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Challenges of *In Vitro* Disposition Modeling: First Insights from the Tox21 Project

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Symposium:
Challenges in the development of *in vitro-in vivo* extrapolation models for next generation risk assessment

Tuesday, March 21, 2023

Conflict of Interest Statement

The authors declare no conflict of interest

US EPA Office of Research and Development

- The Office of Research and Development (ORD) is the scientific research arm of EPA
- 539 peer-reviewed journal articles in 2021
- Research is conducted by ORD's four national centers organized to address:
  - Public health and environmental assessment
  - Computational toxicology and exposure
  - Environmental measurement and modeling
  - Environmental solutions and emergency response
- 13 facilities across the United States
- Research conducted by a combination of Federal scientists, including uniformed members of the **Public Health Service**; contract researchers; and postdoctoral, graduate student, and post-baccalaureate trainees

ORD Facility in
Research Triangle Park, NC

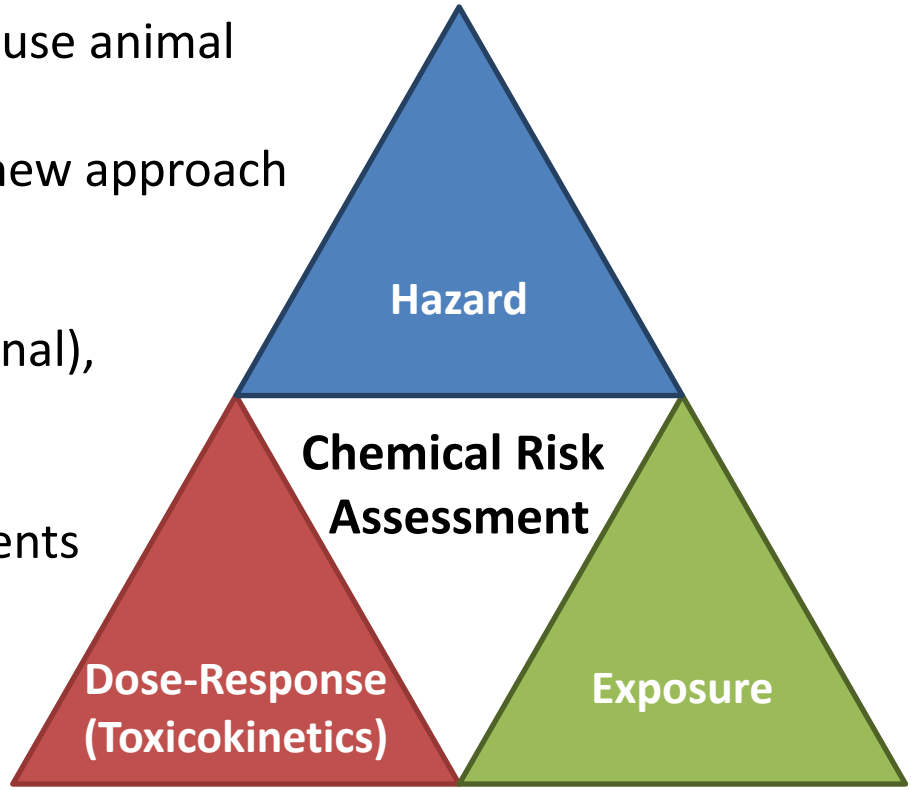
Chemical Risk Assessment Requires Understanding Dose-Response

- NRC (1983): Risk is a function of inherent chemical hazard, extent of exposure, and the dose-response relationship (including toxicokinetics)

- **Hazard:** To estimate the impact of potentially harmful chemicals we use animal and *in vitro* studies and extrapolate to humans
  - Next generation risk assessment (NGRA) is working to develop new approach methodologies (NAMs) that cover key biological pathways

- **Exposure:** Must consider the context (consumer/ambient/occupational), route, frequency, and extent of contact with the chemical
  - Concurrent development of NAMs for exposure includes high throughput toxicokinetics and exposure models and measurements

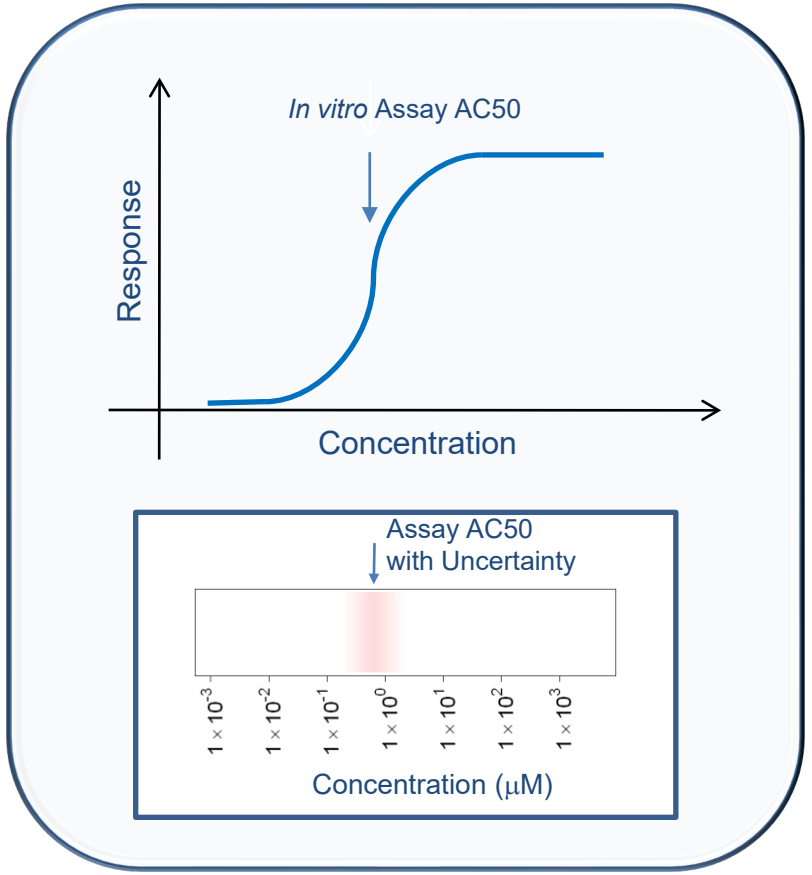
- **Dose-response:** Must understand quantitative relationship between magnitude of exposure and amount of effect
  - NGRA requires tools for *in vitro-in vivo* extrapolation (IVIVE)



NRC, 1983

Next Generation Risk Assessment (NGRA) is Built Upon New Approach Methodologies (NAMs)

- We attempt to estimate points of departure *in vitro* using high throughput screening (HTS) for bioactivity as a surrogate for hazard
- **Tox21**: Examining >8,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast** (Toxicity Forecaster): >4000 chemicals (including a subset of Tox21) for >2000 additional assay endpoints (invitrodb version 3.5) (Kavlock *et al.*, 2012)
- To use HTS assays as an alternative to traditional animal studies we must link *in vitro* bioactivity concentrations and potentially toxic doses via *in vitro-in vivo* extrapolation (IVIVE).

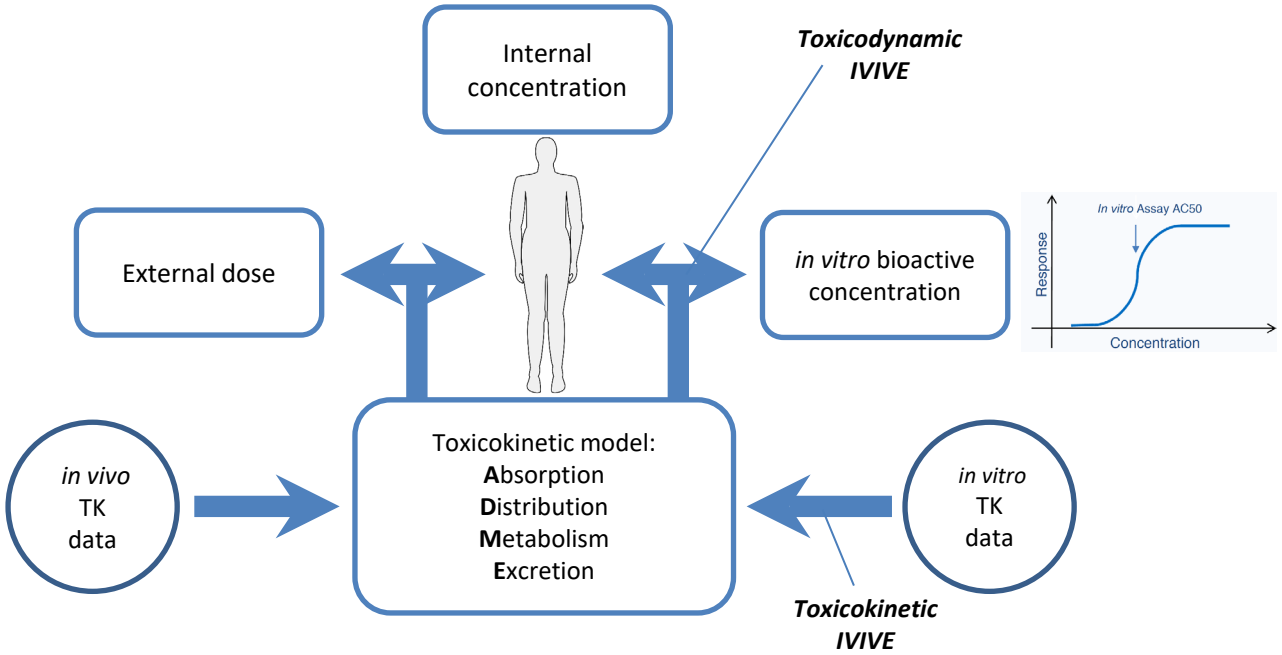


In Vitro - *In Vivo* Extrapolation (IVIVE)

IVIVE is the use of *in vitro* experimental data to predict phenomena *in vivo* (Coecke et al., 2013, Wetmore, 2015)

- *In Vitro* Disposition:
  - Difference between nominal and effective concentration of chemical
  - Partitioning to plate wall, nutrients, volatilization

In vitro to *in vivo* extrapolation (IVIVE)



Breen et al., 2021

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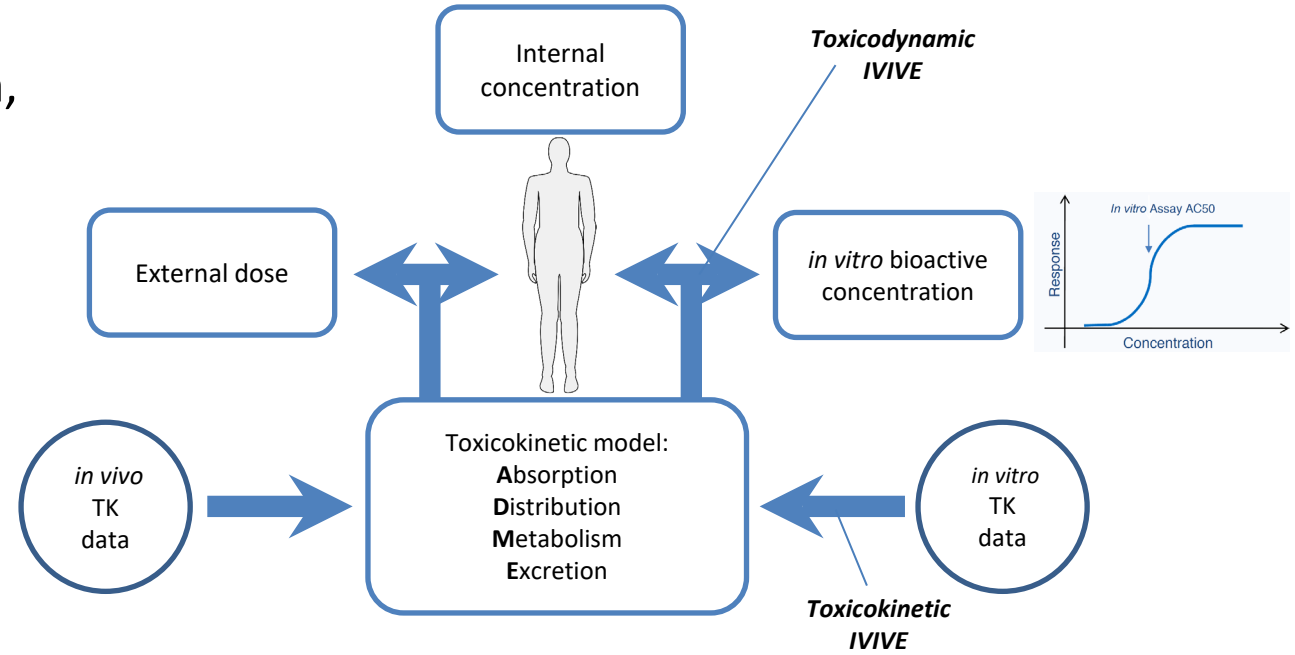

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- IVIVE-PD/TD (**Pharmacodynamics/Toxicodynamics**):
  - Effect of molecules/chemicals at biological target *in vivo*
  - Assay design/selection important
  - Perturbation as adverse/therapeutic effect, reversible/irreversible effects

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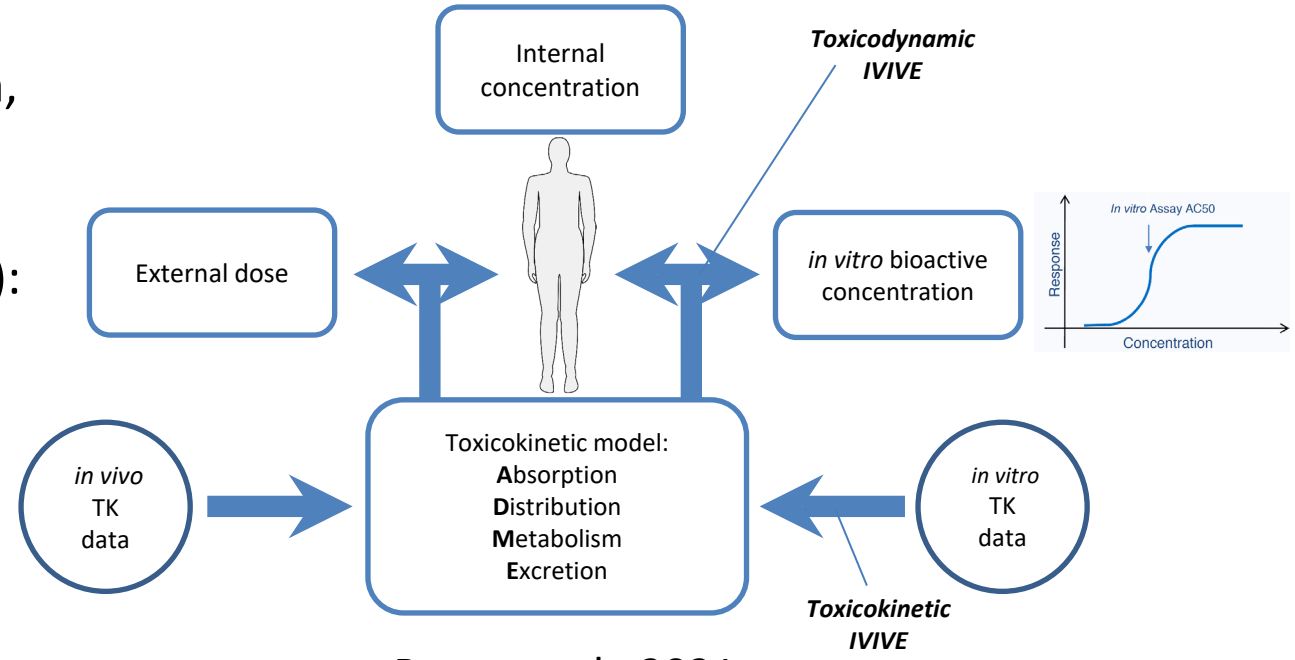

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Our focus today

- IVIVE-PK/TK (**Pharmacokinetics/Toxicokinetics**):
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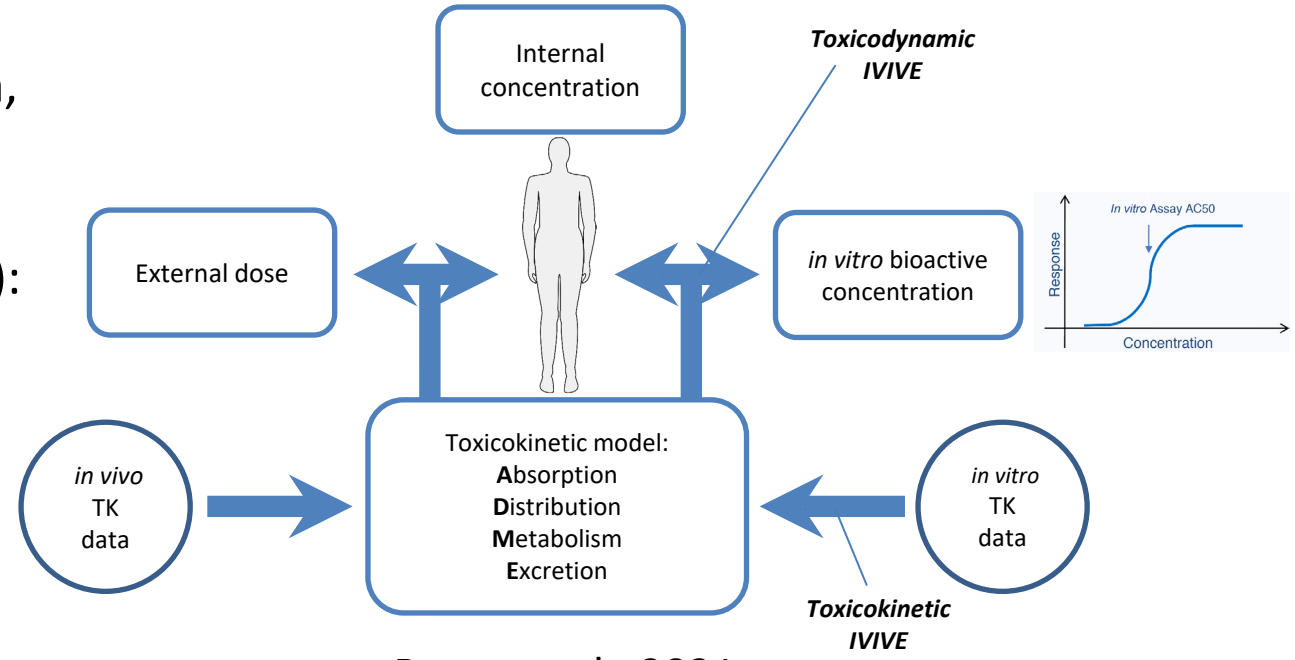


Breen et al., 2021

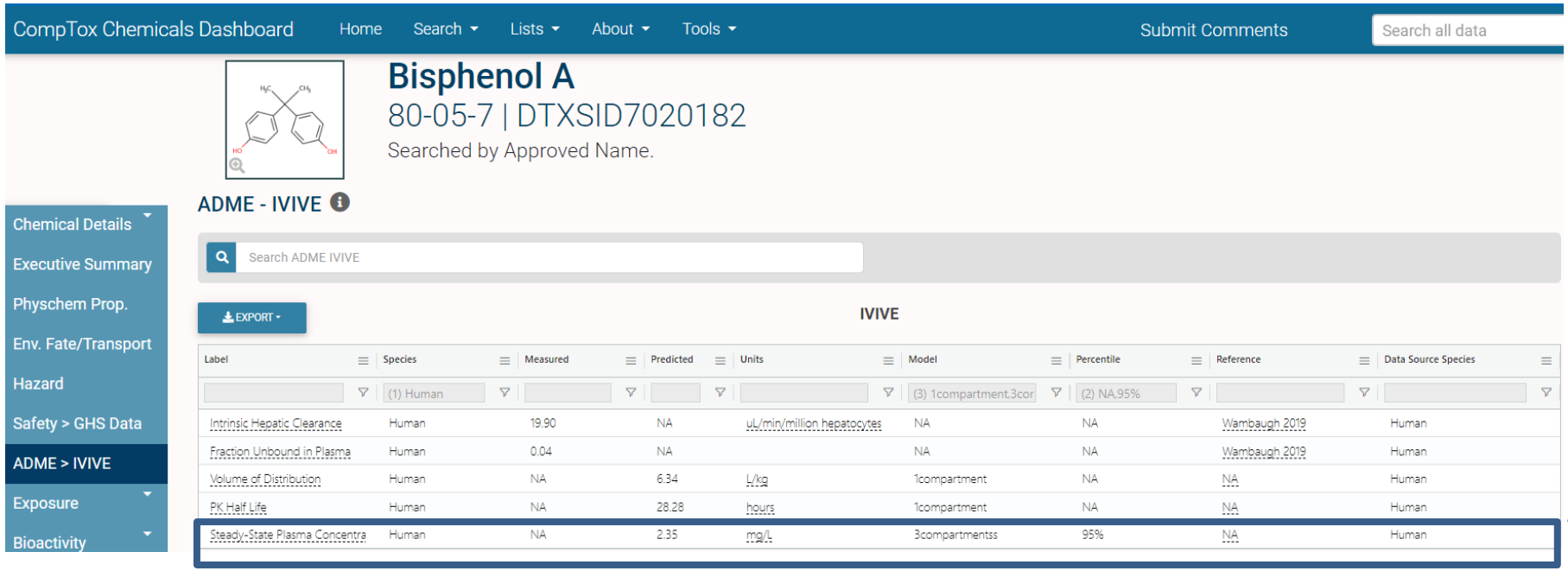
IVIVE via High-Throughput Toxicokinetics (HTTK): Administered Equivalent Doses (AEDs)

Identification of a potency value to use for IVIVE of a threshold dose

- Operationally, the httk R package (v 2.2.2) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs).
- AC50 or LEC (micromolar) * (1 mg/kg/day/C_{ss} (micromolar)) = AED prediction

$$\frac{2.35\ \cancel{mg}}{L}\ \frac{\cancel{g}}{1000\ \cancel{mg}}\ \frac{\cancel{mol}}{228.291\ \cancel{g}}\ \frac{1e6\ \mu mol}{\cancel{mol}} = 10.294\ \mu mol/L = \mu M$$

$$\frac{0.1\ \cancel{\mu M}}{}\ \frac{1\ mg/kg/day}{10.294\ \cancel{\mu M}} = \mathbf{0.010\ mg/kg/day = AED95}$$

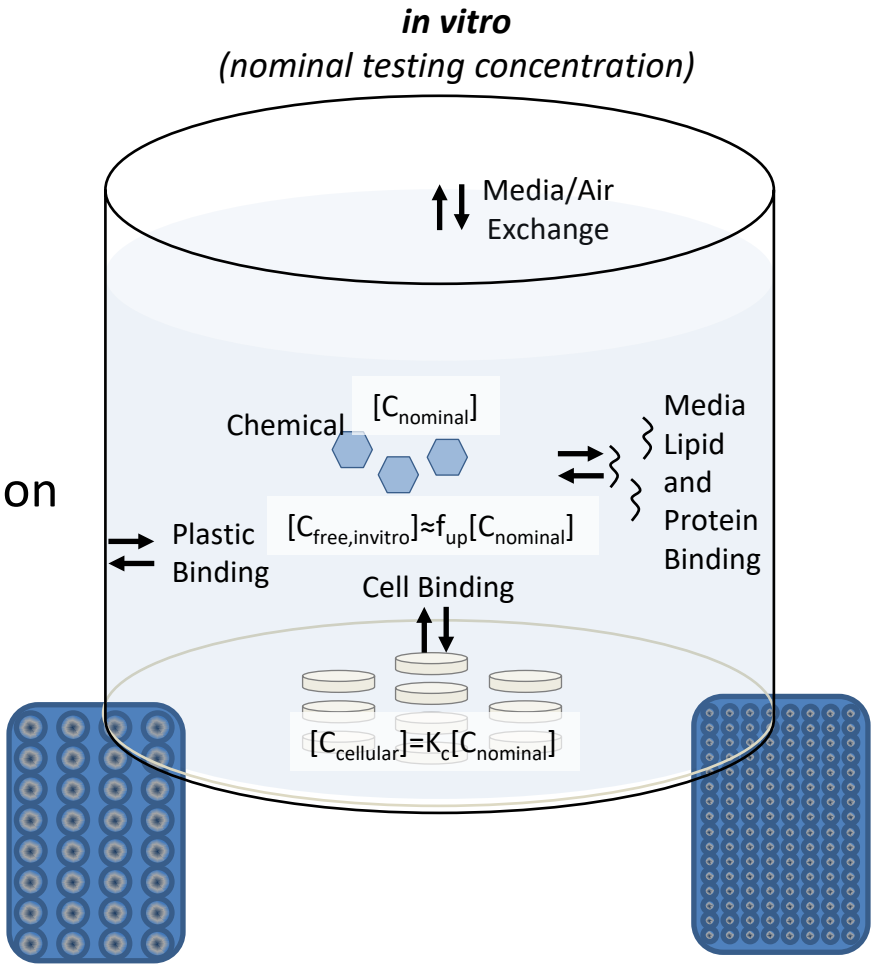


Steady-state plasma concentration (C_{ss}) here is from 95th population quantile (higher plasma conc. for same dose)

Slide from Katie Paul Friedman

Distribution Considerations for IVIVE

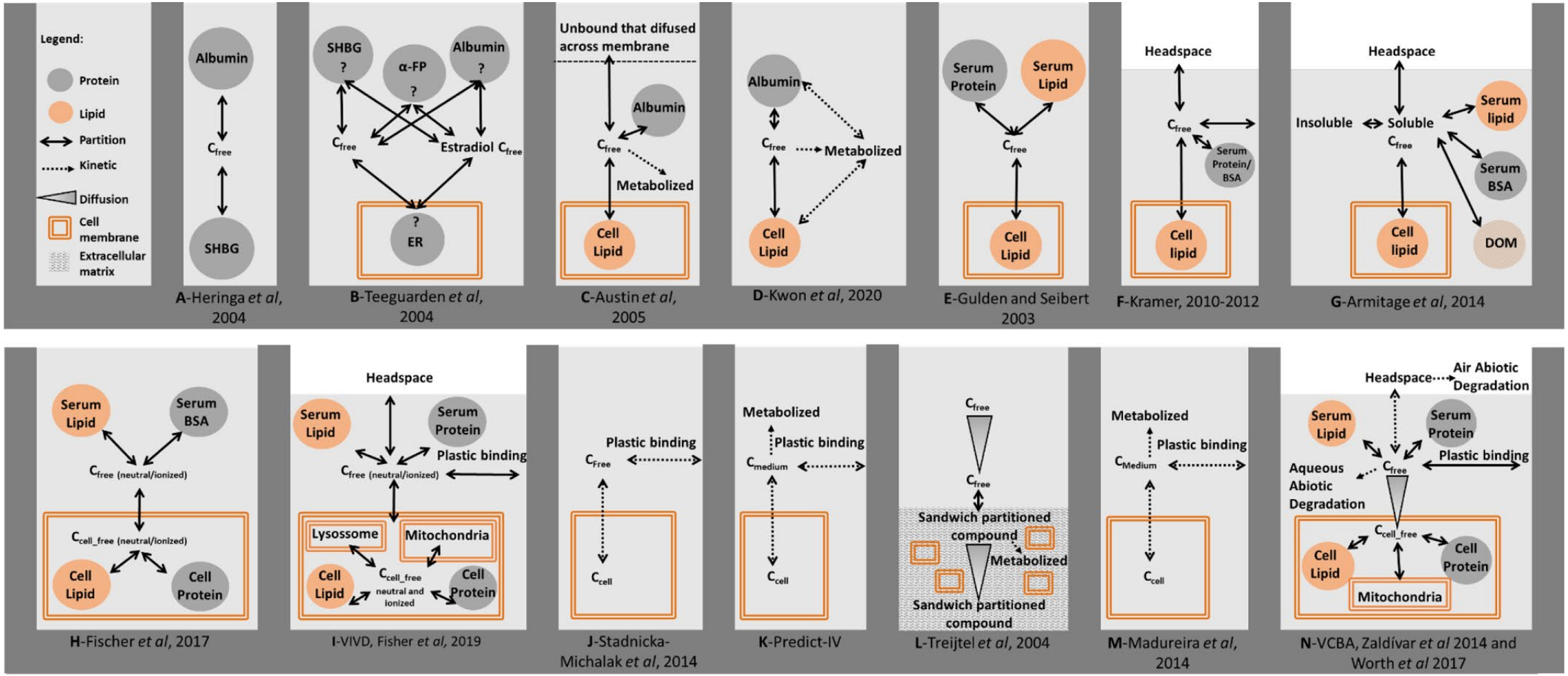
- IVIVE predictions currently rely on $C_{nominal}$, can also consider concentration in the cells ($C_{cellular}$), and the free concentration in the *in vitro* media ($C_{free,invitro}$)
  - Differences of $C_{free,in\ vitro}$ based on physico-chemical properties and composition of well and media
  - *In vitro* cells (C_{cell}) differ from *in vivo* tissue (C_{tissue})
- EPA's IVIVE software "httk" uses for four different models for distribution to get administered equivalent dose (AED) from bioactive in vitro concentrations
  - Schmitt (2008) – Mechanistic tissue model, empirically calibrated
  - Armitage et al. (2014)– Mechanistic mass-balance in hazard NAMs
  - Kilford et al. (2008) – Empirical hepatocyte assay binding
  - Pearce et al. (2017) – Empirical plasma protein assay binding



- Domain of applicability – experimental conditions baked into empirical models
- Armitage could be more general theory – needs empirical evaluation to establish confidence

Models for *In Vitro* Distribution

- Proença et al. (2021) "Effective exposure of chemicals in *in vitro* cell systems: A review of chemical distribution models":



What factors influence *in vitro* partitioning?

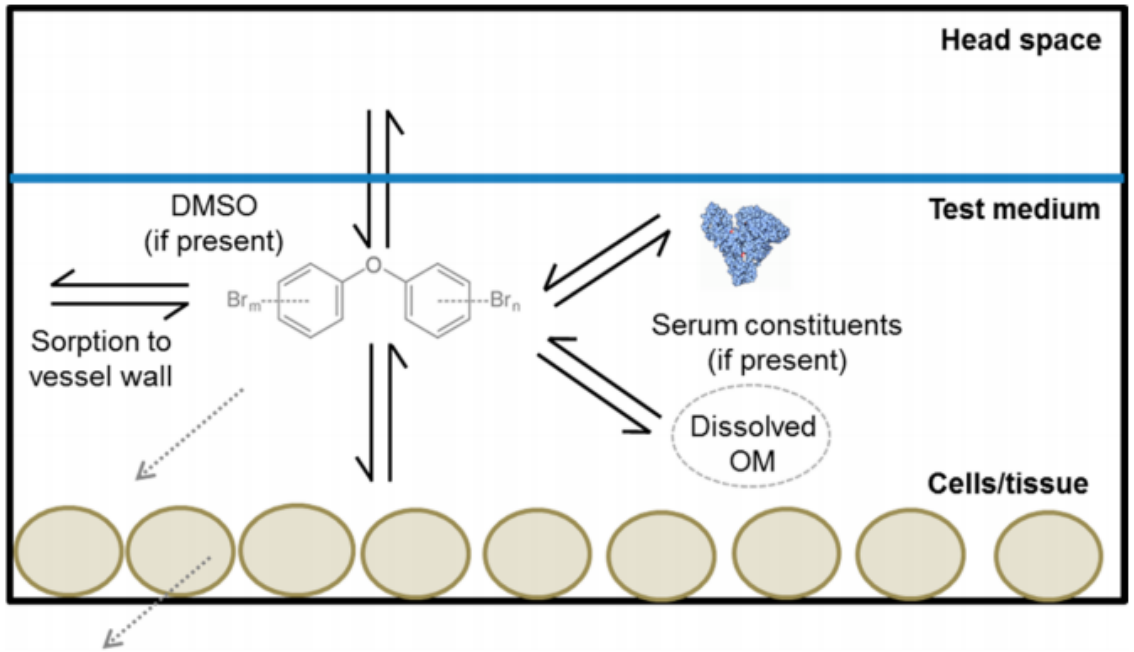
- Armitage et al. (2014) suggest that *in vitro* partitioning relates strongly to logKow and serum in the medium
- Note, Armitage model expanded to ionizable compounds by Fischer et al. (2017) (see also Armitage 2021) – ionization added to httk implementation
- To date, *in vitro* partitioning has been empirically evaluated for very few chemicals; thus, it is unknown for how many chemicals and to what degree differential chemical partitioning affects the accuracy of IVIVE predictions made across the ToxCast and Tox21 chemical library.

Mass-balance model

$$C_W = \frac{M_T}{K_{AW}V_A + V_W + K_{SaW}V_{Sa} + K_{SlW}V_{Sl} + K_{DW}V_D + K_{CW}V_C} \quad (1)$$

Armitage et al. 2014 PMID 25014875

Diagram of *in vitro* compartments



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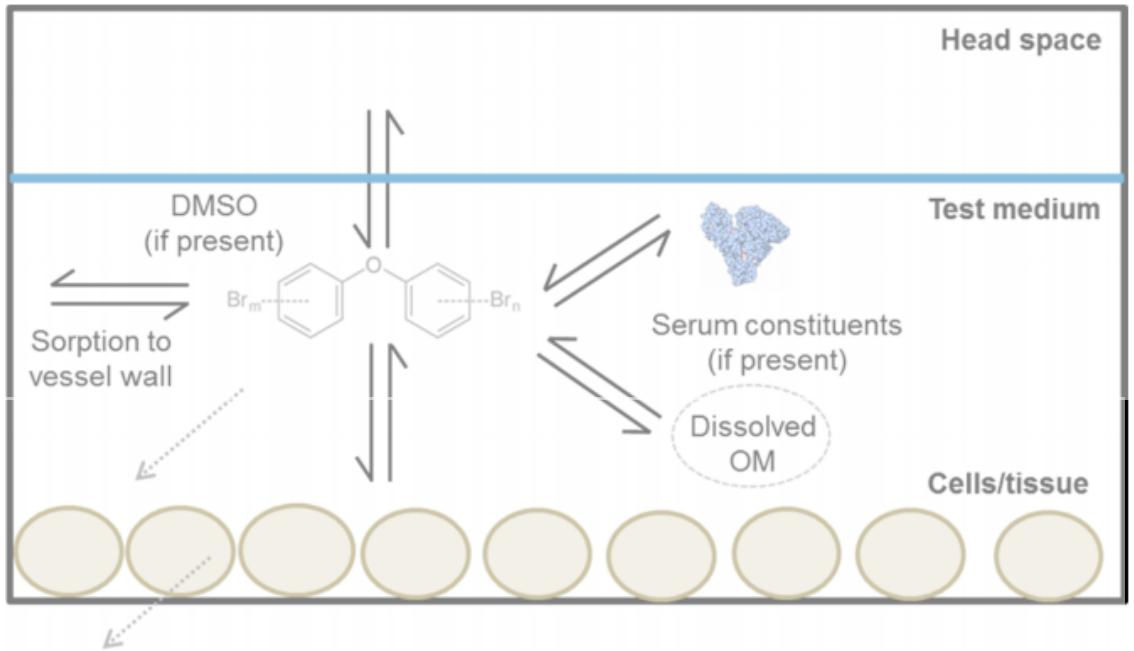

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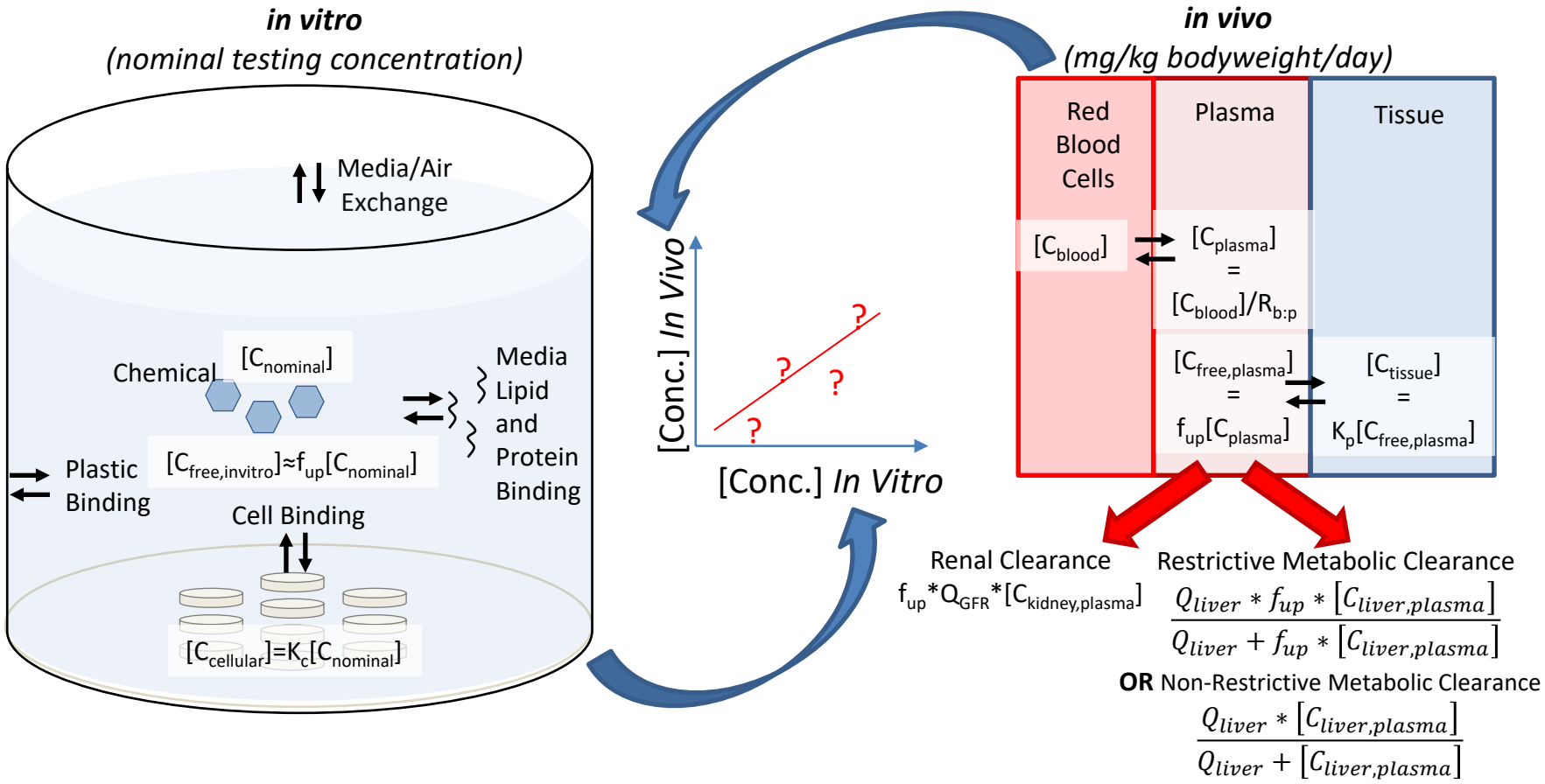
Additional IVIVE Considerations

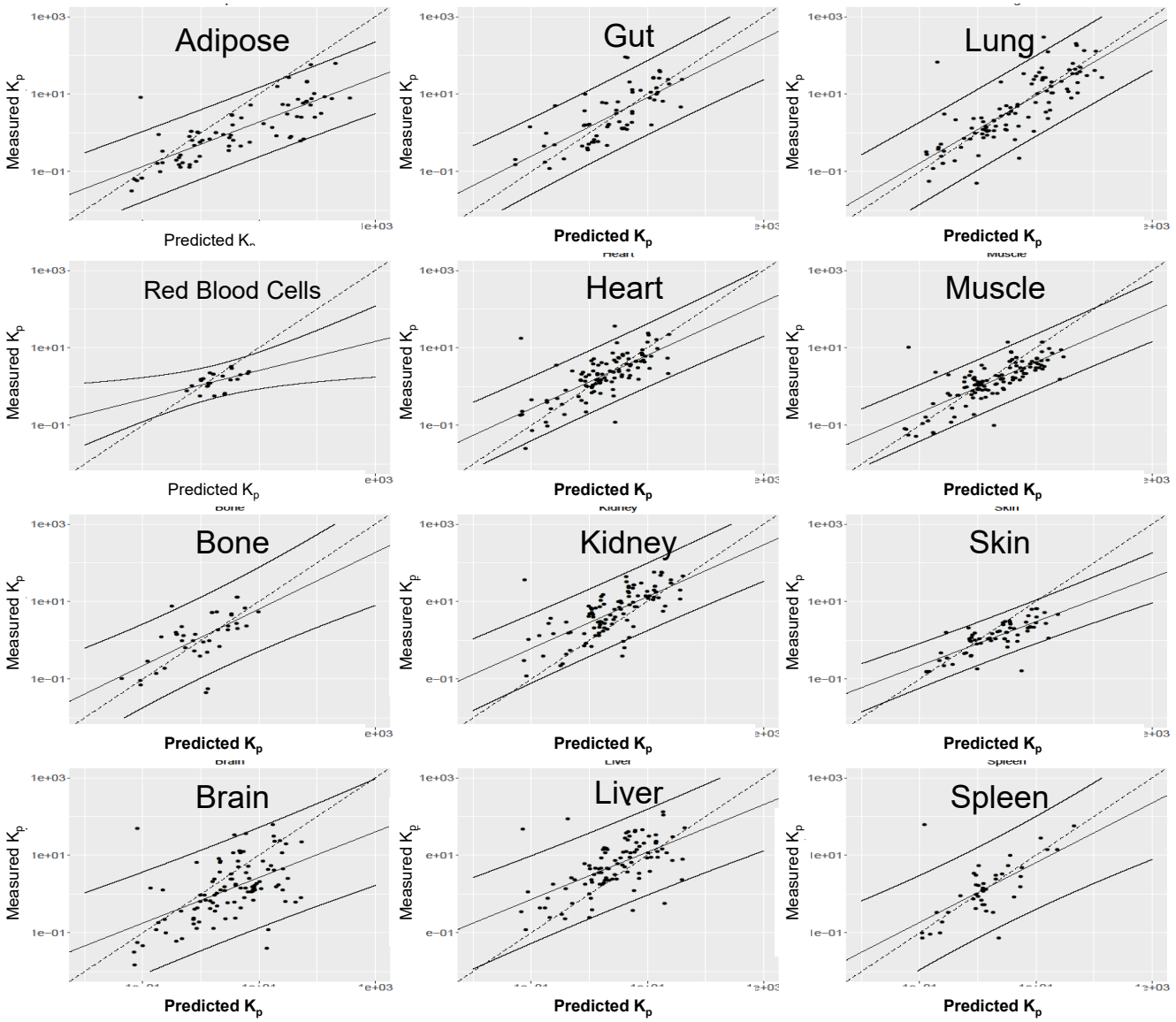


How do you select the appropriate *in vitro* and *in vivo* concentrations for extrapolation?

Tissue:Plasma Partition Coefficients

Pearce et al. (2017b)

- "httk" uses a modified Schmitt (2008) method with elements of Peyret et al. (2010)
- Pearce et al. (2017b) calibrated the Schmitt method using literature measurements of chemical-specific partition coefficients (PC) in rat
  - 945 tissue-specific PC
  - 137 unique chemicals
- Pearce et al. (2017b) evaluated the calibrations with human measured volumes of distribution for 498 chemicals from Obach (2008) – root mean squared error was 0.48
- **We would like to similarly evaluate *in vitro* disposition models**

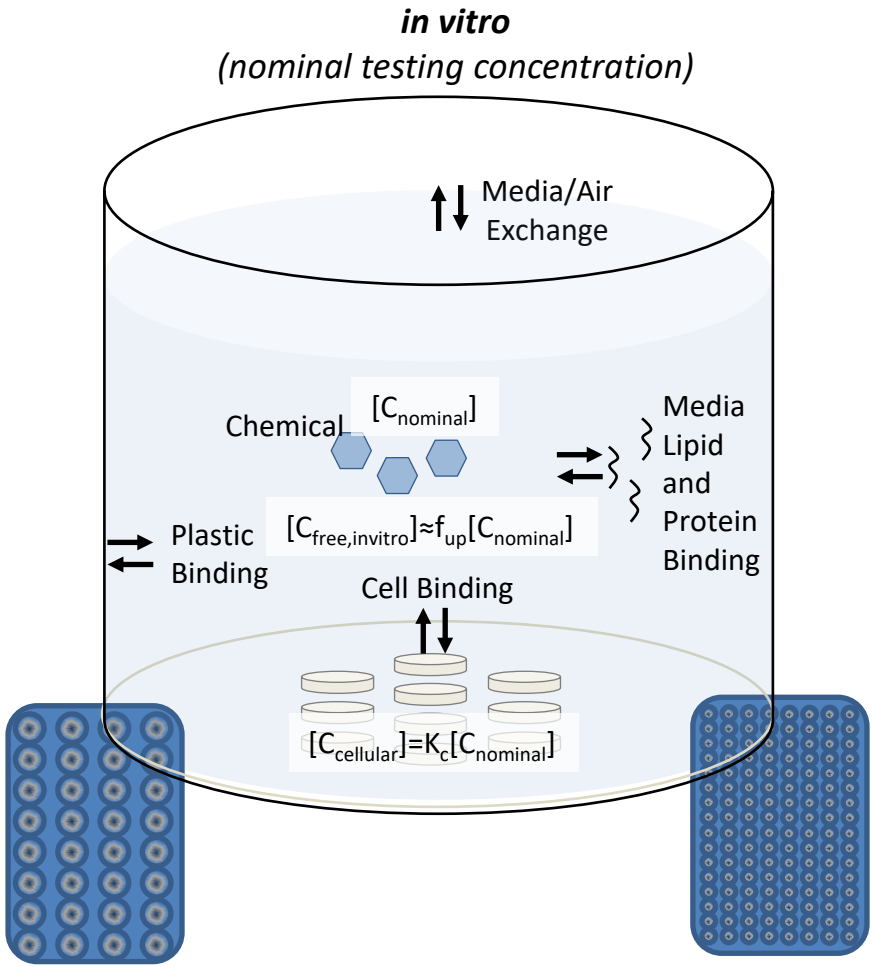


Assay Conditions Influence *In Vitro* Distribution

- ToxCast: >4000 chemicals (including a subset of Tox21) for >2000 additional assay endpoints

- Each technology can vary with respect to plate composition, well number, media composition, etc.

- *In vitro* chemical partitioning between media and cells (in metabolically-incompetent cells) is dependent on:
  - amount of serum in the media;
  - the relative binding of the chemical to serum binding proteins;
  - Log Kow of the chemical;
  - Chemical binding to plastic.



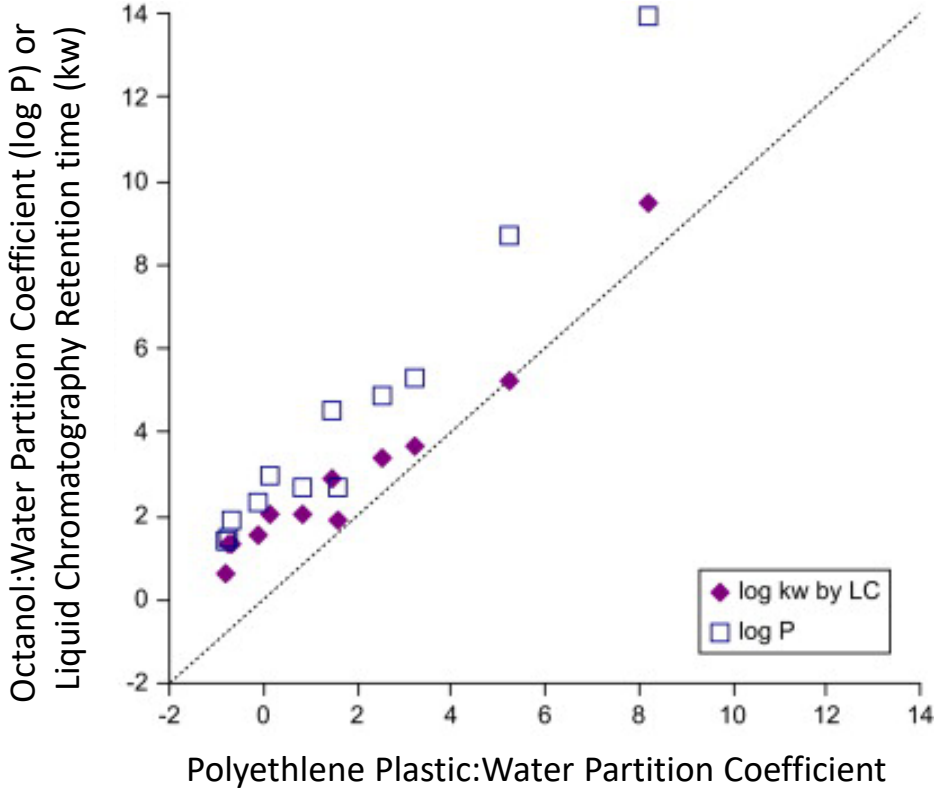
- Madison Feshuk and Katie Paul Friedman have annotated the conditions of all ToxCast assays for use with *in vitro* distribution models

Evaluation data for polymer-water partition coefficient:

Gasslander et al. (2007)

12 chemicals

- Measurement of equilibrium polymer–water partition coefficients using liquid chromatography

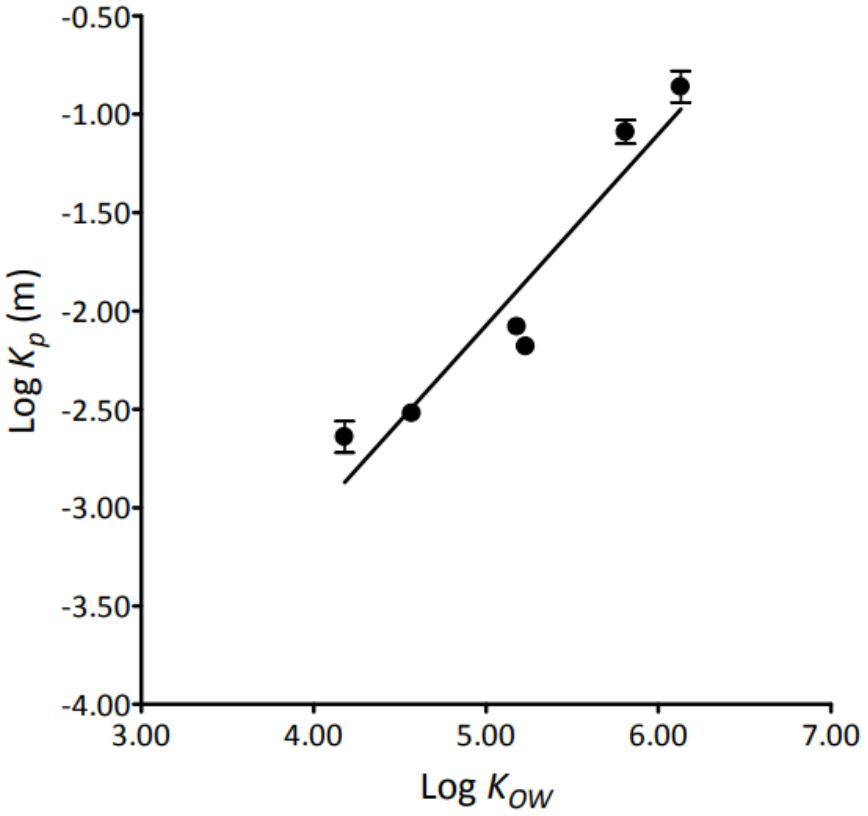


Chemical name	Log P_{ow}
4-Hydroxybenzoic acid	1.42
Methyl-4-hydroxybenzoate	1.86
4-Methylbenzyl alcohol	1.49
2-Methylbenzoic acid	2.35
Propyl-4-hydroxybenzoate	2.93
Diethyl phthalate	2.70
Toluene	2.68
3,5-Di-tert-butyl-4-hydroxyphenyl propanoic acid	4.48
2,4-Di-tert-butyl phenol	4.86
2,6-Di-tert-butyl-4-methyl phenol	5.32
Bis(2-ethylhexyl) phthalate	8.71
Octadecyl-3-(3’,5’-di-tert-butyl-4’-hydroxyphenyl) propionate	13.9

Evaluation data for polymer-water partition coefficient:

Kramer (2010)

- Measurement of equilibrium polymer–water partition using polystyrene culture dishes in an orbital shaker for 48h



7 polycyclic aromatic hydrocarbons

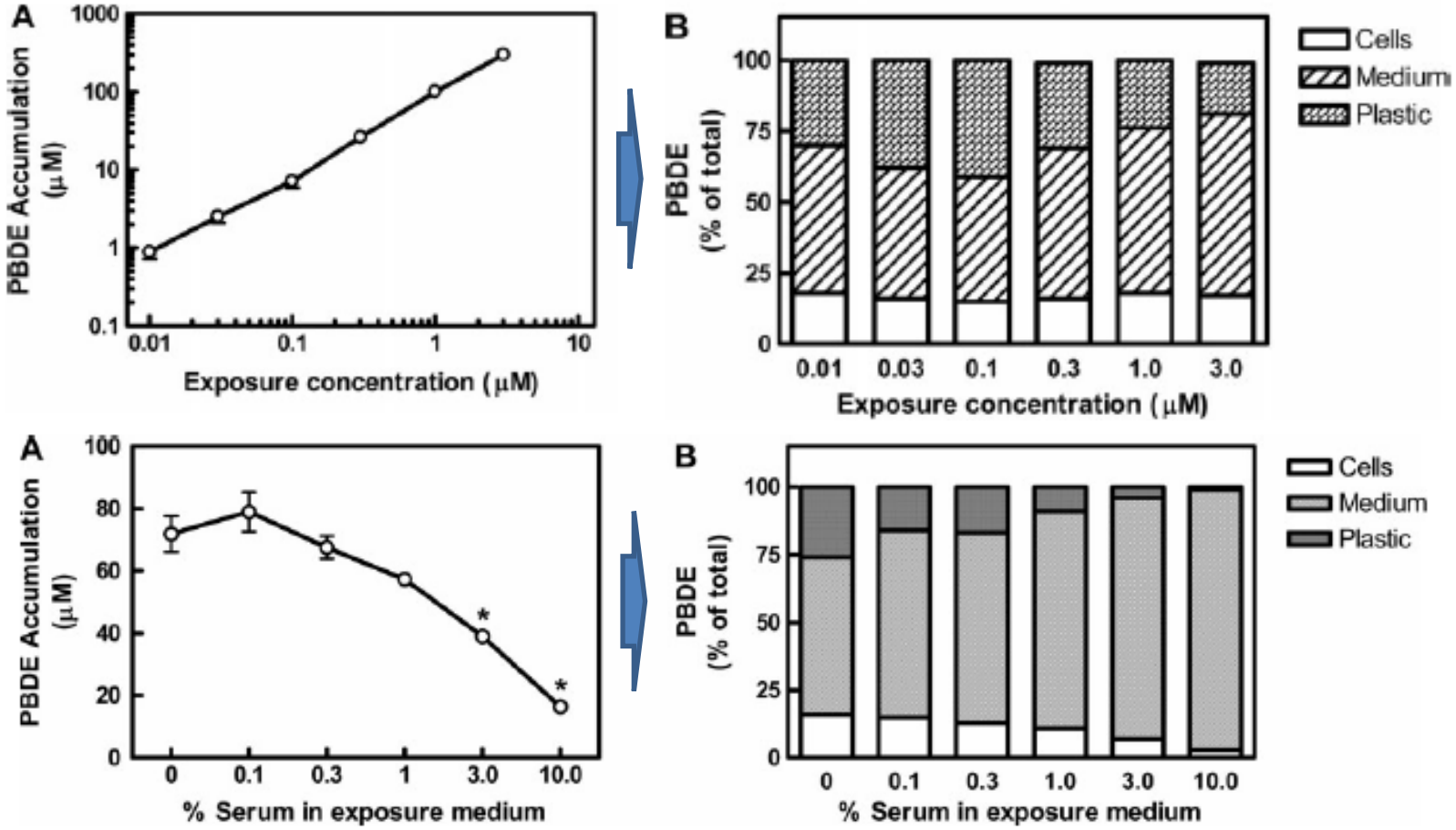
Table 5.1: Physicochemical properties and partition coefficients of PAHs to the various compartments in a Balb/c 3T3 basal cytotoxicity assay. Log K_f, log K_s, log K_p, and n have been measured in this study. Log K_c values were obtained from Jonker and Van der Heijden (2007). Log K_a values were calculated from the Henry's law constants measured in De Maagd et al. (1998), unless otherwise stated. Log K_{OW} and water solubilities were also obtained from De Maagd et al. (1998), unless otherwise stated. * Billington et al. 1988, ** Rossi and Thomas 1981, *** Eastcott et al. 1988, (a) Ten Hulscher et al. 1992, (b) Yalkowsky et al. 1983, (c) Karickhoff et al. 1979.

PAH	Naphtha-lene	Fluorene	Phenan-threne	Fluoran-thene	Pyrene	Chrysene	Benzo(a)-pyrene
CAS	91-20-3	86-73-7	85-01-8	206-44-0	129-00-0	218-01-9	50-32-8
Mol. weight (g/mol)	128.18	166.23	178.24	202.26	202.26	228.30	252.32
Water solubility (g/mL @ 25°C)	34.8	1.95*	0.823	0.207	0.130 **	0.0015	0.00182
H (Pa m^3 mol^{-1})	45.0	6.50	2.90	1.10	2.0	0.45 ***	0.034[a]
Log K_{OW}	3.33	4.18[b]	4.57	5.23	5.18[c]	5.81	6.13
Log K_f in bare medium @ 20°C ± SE	3.63 ± 0.05	3.84 ± 0.01	3.91 ± 0.01	4.33 ± 0.02	4.28 ± 0.01	4.40 ± 0.02	4.51 ± 0.03
Log K_s @ 20°C (m^3/mol BSA) ± SE	2,76 ± 0.08	3,09 ± 0.01	3,14 ± 0.02	3,42 ± 0.04	3,56 ± 0.02	3,64 ± 0.03	3,78 ± 0.06
Log K_p (m) @ max. water solubility/100 ± SE	No data	-2.64 ± 0.08	-2.52 ± 0.04	-2.18 ± 0.04	-2.08 ± 0.02	-1.09 ± 0.06	-0.86 ± 0.03
n of K_p @ 20°C ± SE	No data	0.76 ± 0.04	0.80 ± 0.02	0.86 ± 0.03	0.77 ± 0.02	0.75 ± 0.06	0.86 ± 0.05
Log K_c @ 20°C (m^3/kg lipid)	0.64	1.60	2.07	2.67	2.78	3.46	3.90
Log K_a	-1.73	-2.57	-2.92	-3.35	-3.09	-3.73	-4.86

Evaluation Data for *In Vitro* Distribution: Mundy et al. (2004)

One chemical

- PBDE-47 is highly lipophilic and $C_{nominal}$ underestimated cellular concentration by up to 2 orders of magnitude



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Evaluation Data for *In Vitro* Distribution: Kramer et al. (2015)

Six chemicals

Table 1
Drugs and their physicochemical properties tested for their distribution in in vitro systems within the Predict-IV project.

Drug	Therapeutic class	% bound in human plasma[a]	Experimental LogP[a]	Log $D_{7.4}$[b]	Assays used for biokinetics analyses (reference)
Chlorpromazine	Antipsychotic, neuroleptic	97.8%	5.41	3.39 (basic)	Single and repeated dose exposure brain, liver, intestinal models (Broeders et al., 2012, 2013, 2015a,b)
Amiodarone	Antiarrhythmic	99.98%	7.80	5.66 (basic)	Single and repeated dose exposure liver, brain models (Pomponio et al., 2015a,b)
Diazepam	Anxiolytic, anticonvulsant, tranquilizer	99%	2.82	Neutral	Repeated dose exposure brain model (Broeders et al., 2015a)
Cyclosporin A	Immunosuppressor	90%	2.95	Neutral	Repeated dose exposure kidney, liver, brain model (Wilmes et al., 2013; Bellwon et al., 2015a,b)
Cisplatin	Anticancer, antineoplastic	97.5%	−2.35	Neutral	Repeated dose exposure kidney model (Wilmes et al., 2015)
Colchicine	Anti-inflammatory	23%	1.30	Neutral	Single and repeated dose exposure BBB model (Fabulas-da Costa et al., 2013)
Adefovir dipivoxil	Anti-viral	4% [c]	2.45[c]	Neutral	Repeated dose kidney model (Crean et al., 2015)
Ibuprofen	Anti-inflammatory	99% [d]	3.97[e]	0.8 (acidic)[e]	Single and repeated dose exposure liver model (Truisi et al., 2015)

[a] Unless otherwise stated, drug properties are taken from Fabulas-da Costa et al. (2013).
[b] Seydel and Wiese (2002).
[c] Dörwald (2012).
[d] Paliwal et al. (1993).
[e] Avdeef et al. (1998).

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Evaluation Data for Fraction Free *In Vitro*: Kilford et al. (2008)

39 drugs

- Empirical regression to experimentally measured unbound (free) fraction in hepatocyte incubation *in vitro* assays, based on distribution coefficient (lipophilicity of neutral fraction of compound)

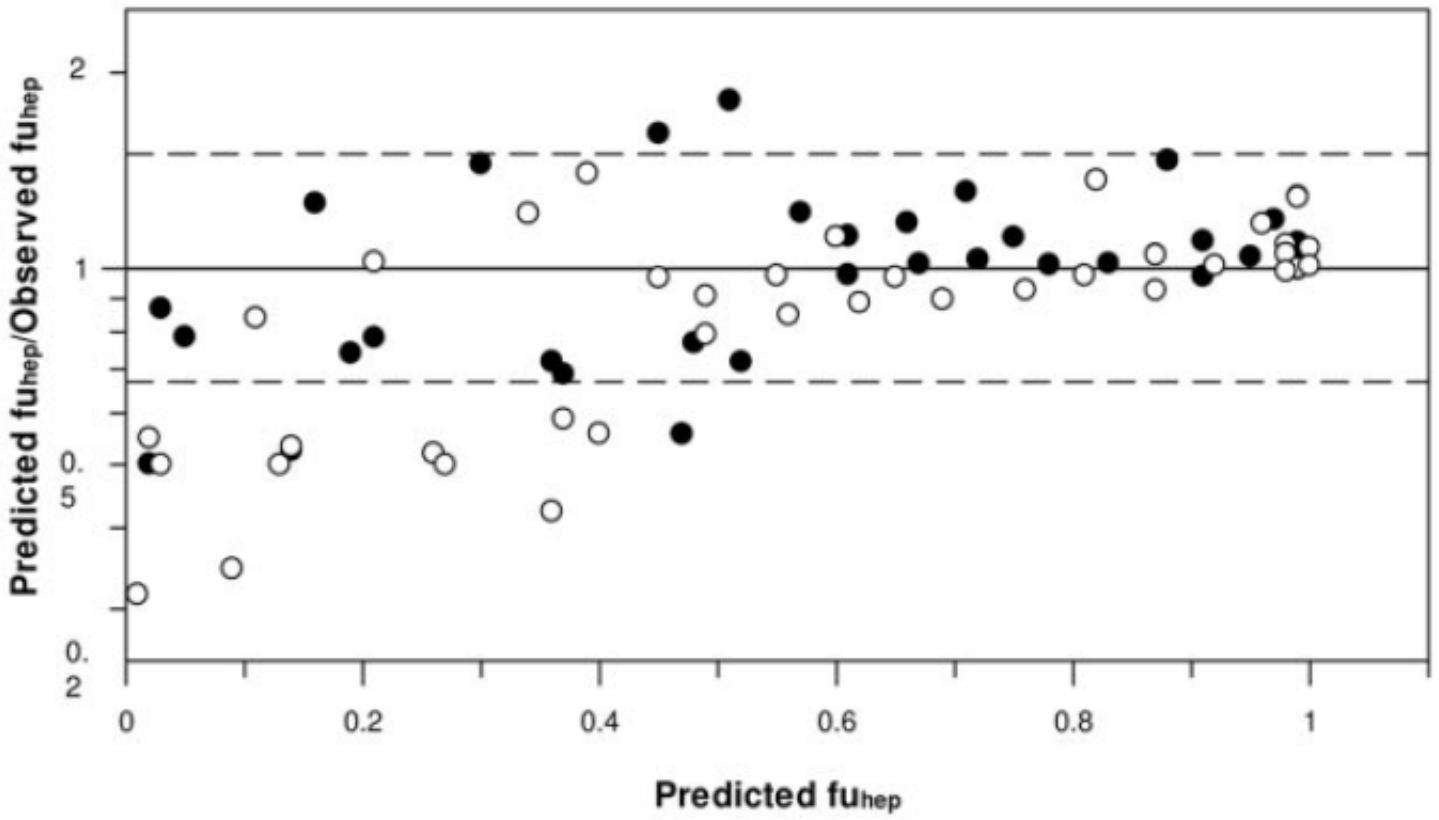


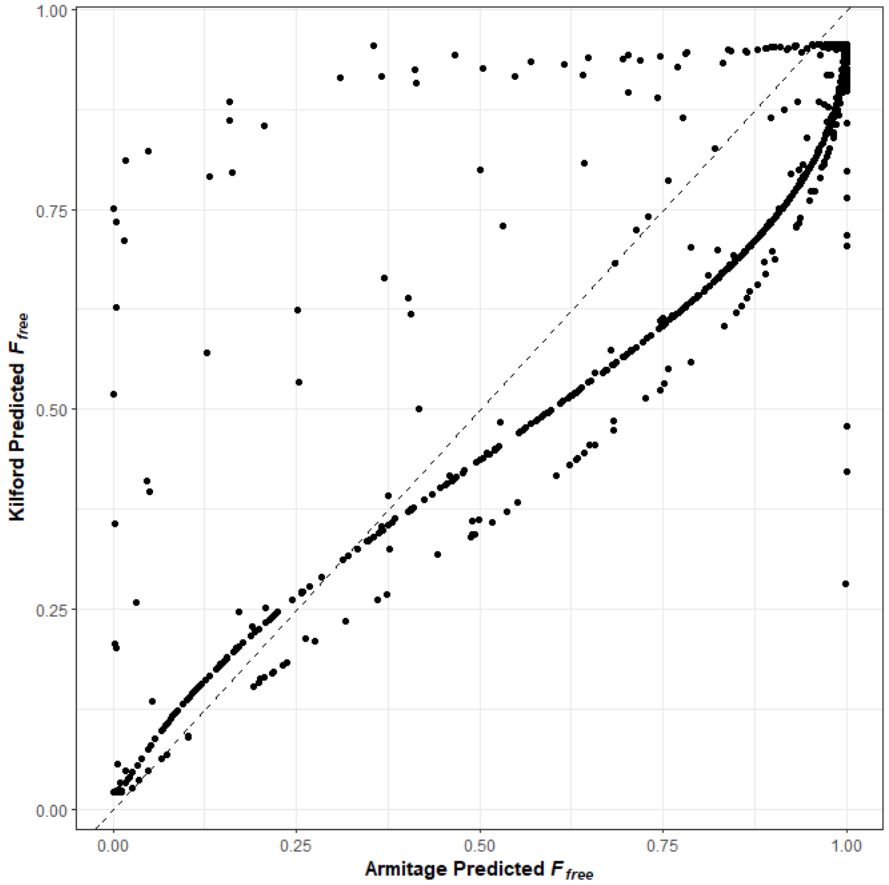
TABLE 1

LogP/D values for the 39 drugs investigated and their respective fu_{mic} and fu_{hep} values at microsomal and cell concentrations of 1 mg/ml and 10^6 cells/ml, respectively

Compound		logP/D	fu_{mic}	fu_{hep}
a-Naphthoflavone[1]	Neutral	4.65	0.02	0.03
2-Ethoxybenzamide[2,3]	Neutral	1.34	0.98	0.90
Albendazole[2,3]	Neutral	3.29	0.56	0.66
Aldosterone[1]	Neutral	0.72	0.60	0.54
Androstenedione[1]	Neutral	2.89	0.49	0.54
Astemizole[2,3]	Base	4.14	0.01	0.04
Betaxolol[2,3]	Base	2.40	0.62	0.70
Bumetanide[2,3]	Acid	0.31	0.92	0.91
Caffeine[1]	Base	−0.13	0.99	0.99
Cerivastatin[2,3]	Acid	1.44	0.65	0.67
Clozapine[2,3]	Base	3.60	0.26	0.50
Cortisol[1]	Neutral	1.42	0.21	0.21
Dextromethorphan[1]	Base	4.19	0.98	0.93
DHEA[1]	Neutral	3.42	0.39	0.28
Diazepam[2,3]	Neutral	2.25	0.69	0.77
Diclofenac[1]	Acid	1.26	0.76	0.82
Flavone[1]	Neutral	3.55	0.11	0.13
Fluconazole[4]	Base	0.50	0.98	0.99
Fluoxetine[1]	Base	4.05	0.09	0.26
Fluvoxamine[1]	Base	3.21	0.27	0.54
Gemfibrozil[1]	Acid	1.80	0.98	0.99
Glyburide[2,3]	Acid	2.19	0.82	0.60
Imipramine[1]	Base	4.80	0.45	0.46
Indapamide[2,3]	Neutral	1.76	0.96	0.82
Isradipine[2,3]	Neutral	3.75	0.34	0.28
Ketoconazole[4]	Base	3.54	0.13	0.26
Metyrapone[2,3]	Neutral	1.37	0.99	0.77
Miconazole[4]	Base	5.93	0.03	0.06
Naloxone[1]	Base	2.09	0.87	0.94
Oxaprozin[2,3]	Acid	1.61	0.87	0.83
Phenytoin[1]	Base	2.52	0.81	0.83
Progesterone[1]	Neutral	4.03	0.14	0.27
Propranolol[1]	Base	3.09	0.55	0.56
Quinidine[1]	Base	3.44	0.40	0.72
Quinine[4]	Base	3.44	0.36	0.85
Testosterone[1]	Base	3.47	0.49	0.62
Tolbutamide[1]	Acid	0.52	1.00	0.99
Verapamil[2,3]	Base	4.10	0.37	0.63
Warfarin[1]	Acid	0.28	1.00	0.93

Kilford $F_{u,hep}$ vs. Armitage $F_{free,in\ vitro}$

- Kilford et al. (2008) model predicts free (unbound) fraction in hepatocyte intrinsic clearance *in vitro* assay
- Dataset compiled from literature experiments largely with dead cells (no metabolism)
- We compared this with the free *in vitro* concentration from Armitage et al. (2014) across the HTTK Library



- Note, Armitage model expanded to ionizable compounds by Fischer et al. (2017) (see also Armitage et al. 2021) – similarly, we have added consideration of chemical ionization to the httk implementation

Kilford $F_{u,hep}$ vs. Armitage $F_{free,in\ vitro}$

- Kilford et al. (2008) model predicts free (unbound) fraction in hepatocyte intrinsic clearance *in vitro* assay
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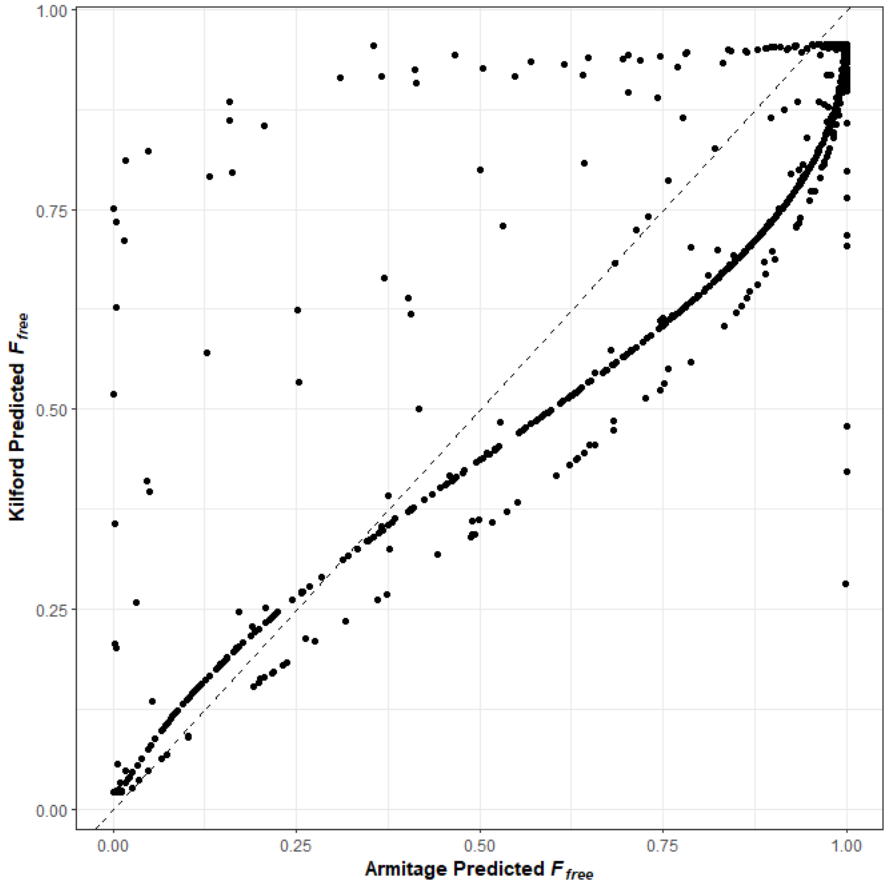

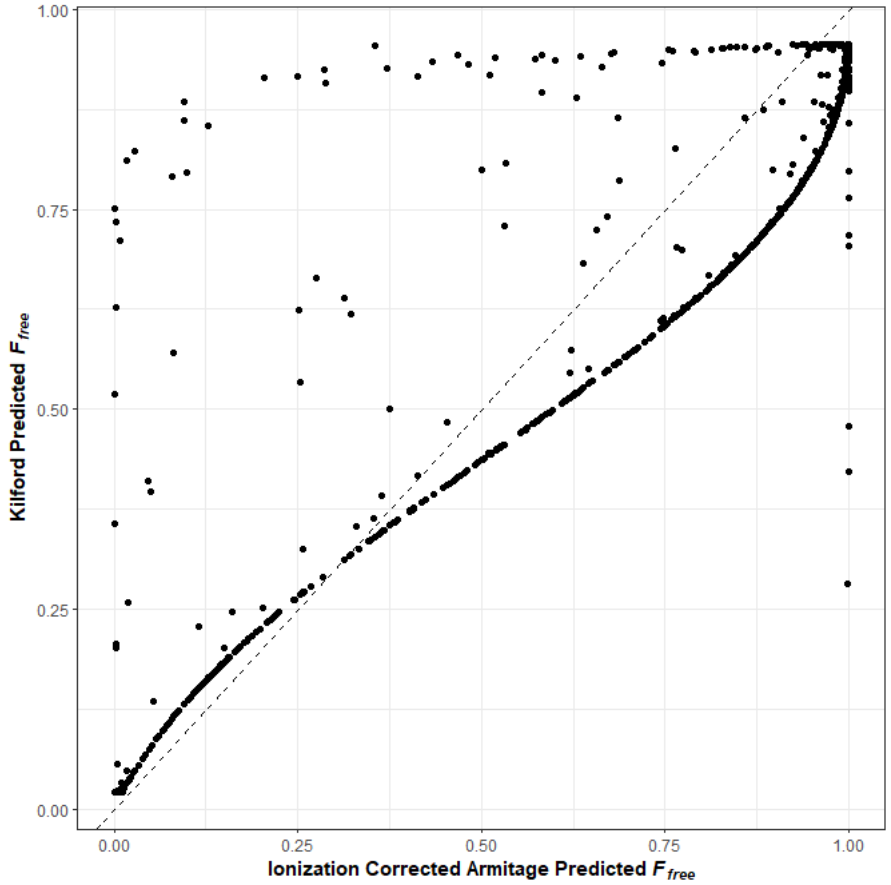

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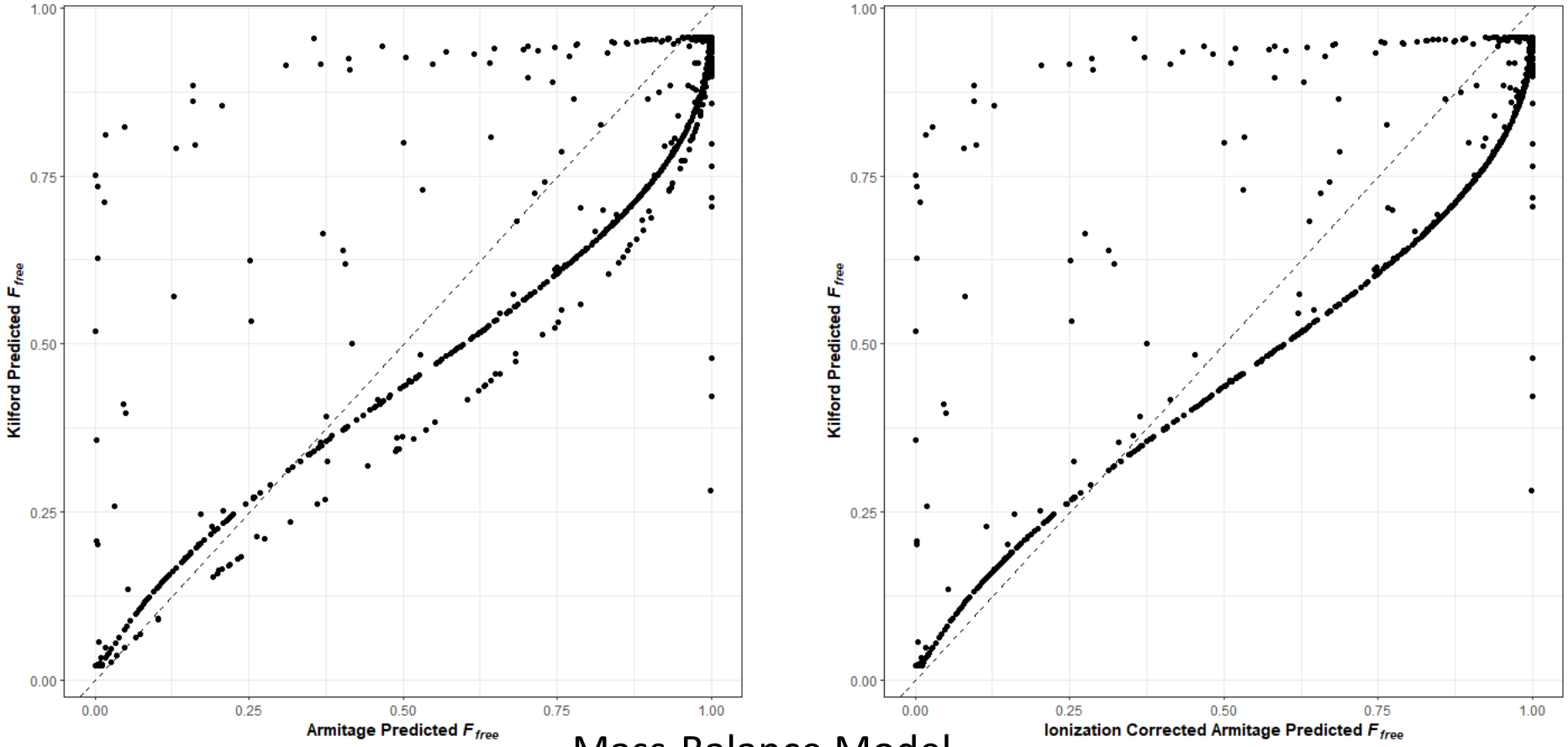
Empirical Fit to Data from 39 Drugs



Mass-Balance Model

Tox21 *In Vitro* Distribution Experiment

- Currently we have ~60 chemicals (Gasslander, Mundy, Kramer, Kramer, Kilford) that are mostly pharmaceuticals, with experimental *in vitro* distribution data in the literature

- We wish to develop larger, standardized set of *in vitro* disposition data -- emphasize non-pharmaceutical chemical space

- 200 chemicals were selected to optimize chemical diversity, overlap with activity data, httk, and other models of biokinetics
  - 44.5% low fraction unbound, 27% moderate, 28.5% high.
  - 50% neutral, 30% anionic, and 17% cationic at pH 7.4.
  - For these chemicals, the Armitage et al. (2014) model predicts that the cells will be 100-fold lower than media for 10.5%, will be 3.2 lower than media for 14.5%, within 3.2-fold of media for 18%, greater than 3.2 the media for 36%, and greater than 100x the media for 18%.

- To date we have completed a pilot study of ten chemicals (presented in following slides)

Tox21 *In Vitro* Distribution Pilot Study Design

United States Environmental Protection Agency

Table 1. Sample Calculations		
Design Parameter:	**Multiplier**	**Comments**
Cell Type(s)	1	MCF7
Number of Plates	--	See Plate Matrix
Technical Replicates	4	See Plate Map
Chemicals	10	See Chemical List
Concentrations	1	10 µM
Time Points	3	1, 6, 24 hours
Media Types	1	10% FBS

Cell Plating | **Chemical Dispensing** | **Media Transfer** | **Acetonitrile Addition**

BioTek MultiFlo FX Peristaltic Dispenser

LabCyte Echo 550 Acoustic Dispenser

Integra ViaFlo 384 Guided Pipetting System

Gyger Certus Flex Solenoid MIcrodispenser

Table 2. Plate Matrix				
Test Plate	**Test Plate Barcode**	**Plating Condition**	**Exposure Duration (hr)**	**Measured Compartment**
A	TC00000013	Medium - cells	1	Medium
		Medium - cells	1	Plastic
B	TC00000014	Medium + cells	1	Medium
		Medium + cells	1	Plastic + Cells
C	TC00000015	Medium + cells	1	Whole Well Crash
D	TC00000016	Medium - cells	6	Medium
		Medium - cells	6	Plastic
E	TC00000017	Medium + cells	6	Medium
		Medium + cells	6	Plastic + Cells
F	TC00000018	Medium + cells	6	Whole Well Crash
G	TC00000019	Medium - cells	24	Medium
		Medium - cells	24	Plastic
H	TC00000020	Medium + cells	24	Medium
		Medium + cells	24	Plastic + Cells
I	TC00000021	Medium + cells	24	Whole Well Crash

For pilot study samples were analyzed both individually and combined (cassette) to enhance throughput

Test Plate

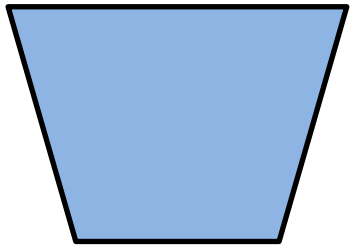
Receiving Plate

Slide from Greg Honda

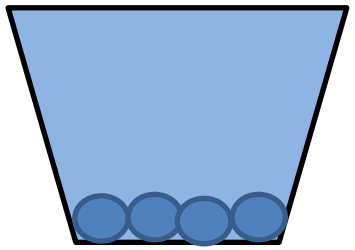
Pilot Study – Plastic Binding

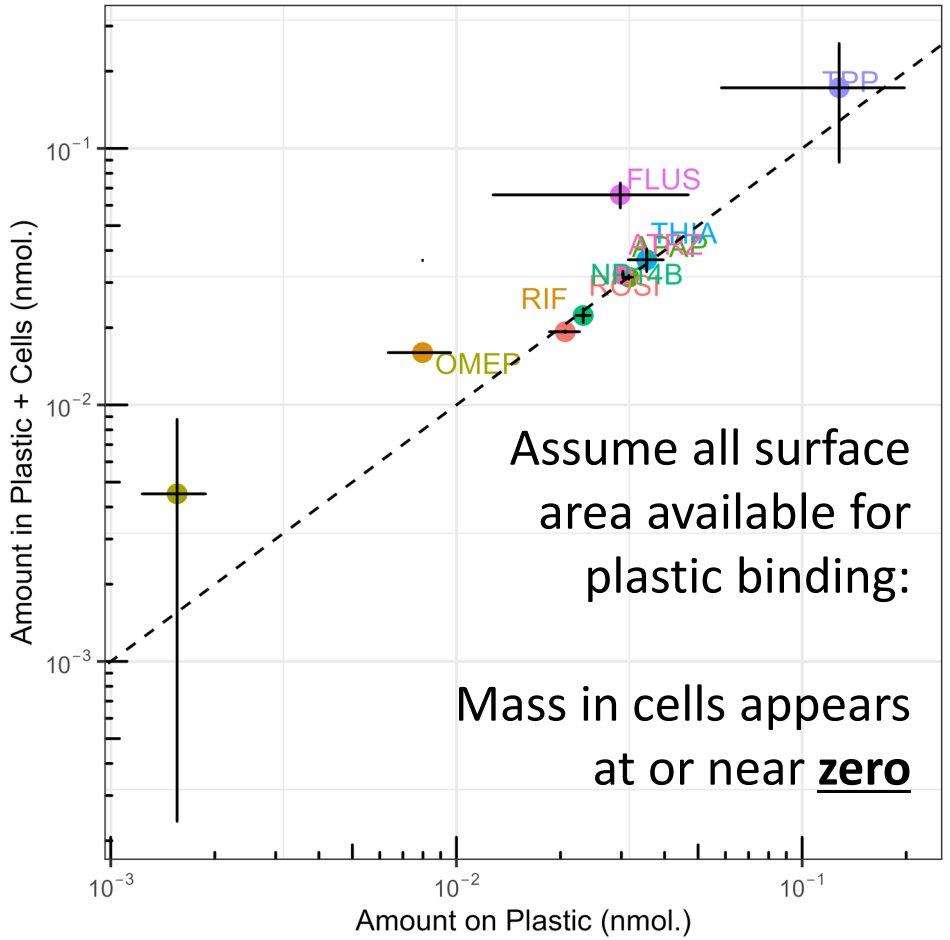
SA total: 137 mm^2

Plastic:



Plastic + Cells:



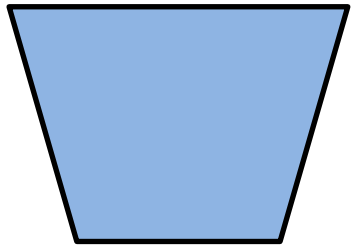


$M_{cells} = M_{Plastic + Cells} - M_{plastic}$

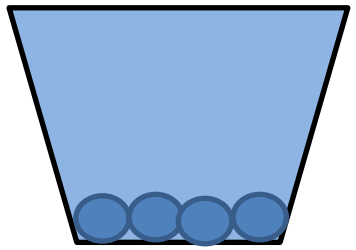
Slide from Greg Honda

Pilot Study – Plastic Binding

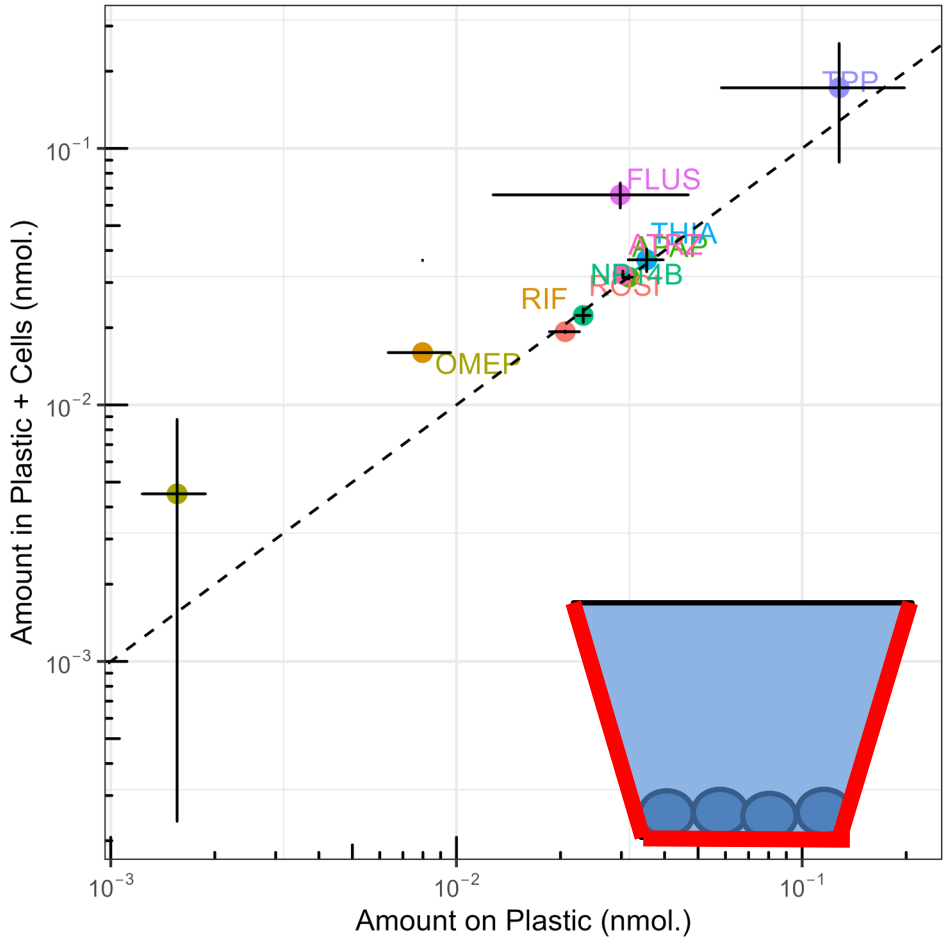
Plastic:



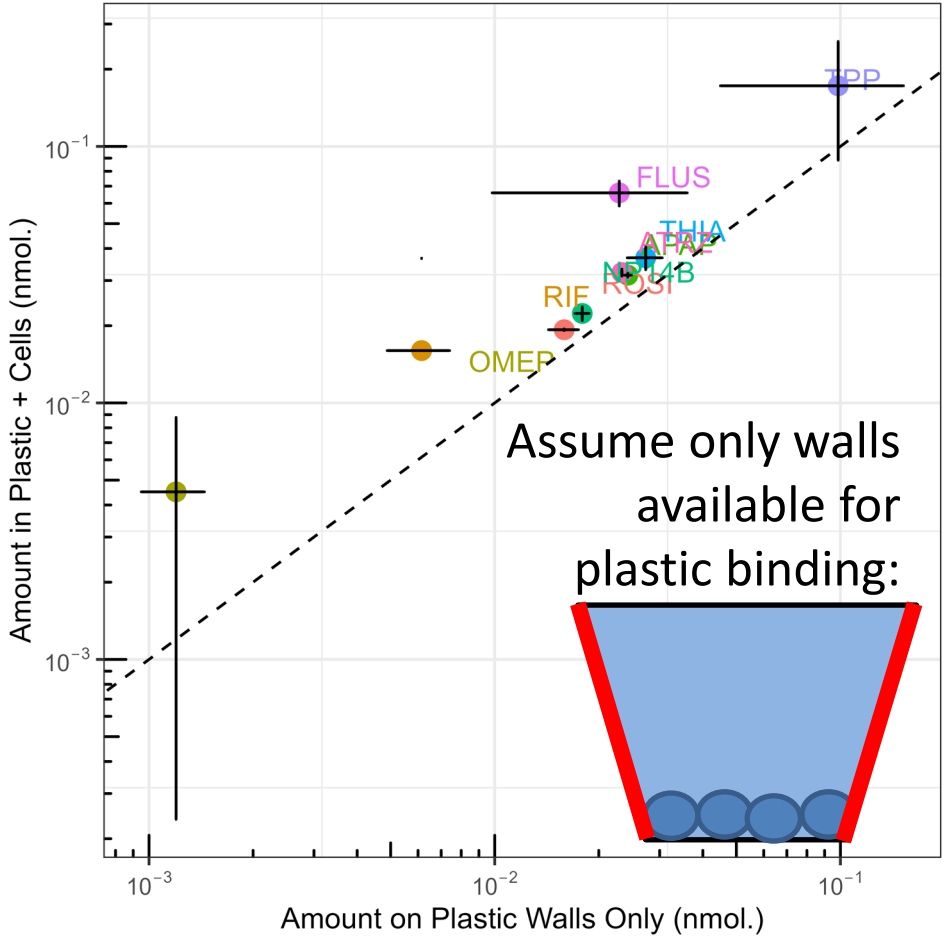
Plastic + Cells:



SA total: 137 mm^2



SA walls: 107 mm^2



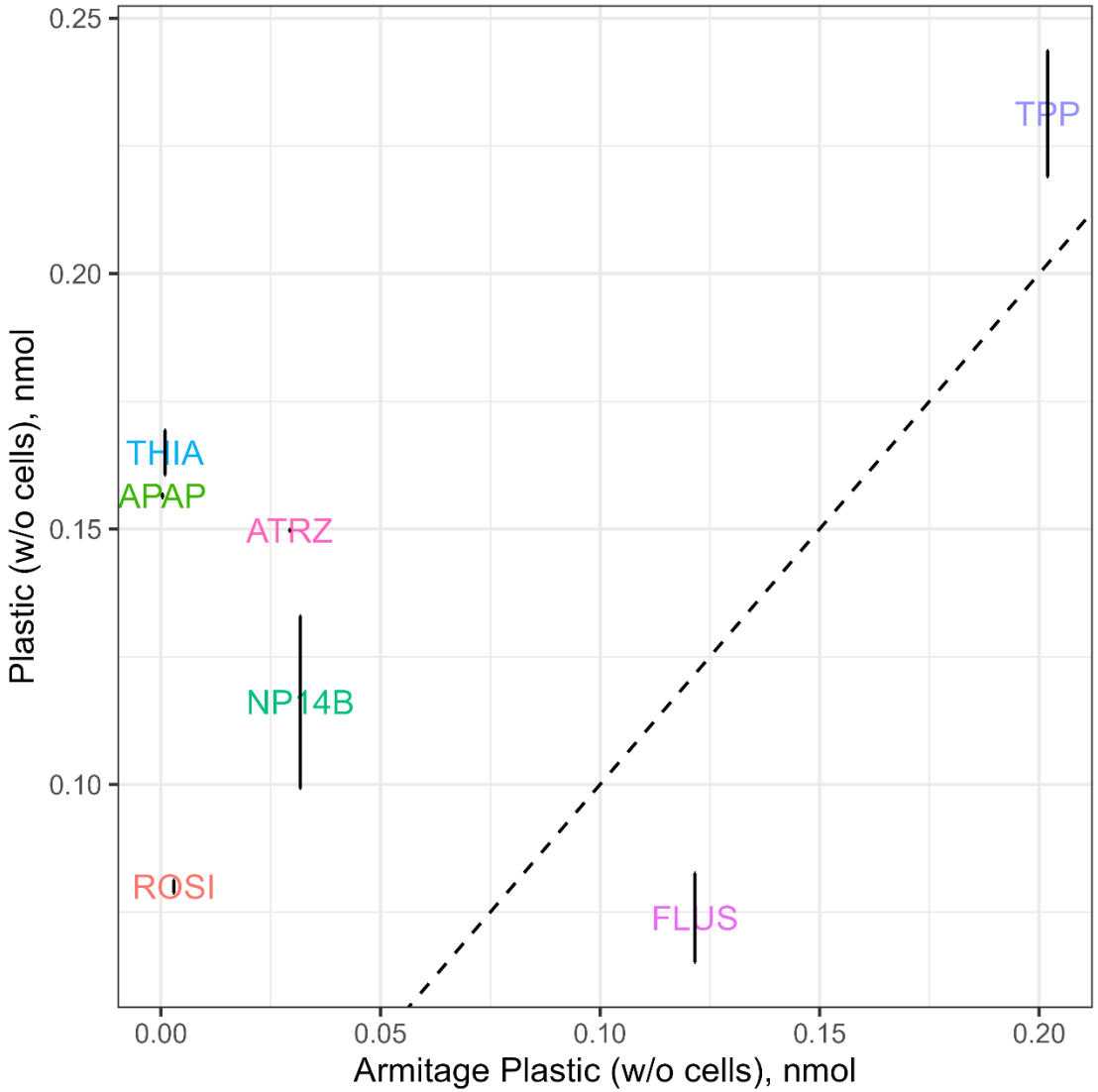
$M_{cells} = M_{Plastic + Cells} - M_{plastic}$

Most cell concentrations are near zero *unless* we assume the bottom surface area is unavailable to plastic binding

Slide from Greg Honda

Armitage Predictions of Plastic Binding

- Armitage model predictions of plastic binding for these 10 chemicals in this design were not very predictive of measured concentrations
- Tendency to under-predict the amount bound to plastic empirically
- Out of the small chemical set, triphenyl phosphate seemed more aligned
- We need more chemical data

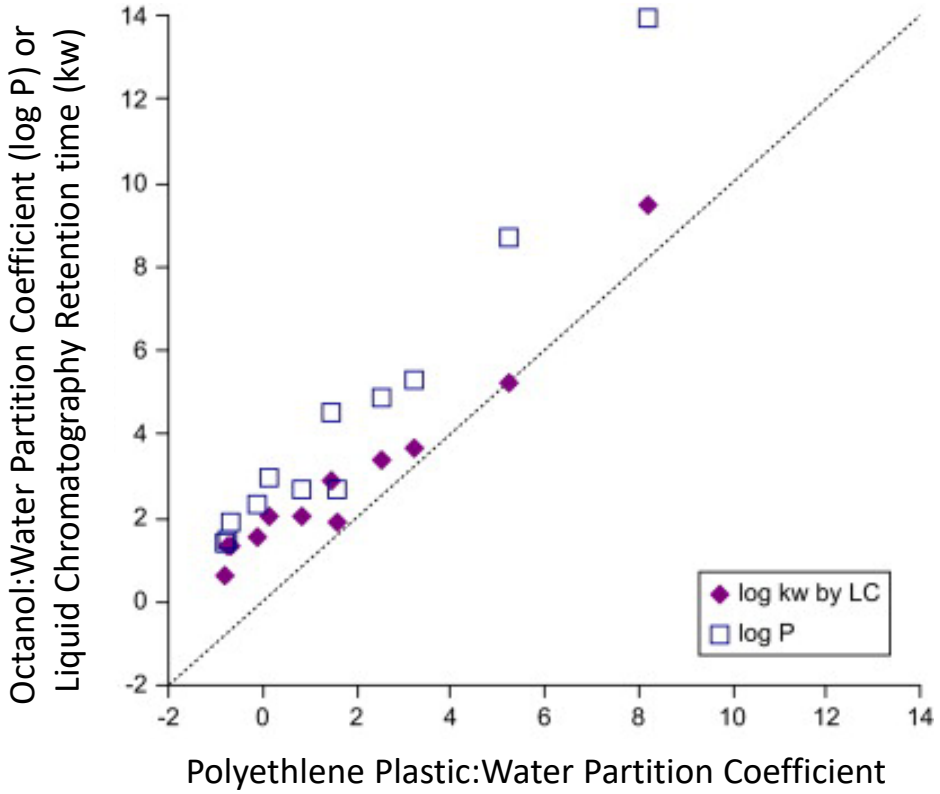


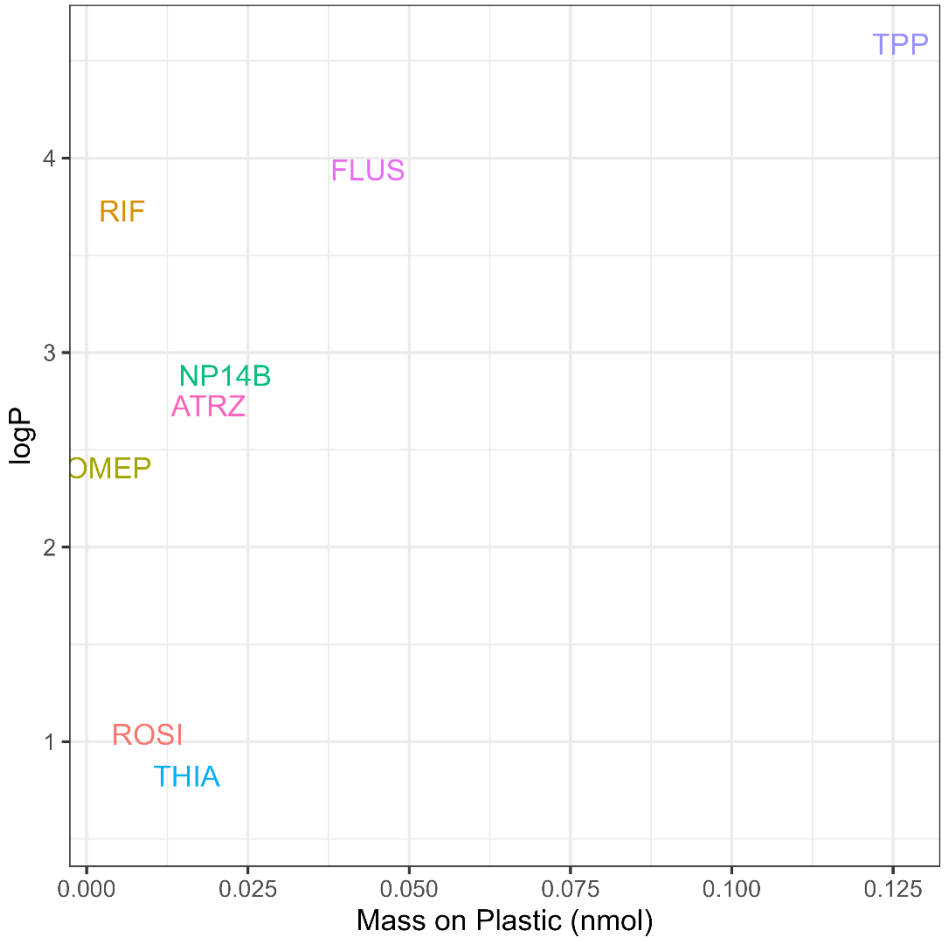
Chemical analysis by David Crizer,
in vitro cell treatment by Josh Harrill

Data analysis by Greg Honda

Armitage Predictions of Plastic Binding

- Data are superficially consistent with Gasslander et al. (2007) – positive correlation but potentially higher Log P than plastic binding



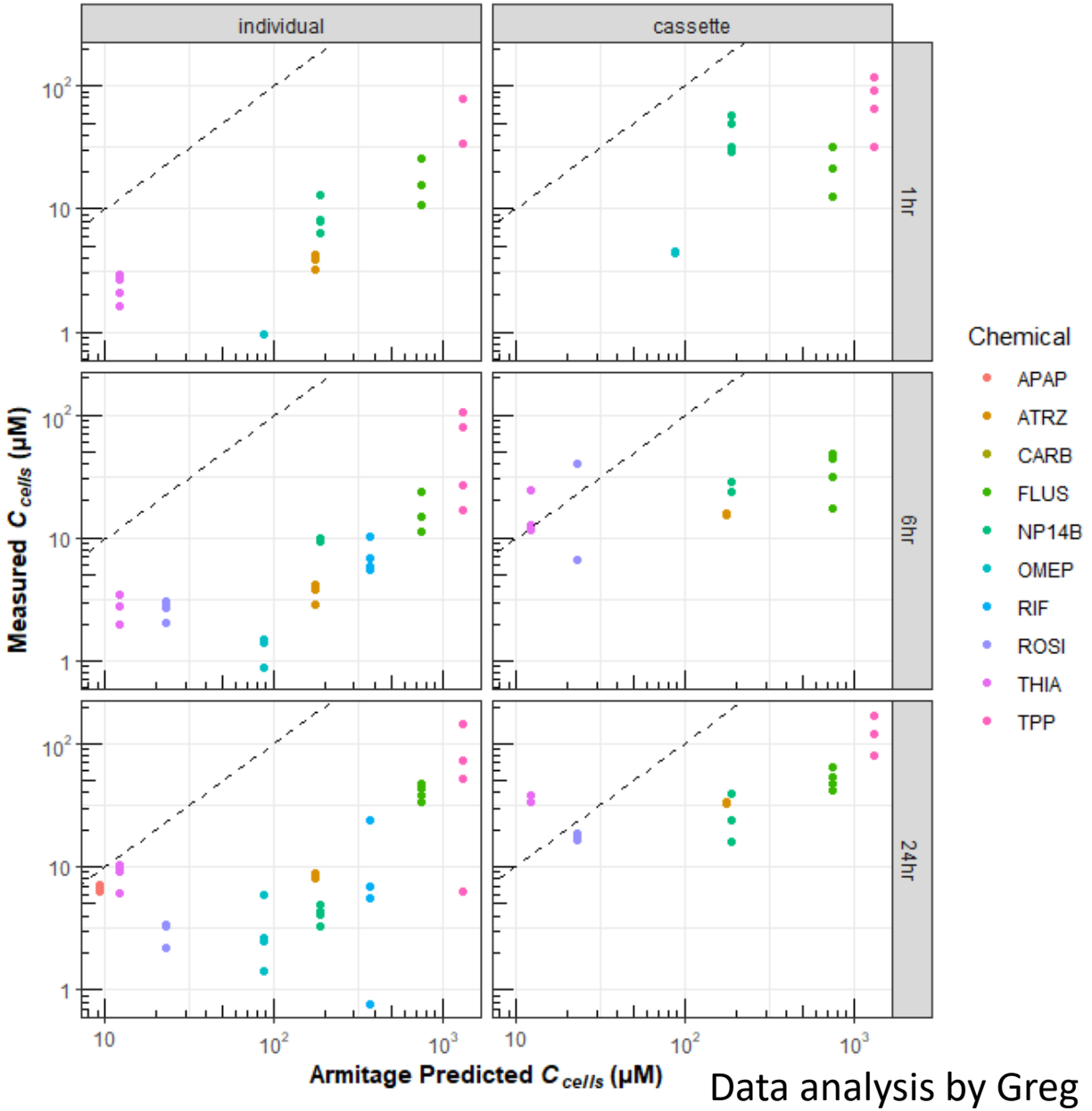


Chemical analysis by David Crizer,
in vitro cell treatment by Josh Harrill

Data analysis by Greg Honda

Armitage Predictions of Cellular Concentrations

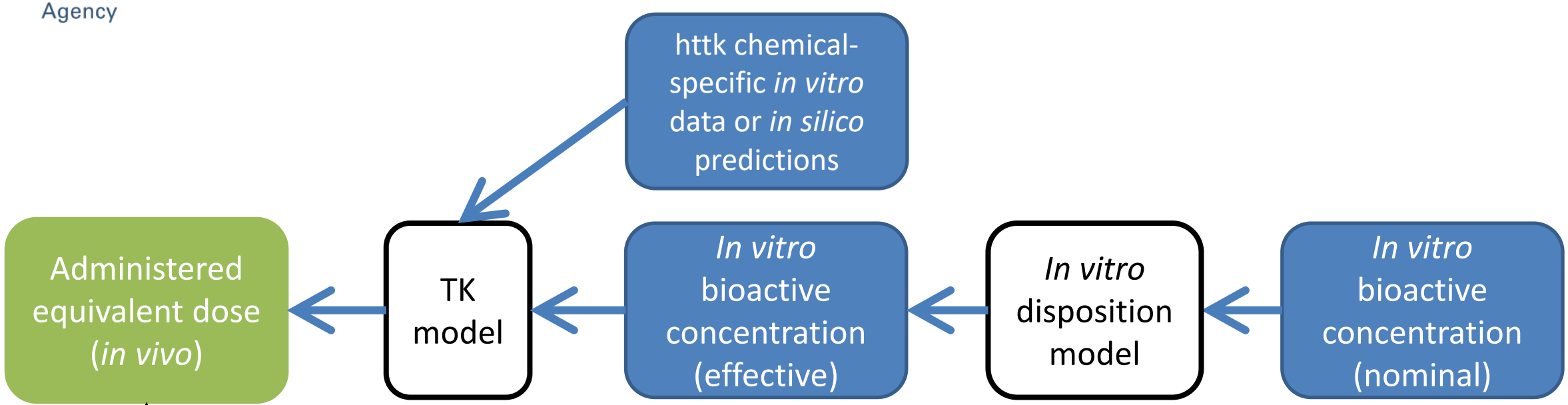
- Armitage et al. (2014) model predictions correlated with measurements, but tend to overestimate the concentrations by ~10x
- Three replicates per chemical, plotted individually
- We can detect more chemicals using individual analysis, but throughput for cassette analysis is much higher
- Distribution seems to be relatively unchanged from 1h to 24 h

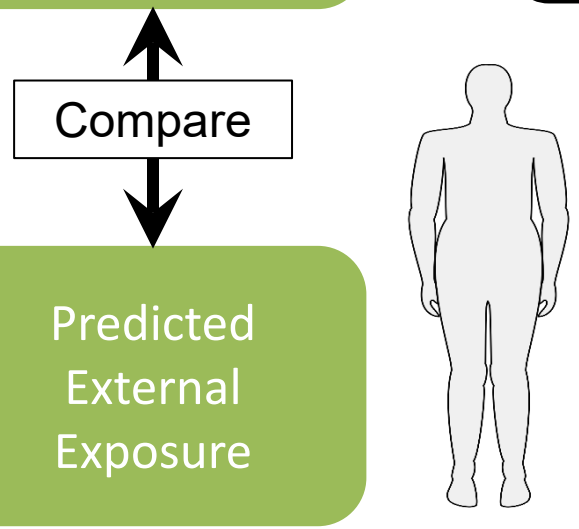


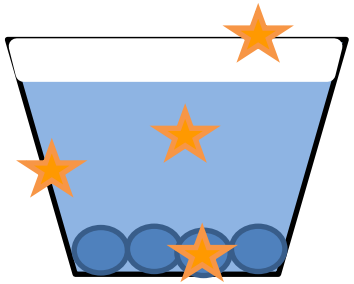
Chemical analysis by David Crizer,
in vitro cell treatment by Josh Harrill

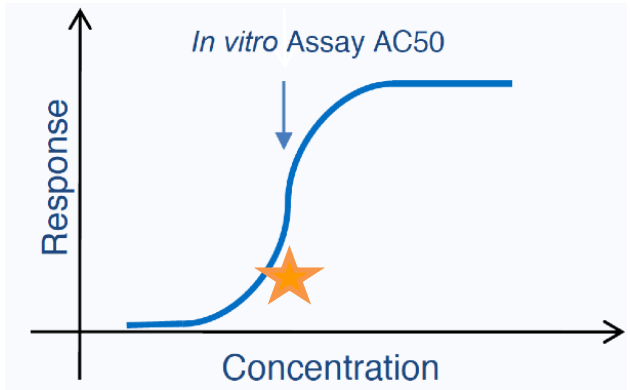
Data analysis by Greg Honda

Impact on *In Vitro* - *In Vivo* Extrapolation (IVIVE)







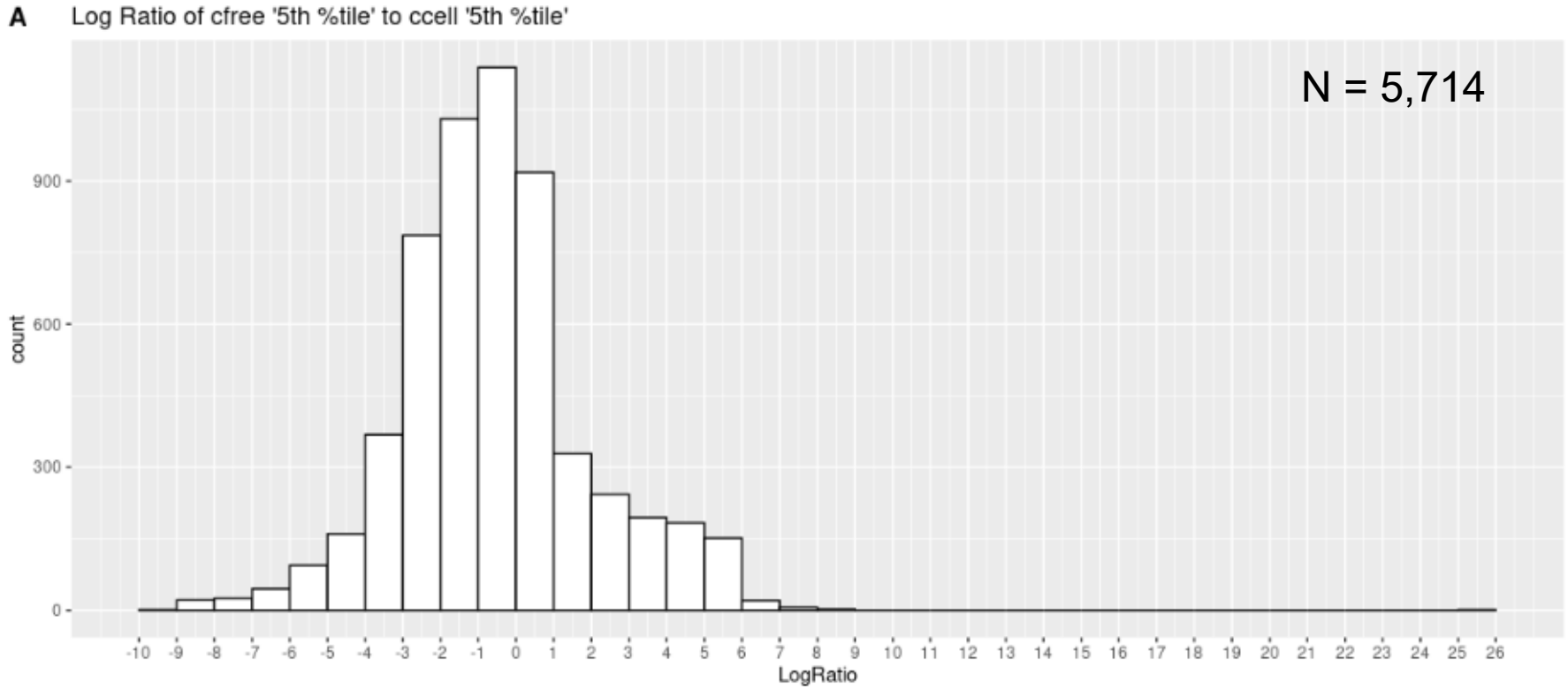


[Tan et al. 2007;
Rotroff et al. 2010;
Wetmore et al. 2012, 2013, 2015]

Slide from Caroline Ring

Impact of *In Vitro* Disposition on NAM-based Point of Departure

- We calculated the 5th percentile ToxCast bioactive concentration (uM) across thousands of tested chemicals
- Used measured HTTK *in vitro* TK data with *in silico* predicted values from ADMet Predictor (Sipes et al., 2017)

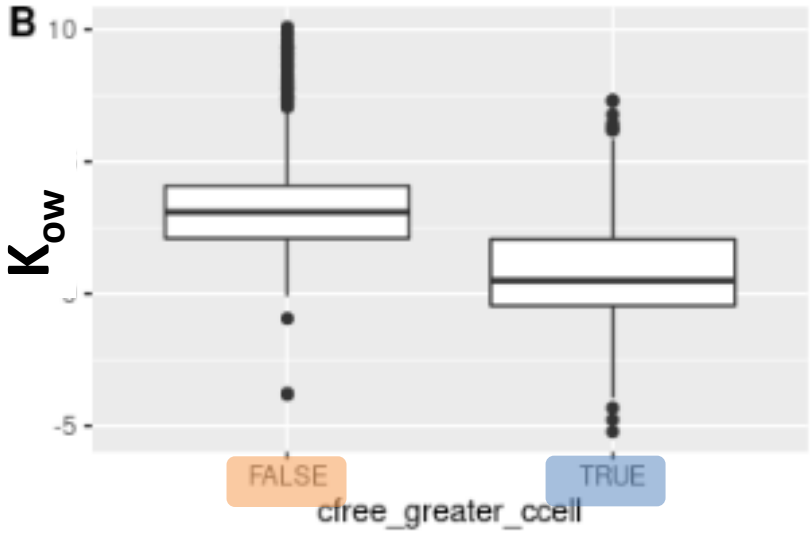


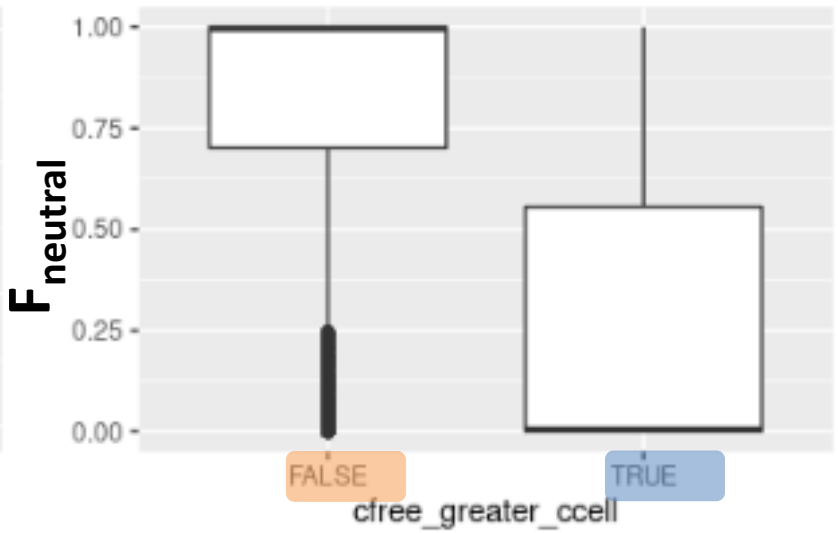
- The central tendency indicates that Armitage et al. (2014) predicted C_{free} in media or C_{cell} are equivalent for most chemicals
- However, values can vary by >6 orders of magnitude in either direction

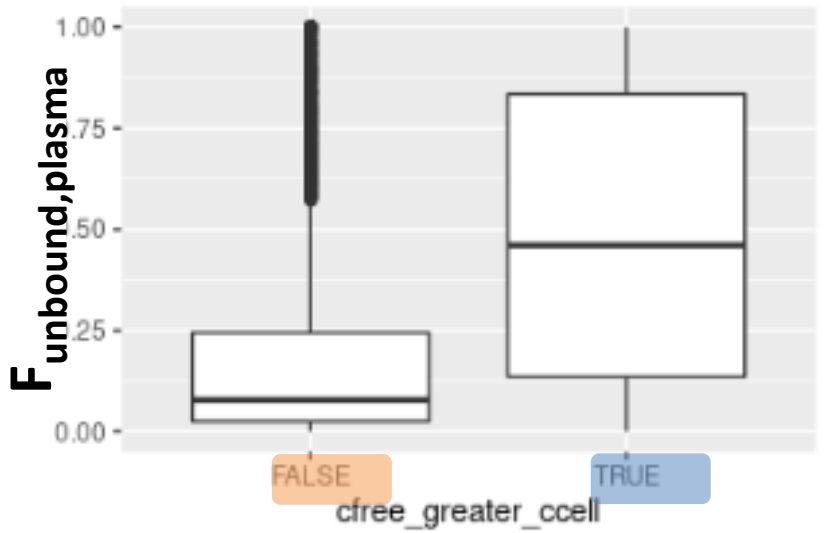
Slide from Ben Savage and Katie Paul Friedman

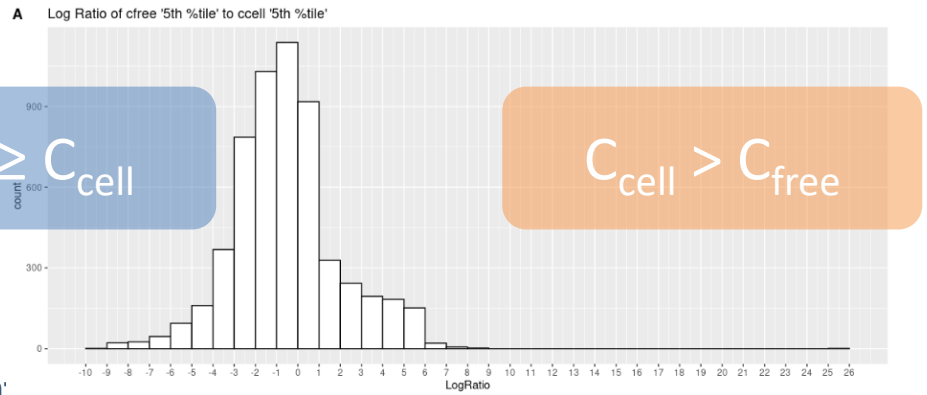
Impact of *In Vitro* Disposition on NAM-based Point of Departure

- Ratio of Cellular to Media concentration depends on octanol:water partition (K_{ow}), ionization at pH 7.4 ($F_{neutral}$) and plasma protein binding ($F_{unbound, plasma}$)









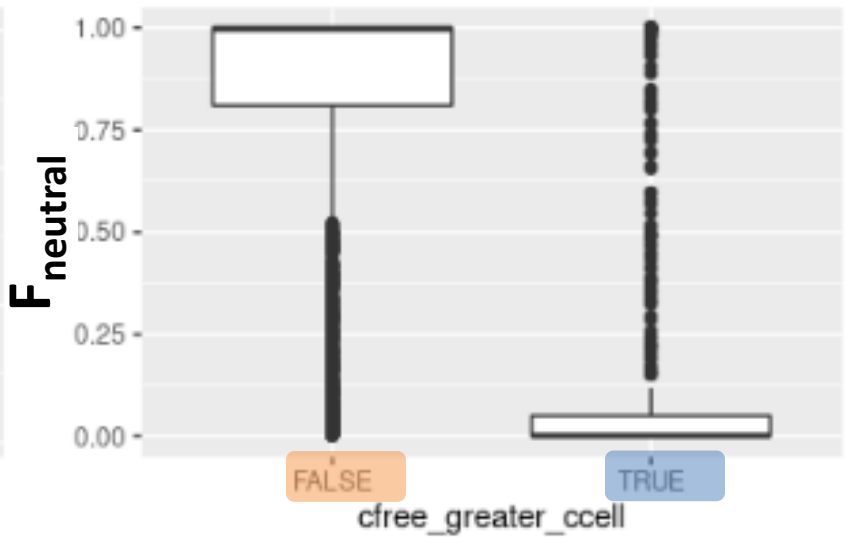
$C_{free} \geq C_{cell}$

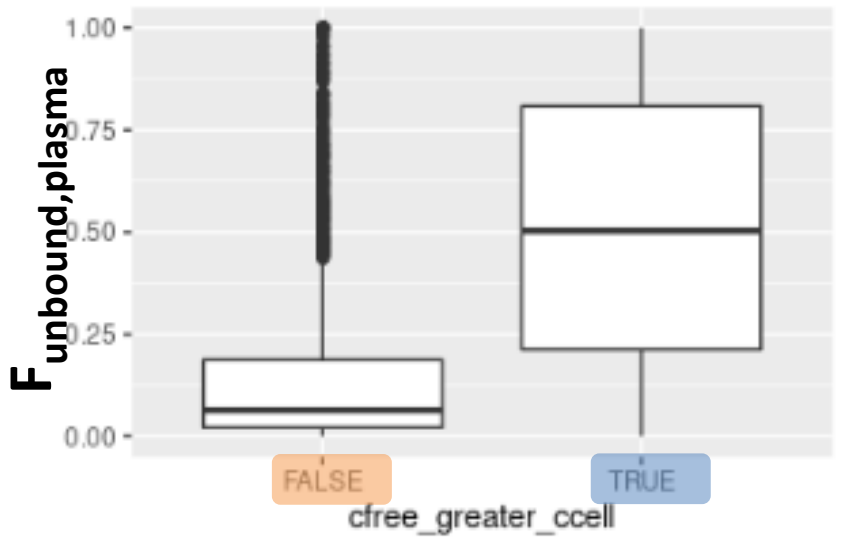
$C_{cell} > C_{free}$

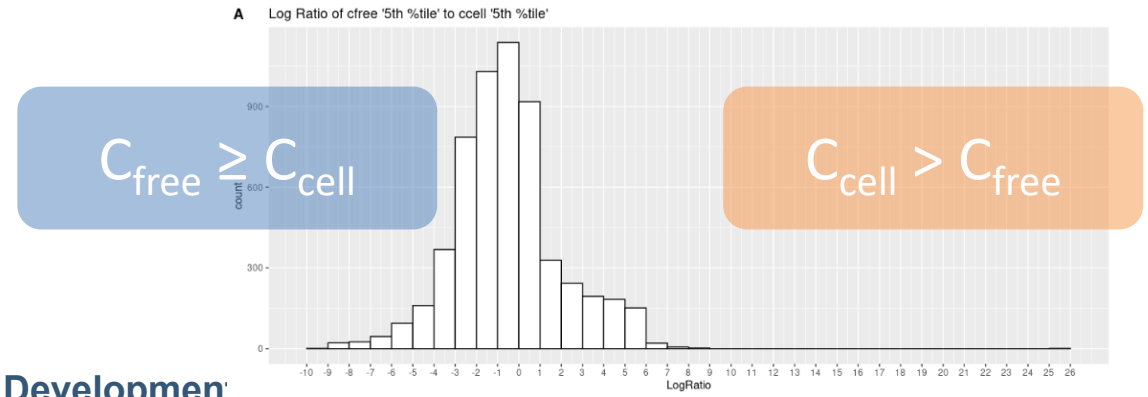
Slide from Ben Savage and Katie Paul Friedman

Impact of *In Vitro* Disposition on NAM-based Point of Departure

- For the chemicals where the differences were extreme (greater than 10-fold) the differences in chemical descriptors are more pronounced





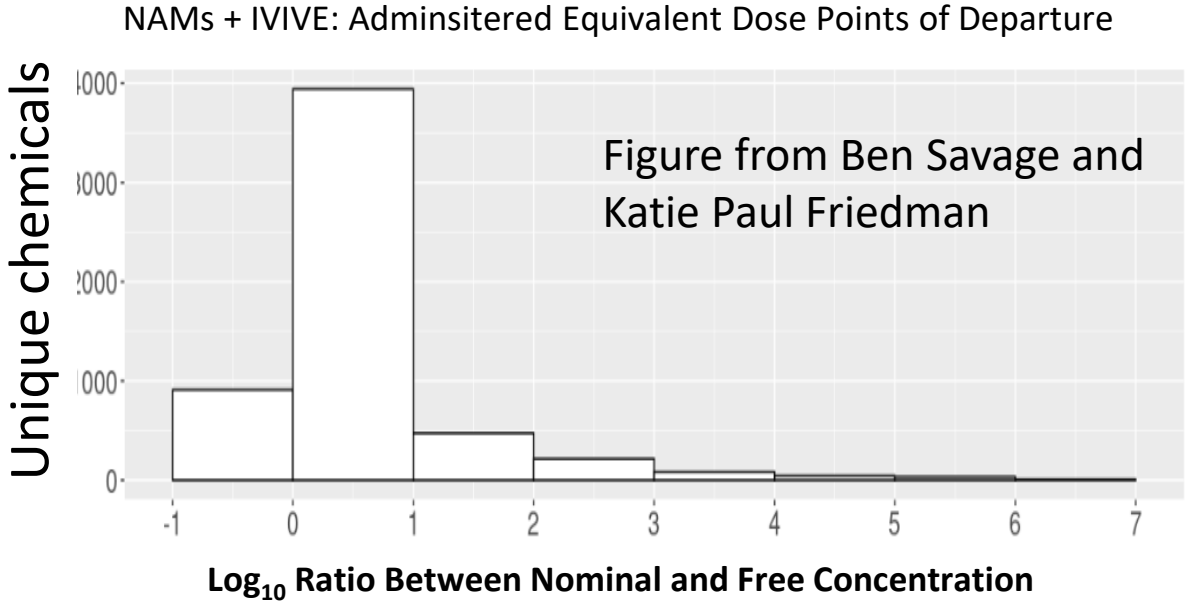


Slide from Ben Savage and Katie Paul Friedman

Summary

- Next Generation Risk Assessment (NGRA) based upon NAMs for hazard and exposure allow risk-based prioritization of large numbers of chemicals if the *in vitro* concentrations can be translated to *in vivo* context (IVIVE)

- Understanding of both toxicokinetics (absorption, distribution, metabolism, and excretion) and *in vitro* disposition within the hazard NAMs are necessary for a quantitative understanding of the dose-response relationship



- While mechanistic and empirical models exist for predicting *in vitro* distribution, data to evaluate these models are limited, especially for non-pharmaceuticals

- The Tox21 collaboration is working to generate new data for non-pharmaceutical chemicals to permit model evaluation and revision

Acknowledgements

- Katie Paul Friedman
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- Michael Devito
- Stephen Ferguson
- Madison Feshuk
- Josh Harrill
- Nisha Sipes
- Russell Thomas
- James Armitage
- Caroline Ring

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