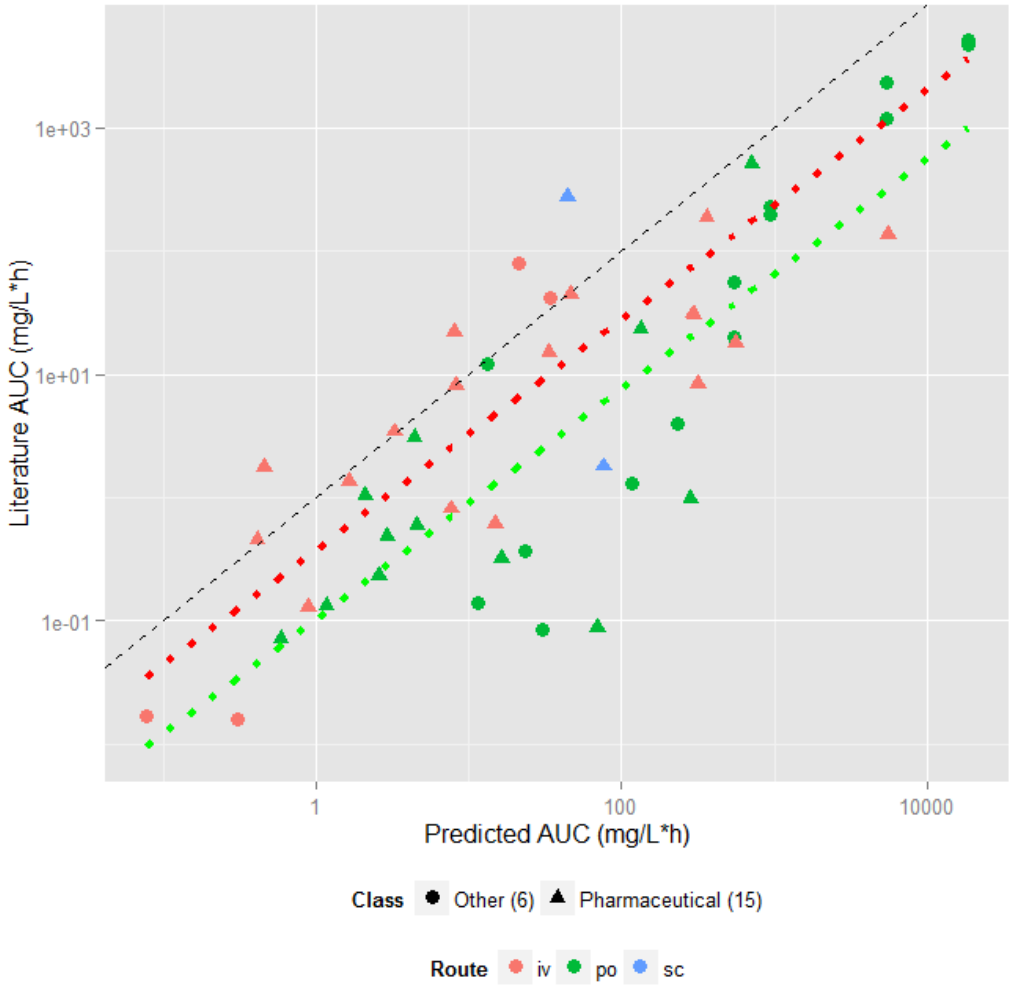


In vivo Predictive Ability and Domain of Applicability

- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)

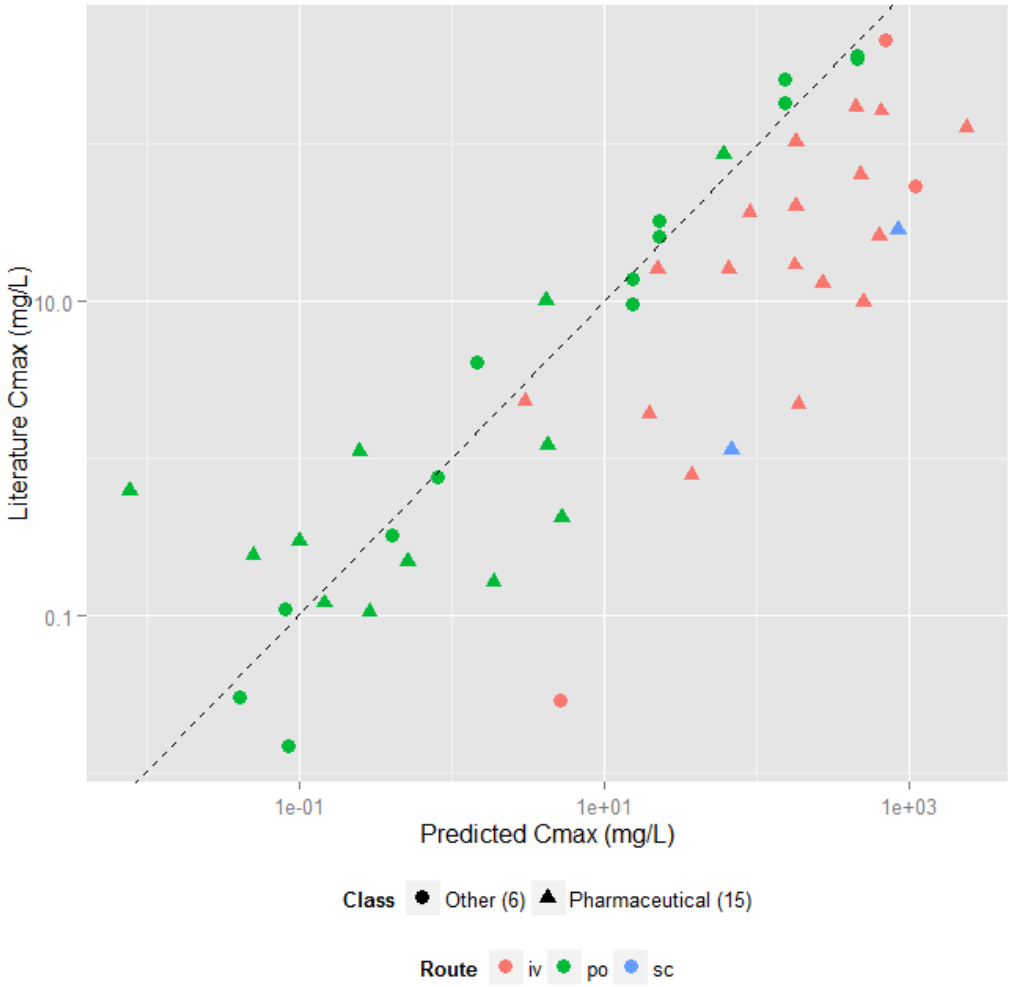
- For environmental compounds, there will be no clinical trials their uncertainty must be well characterized ideally with rigorous statistical methodology
  - We will use direct comparison to *in vivo* data in order to get an empirical estimate of our uncertainty
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Evaluating HTPBPK Predictions from *In Vitro* Data



- HTPBPK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat.
- Predictions are generally conservative – *i.e.*, predicted AUC higher than measured
- Oral dose AUC ~3.6x higher than intravenous dose AUC (p-Value 0.021)

Evaluating HTPBPK Predictions from *In Vitro* Data



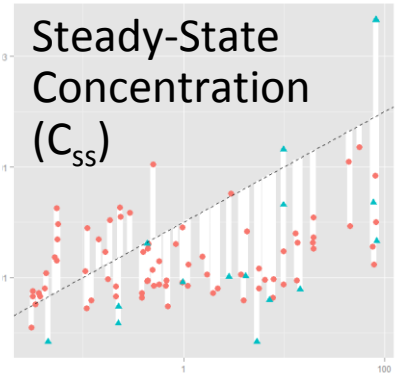
- C_{max} predictions relatively decent (R^2 ~ 0.69)

Predicting When RTK Will Work

- We can use computer algorithms to analyze chemical descriptors to try to predict when the residual will be small
- Factors included are:
  - Physico-chemical properties
    - Log(Kow), molecular weight, acid/base association constants (pKa), general pharmaceutical or perfluorinated compound classification
  - *In vitro* HTTK data
    - Plasma protein binding (F_{ub}) and hepatic clearance
  - Active chemical transport
    - Use quantitative structure activity relationships (QSARs) to predict likelihood each compound is a substrate for 17 different transporters (From Alexander Sedykh and Alex Tropsha (UNC) and Sieto Bosgra (TNO))

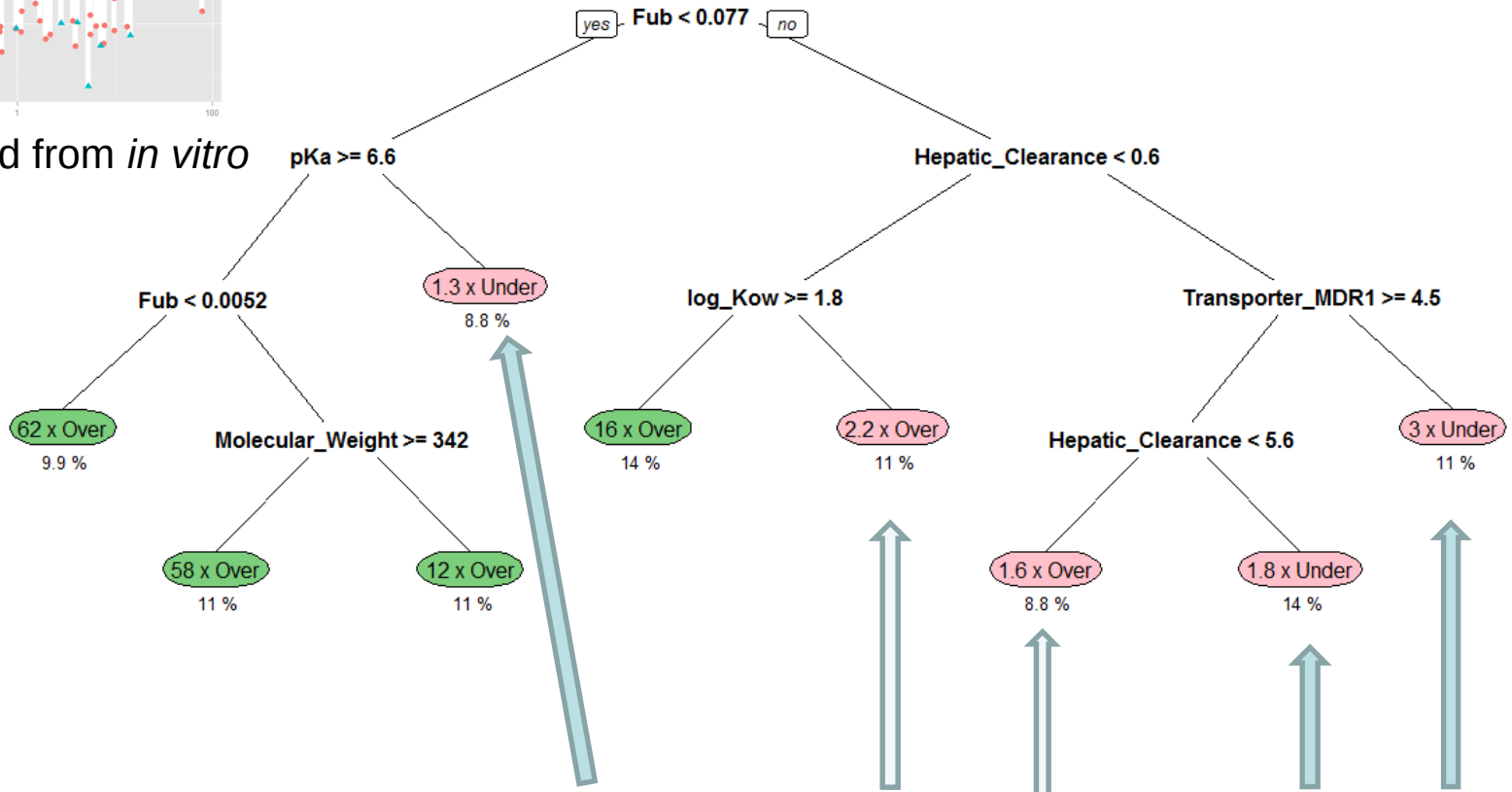
Recursive Partitioning Tree for (Log) Residuals

United States Environmental Protection Agency



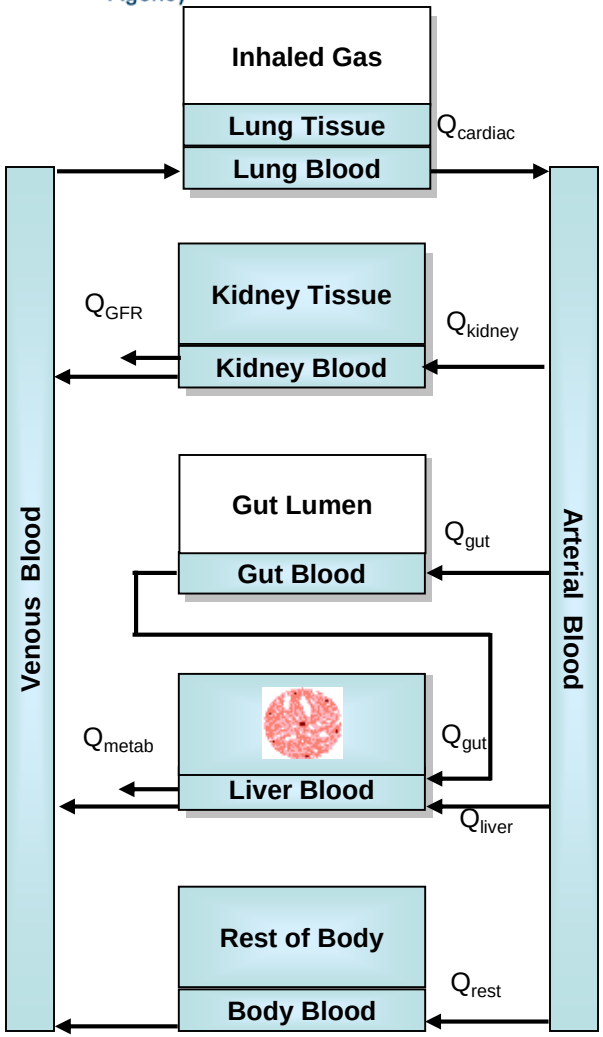
Literature *in vivo*

Predicted from *in vitro*



- If the predicted C_{ss} underestimates the literature value, the necessary exposure predicted with RTK will be higher

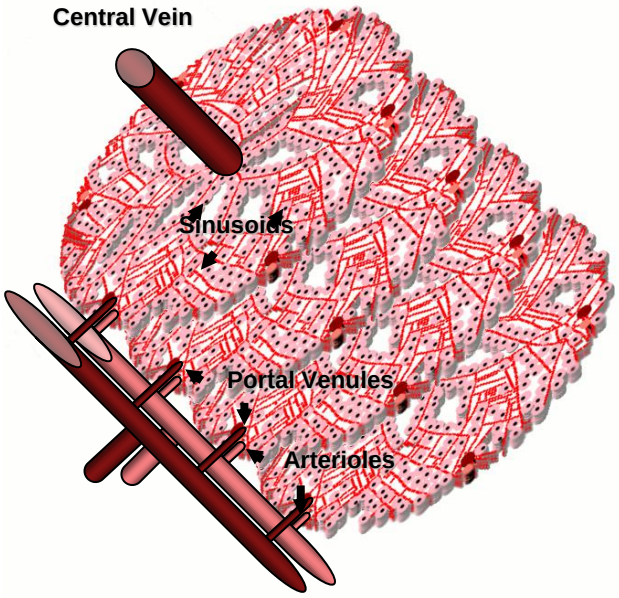
A Virtual Tissues Proof of Concept



We have approximated the micro-anatomic architecture of the hepatic lobule with a discrete topology by a graphical model that can be connected to a chemical-specific physiologically-based TK (PBPK) model

Tissue microdosimetry model (Wambaugh and Shah, 2010) bridges PBPK models and cell-level doses

Allows simulation of cell-based pathway (*e.g.,* Jack *et al.*, 2011) in a local, chemical environment



In vitro Dose-Response Curves

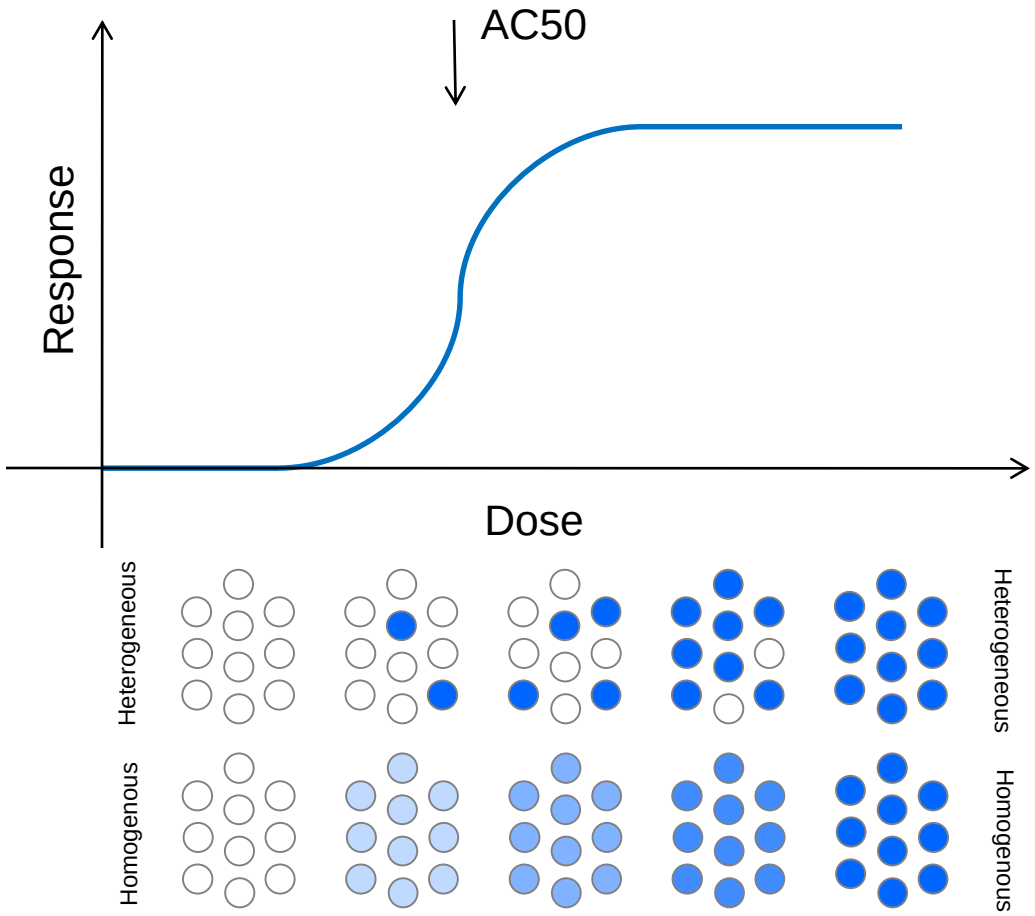


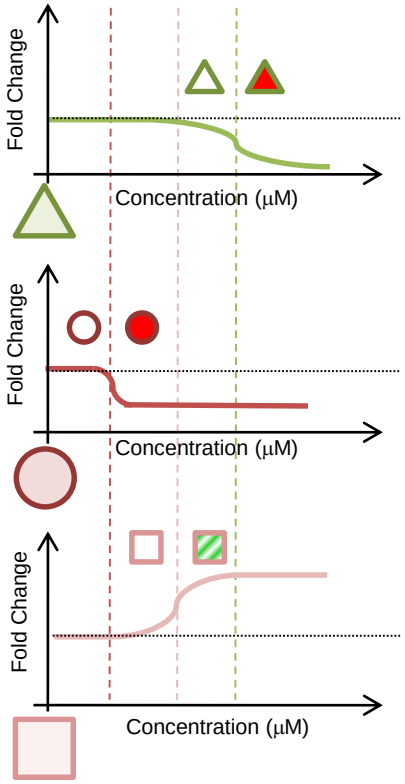
Figure adapted from Luke *et al*., (2010) Dose Response **8,** 347-367.

The **dose-response curve is central** because many of these chemicals are useful and we know that, generally, a sufficient concentration will cause some toxic effect

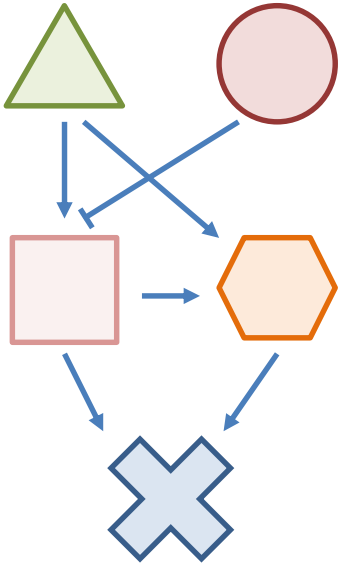
We assume individual cells respond discretely and stochastically as **independent agents**

Chemical Specific Predictions from *in vitro* Activity

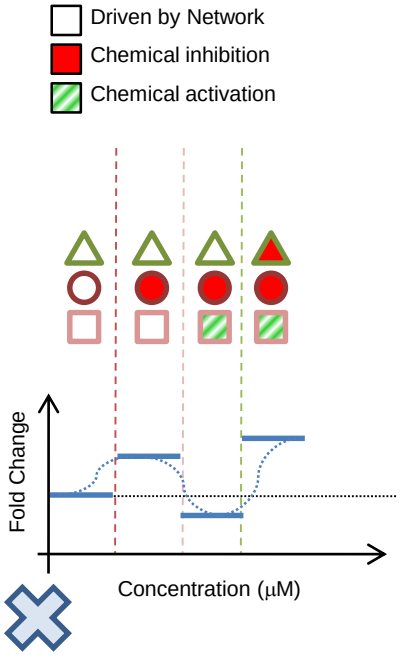
Activity Measured *In vitro*



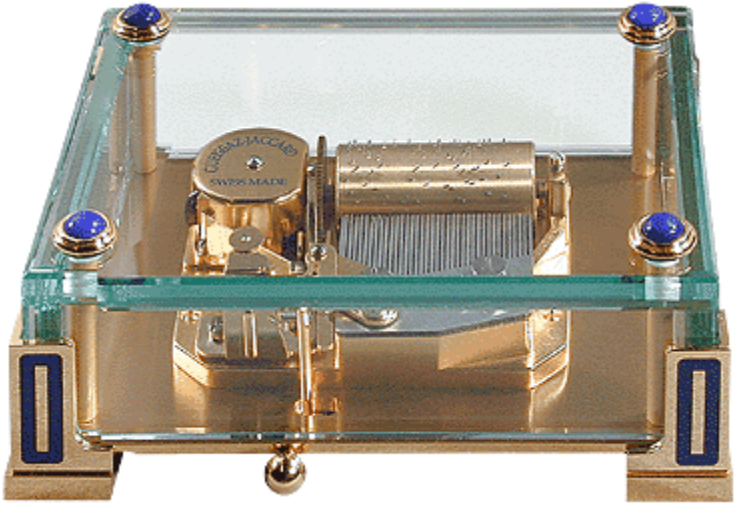
Putative Pathway

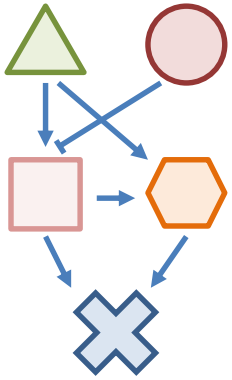


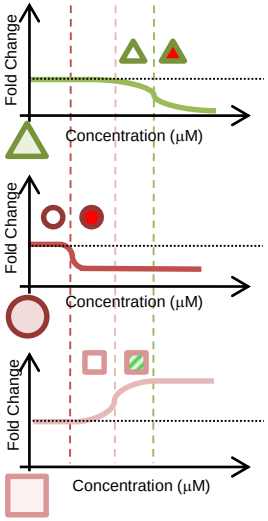
Activity Predicted By Pathway



In vitro Data Gives the Order of Perturbations







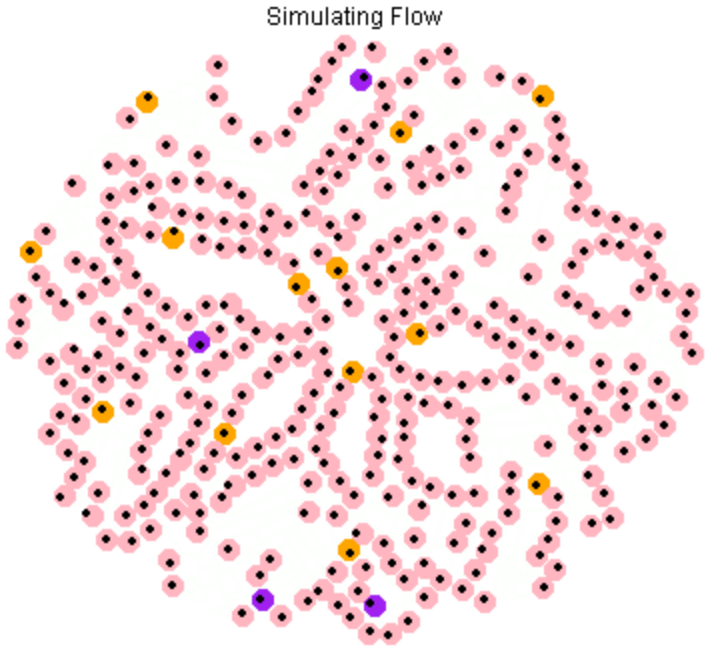
Other applications: Virtual Tissues

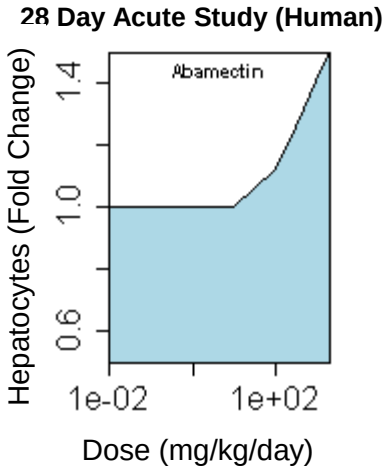
2 Hours After Oral Exposure to 100 mg/kg Abamectin

$$r_{apop} = 0.0015 \frac{1}{h}$$

$$\frac{N^{apop}}{N^{0}} = 0.0087$$

$$\frac{N^{prol}}{N^{0}} = 0.052$$





- H^{o} (Normal/Quiescent)
- H^{prol} (Proliferative)
- H^{apop} (Apoptotic)

High-throughput PBPK and Microdosimetry

- Using in vitro TK methods developed for pharmaceuticals, we can parameterize HTPBPK models
- We can model the difference between *in vivo* measurements and HTTK predictions (*i.e.*, the residuals or errors)
- We can connect HTPBPK models to tissue simulations to provide simulated in vivo context for assessing the impact of chemical perturbations identified by high throughput screening assays

High Throughput Toxicokinetics Researcher Collaborators

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