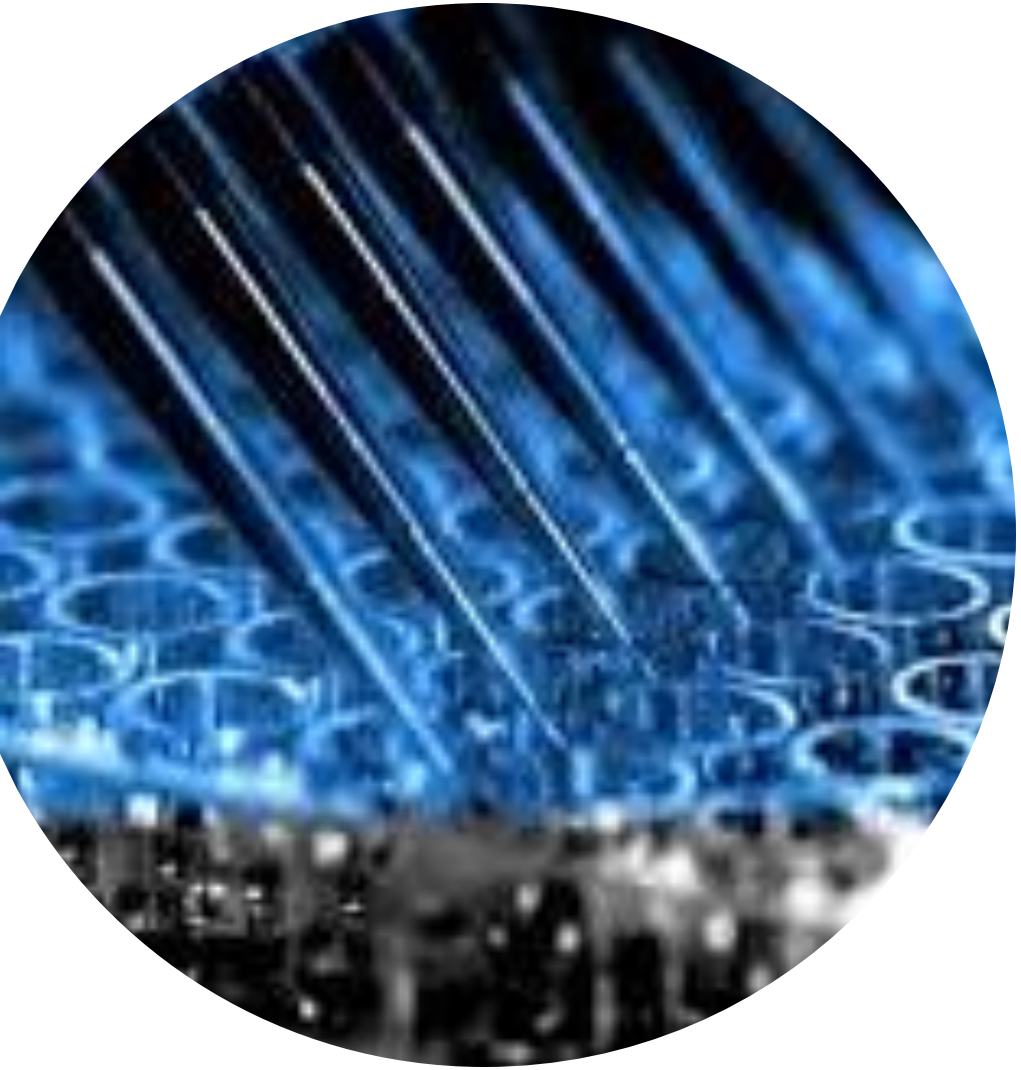
ASCCT Webinar
30 08 2022

Measuring and modeling the distribution of test chemicals in in vitro toxicity assays

1. Toxicology Division, Wageningen University, Wageningen, The Netherlands

Presentation Aim

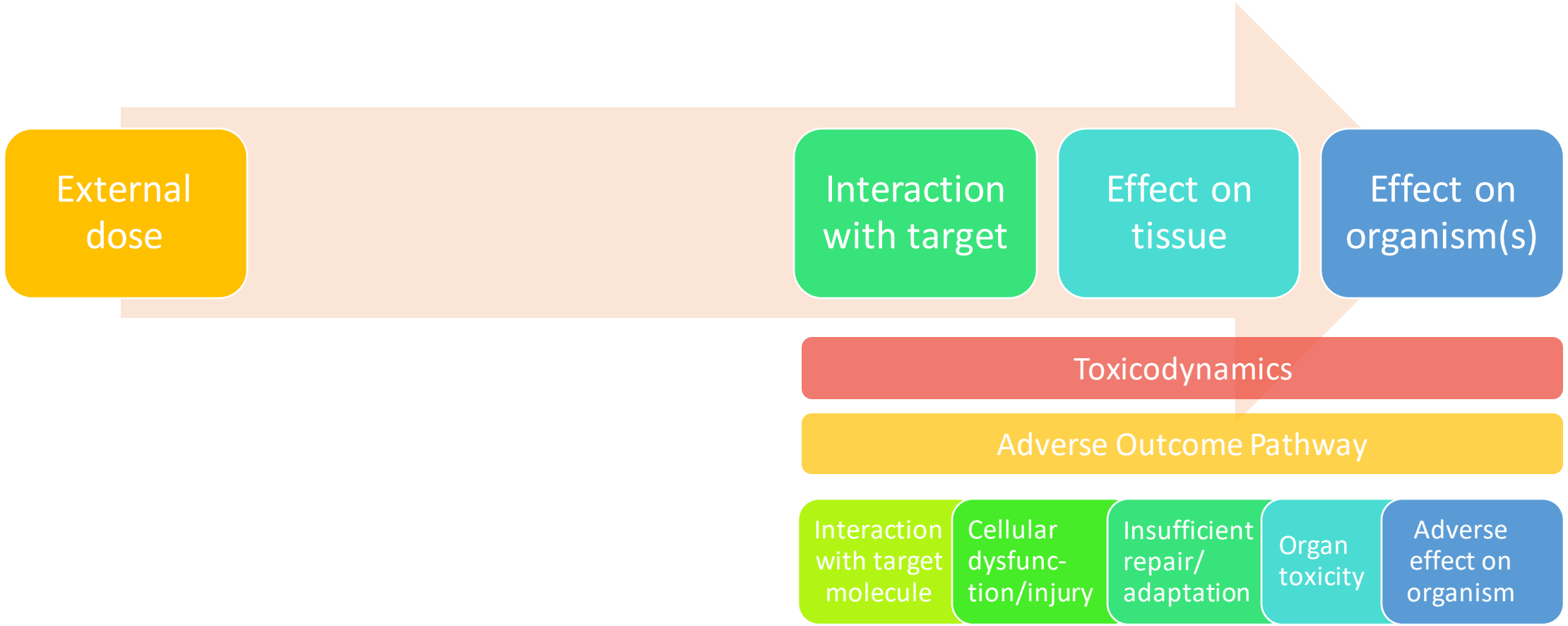
How and why do we account for the freely available fraction of chemicals in *in vitro* assays for QIVIVE?



Basis of Toxicity Testing in the 21st Century: *In Vitro* Cell Assays

- Mechanistic approach
- Human tissue/tissue of species of interest
- High throughput
- Little waste
- (Ethically) sound science
  - 3Rs: replacement, reduction, refinement

In Vitro Assays in Toxicity Testing in the 21st Century



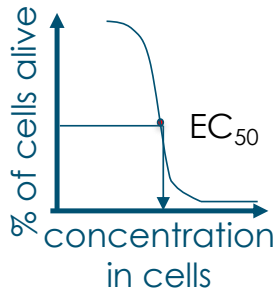
Toxic Concentration *In Vitro* ≠ Toxic Applied Dose

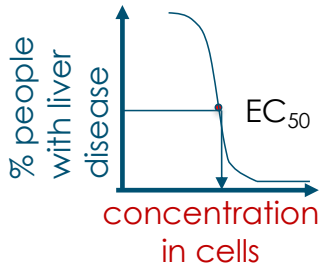
External dose | Internal dose | Target dose | Interaction with target | Effect on tissue | Effect on organism(s)

Toxicokinetics

Toxicodynamics

AOP





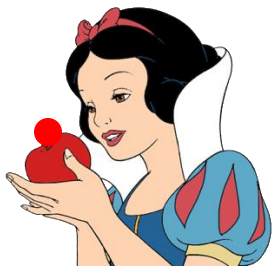
Need to Account for Kinetics *In Vivo*

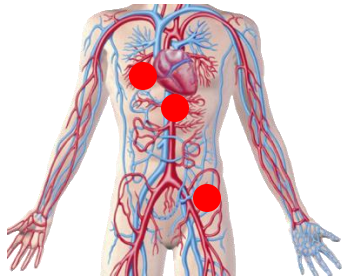
External dose | Internal dose | Target dose | Interaction with target | Effect on tissue | Effect on patient

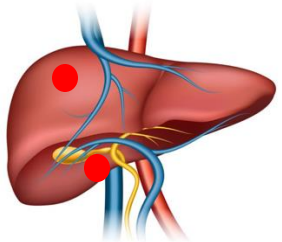
Toxicokinetics | Toxicodynamics

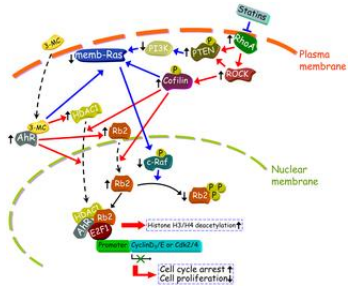
AOP

oral dose → C_{plasma} → C_{tissue} → BED









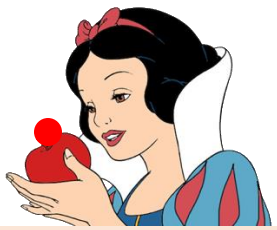
Need to Account for Kinetics *In Vivo* and *In Vitro*

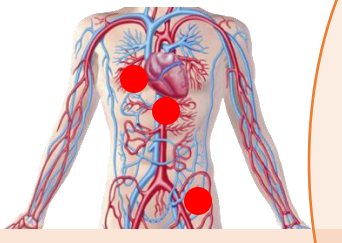
External dose | Internal dose | Target dose | Interaction with target | Effect on tissue | Effect on patient

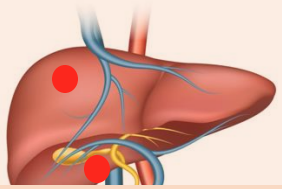
Toxicokinetics | Toxicodynamics

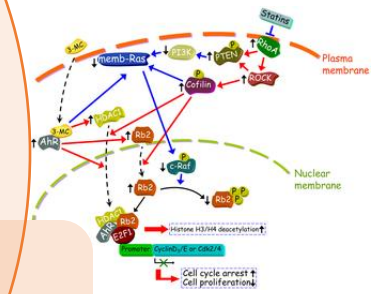
AOP

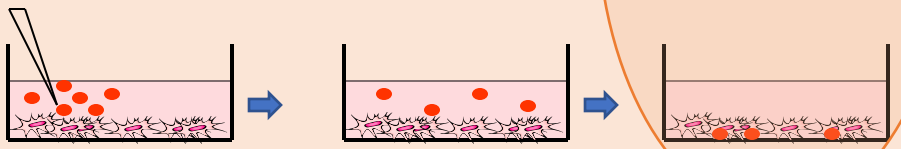
oral dose → C_{plasma} → C_{tissue} → BED







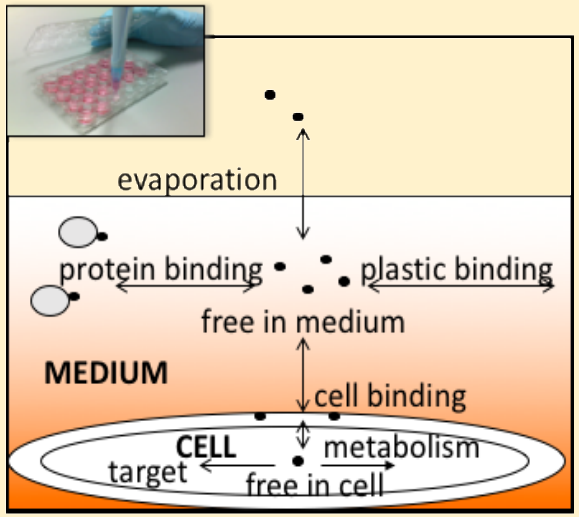


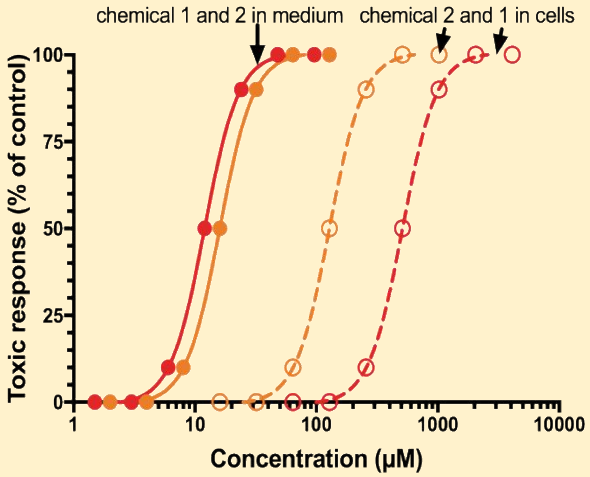


$C_{nominal}$ C_{free} C_{cell}

Tools for Accounting for Kinetics

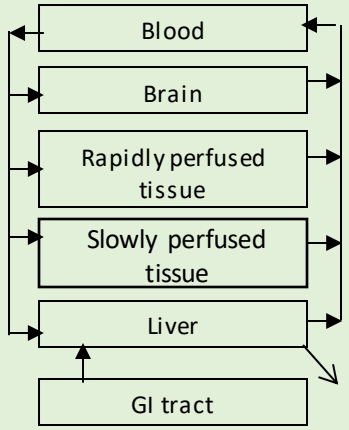
In vitro kinetics

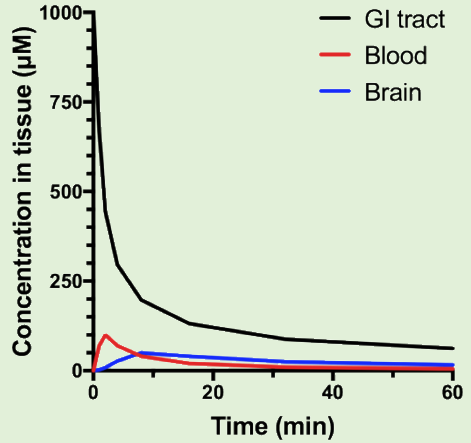




In Vitro Distribution Kinetics Models

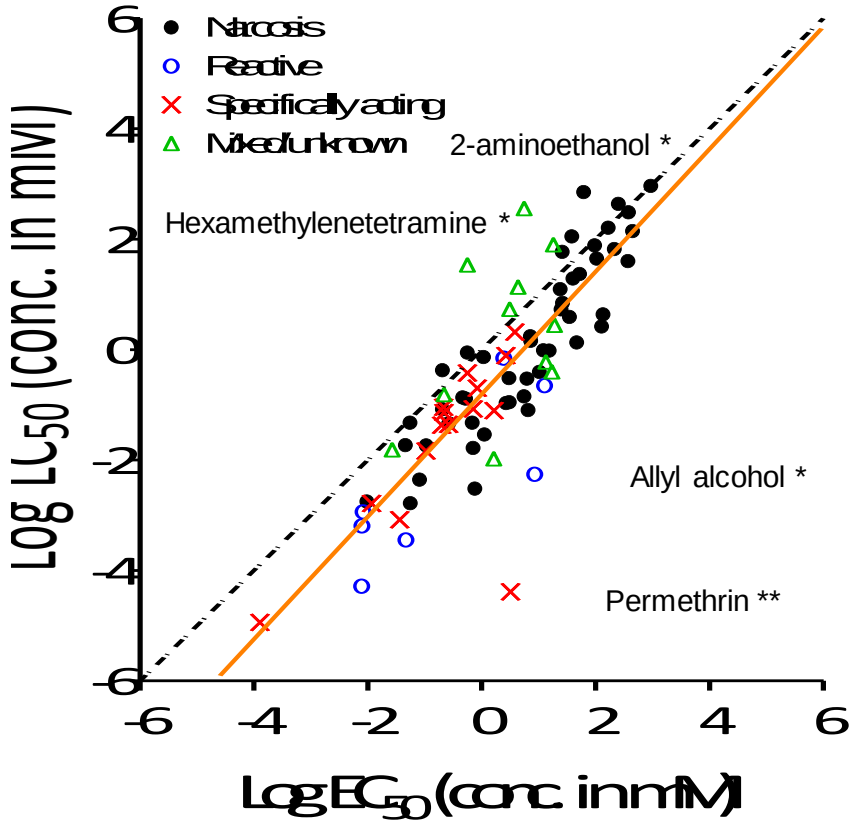
In vivo kinetics





Physiologically Based Kinetic Models

More simple forms of QIVIVE illustrate importance of understanding *in vitro* kinetics



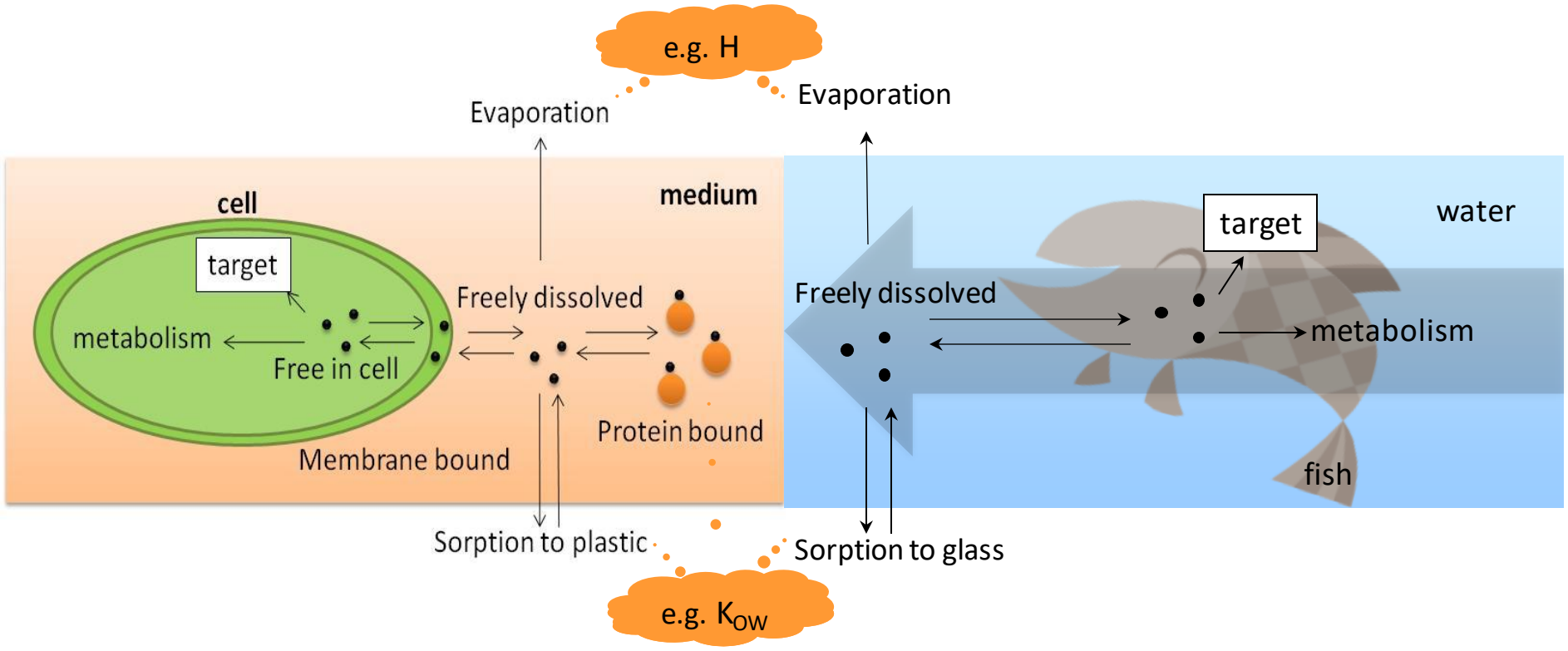
- *In vitro* basal cytototixicity assay (Halle Database) vs. acute fish bioassay (EPA Duluth FHM Database)
- Good correlation, but high variability and poor sensitivity
- Outliers specifically acting, bioactivated chemicals
- Correlation is improves when only narcotic chemicals are used in regression
- *In vitro* still less sensitive than fish acute bioassay

Log LC50 (mM) = 1.10 ± 0.08 Log IC50 (mM) – 0.81 ± 0.11, R^2 = 0.70.

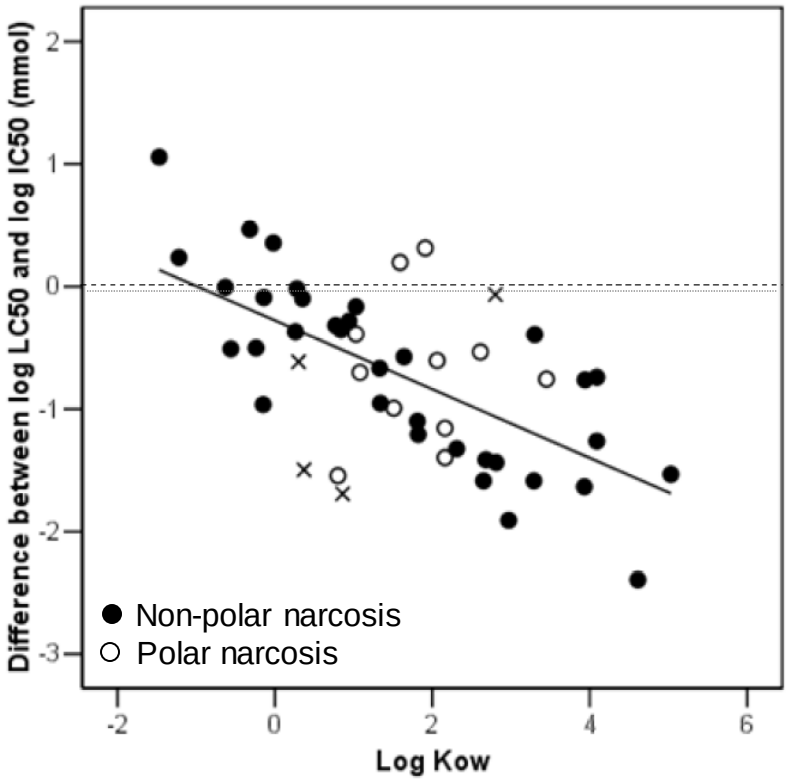
Log LC50 (mM) = 1.11 ± 0.08 Log IC50 (mM) – 0.81 ± 0.12, R2 = 0.80.

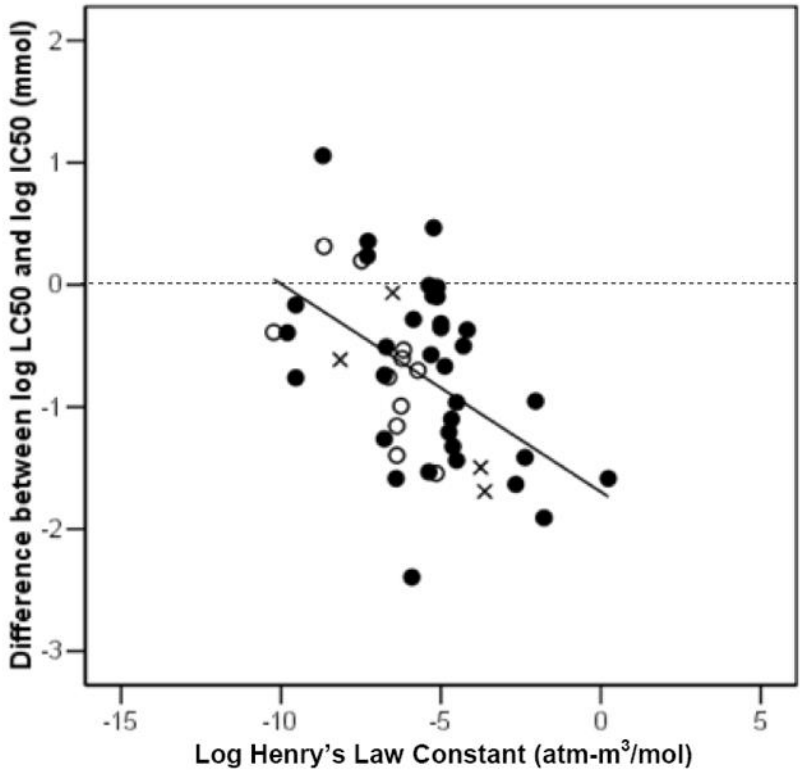
Kramer et al. (2009) Toxicology *In Vitro* 23, 1372-1379.

Importance of *In Vitro* Kinetics in QIVIVE



Role of Physicochemical Properties



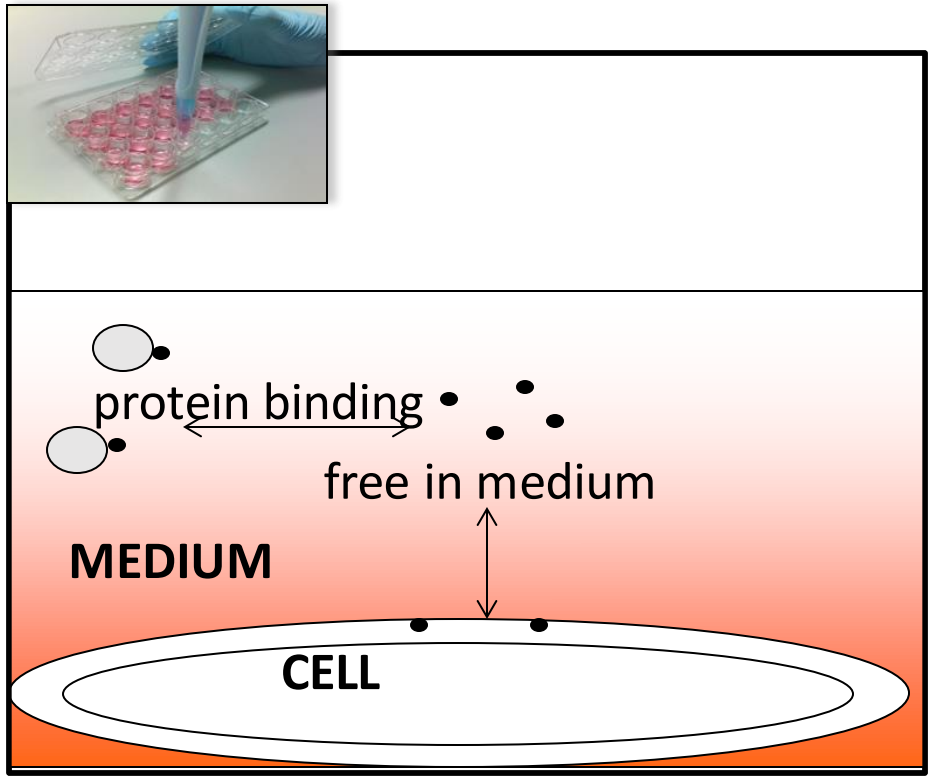


Log LC50 – Log IC50 (mM) = -0.26 (± 0.05) Log *Kow* - 0.30 (± 0.11), R^2 = 0.38

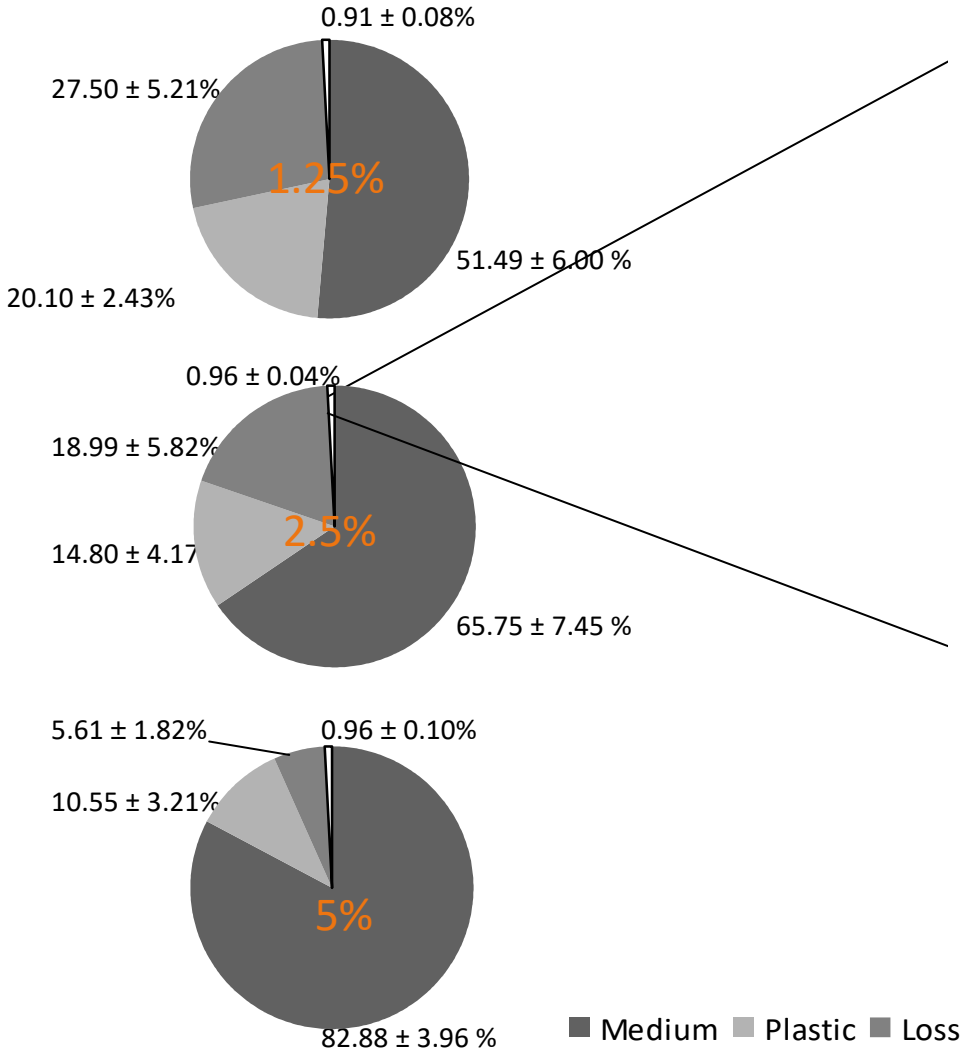
Log LC50 – Log IC50 (mM) = -0.17 (± 0.04) Log *H* (atm-m^3/mol) – 1.70 (± 0.26), R^2 = 0.25

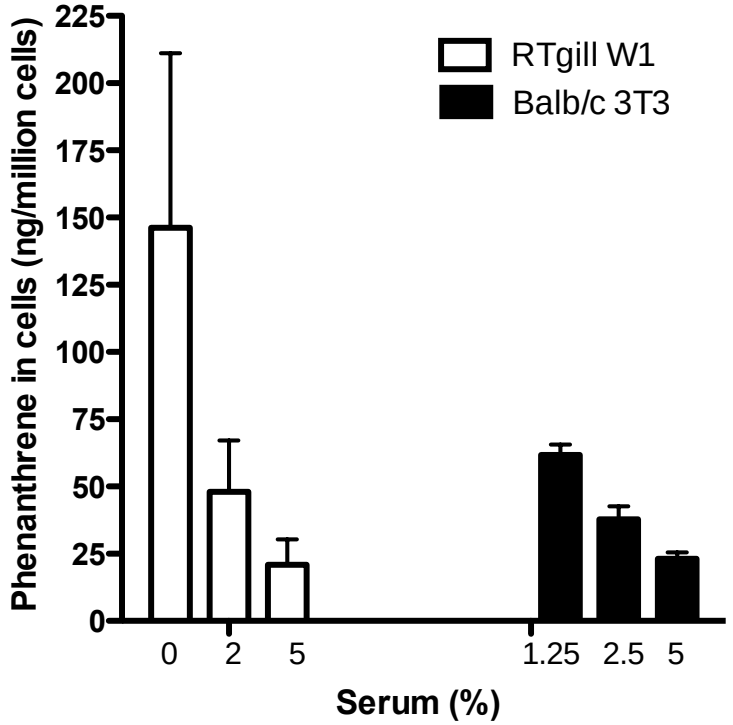
Kramer et al. (2009) Toxicology *In Vitro* 23, 1372-1379.

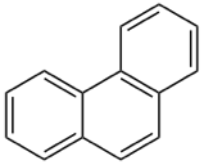
Determinants of *In Vitro* Distribution Kinetics: Serum Constituent Binding



Serum Constituent Binding

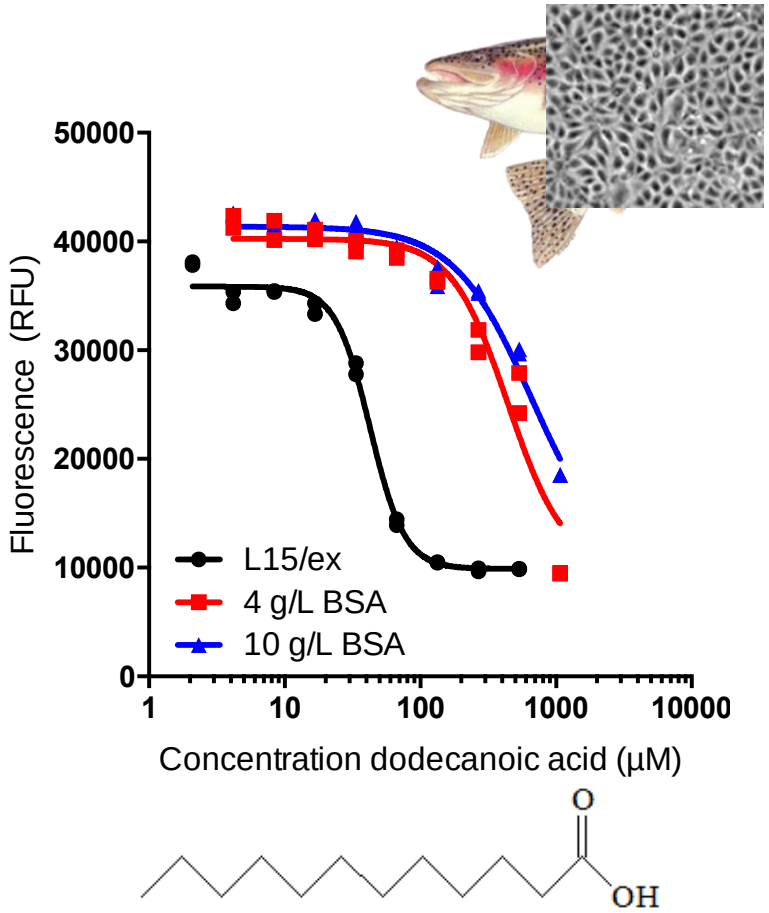


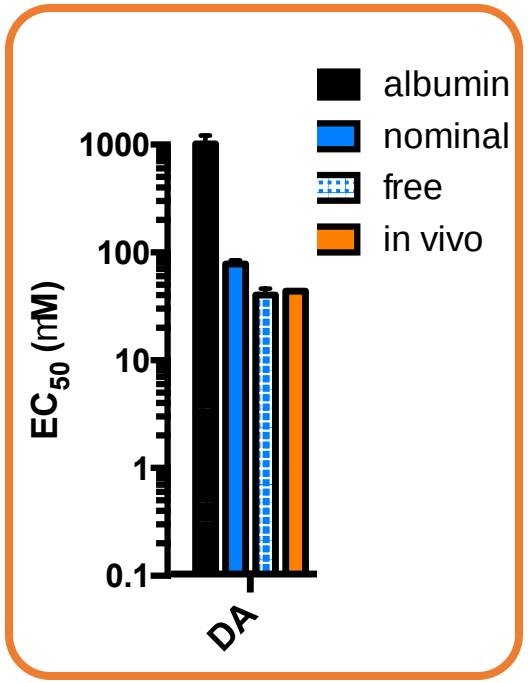




Kramer et al. (2012) CRT 25, 436

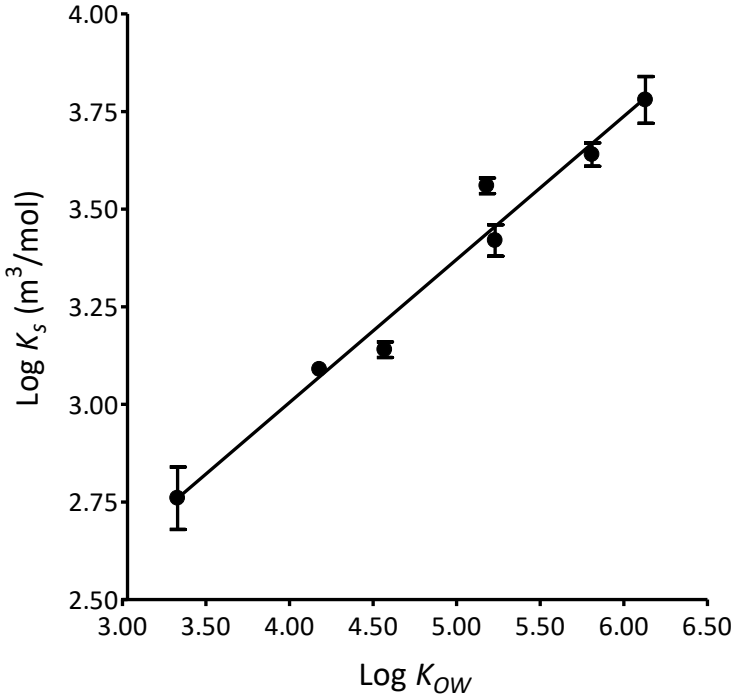
Serum Constituent Binding





Groothuis et al., manuscript in preparation

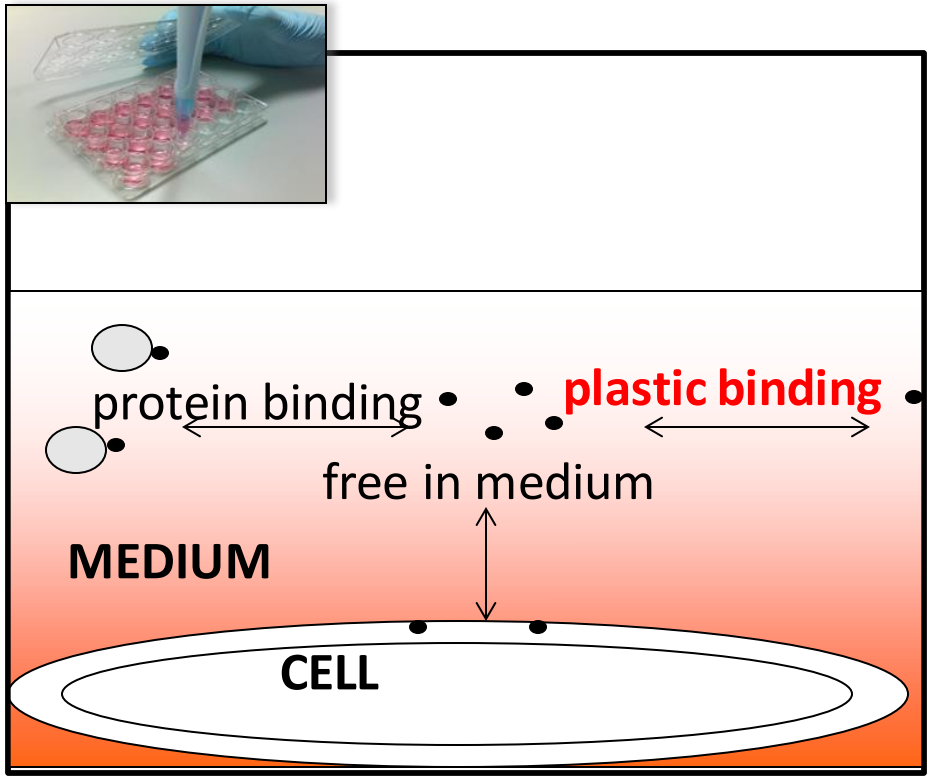
Serum Constituent Binding

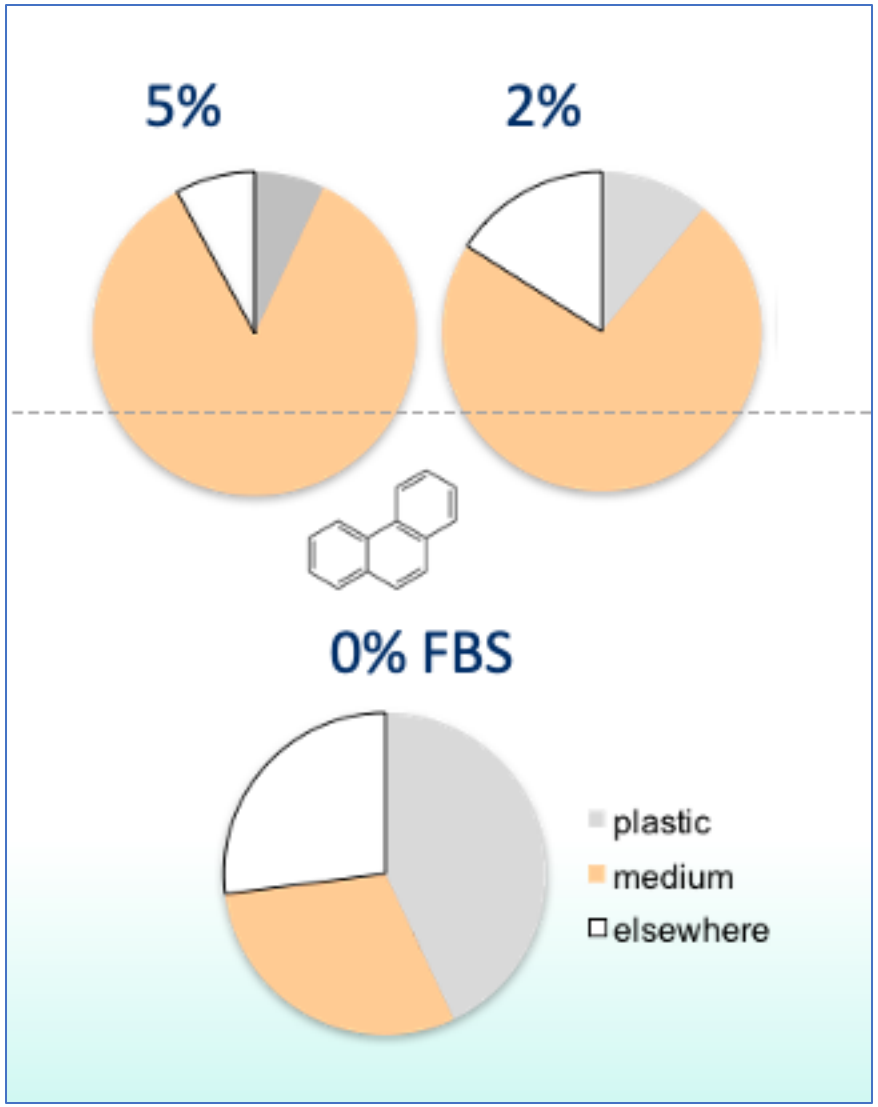


Quantitative Structure Property Relationships (QSPR)

Schaap et al., manuscript in preparation

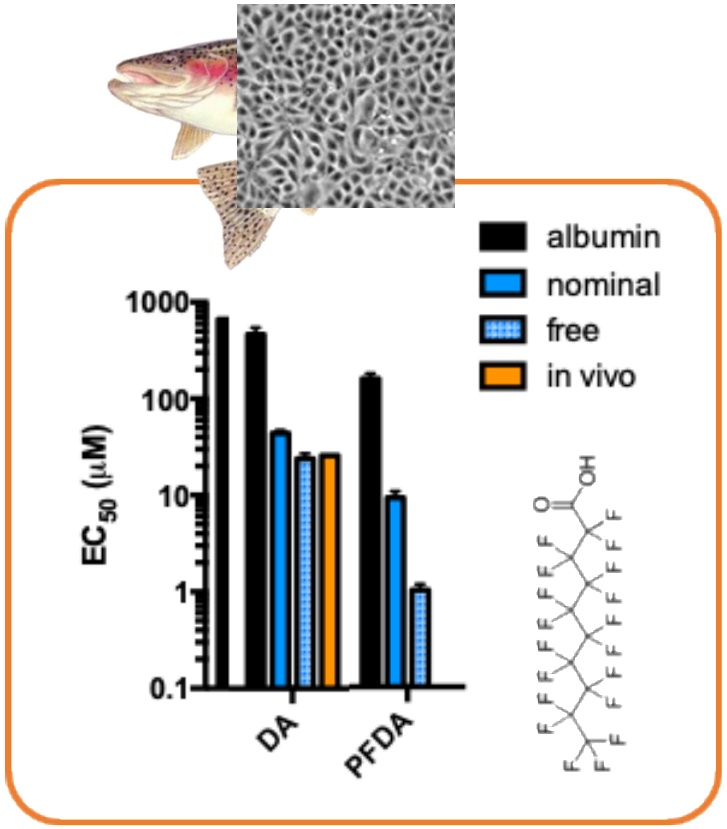
Well Plate Plastic Binding

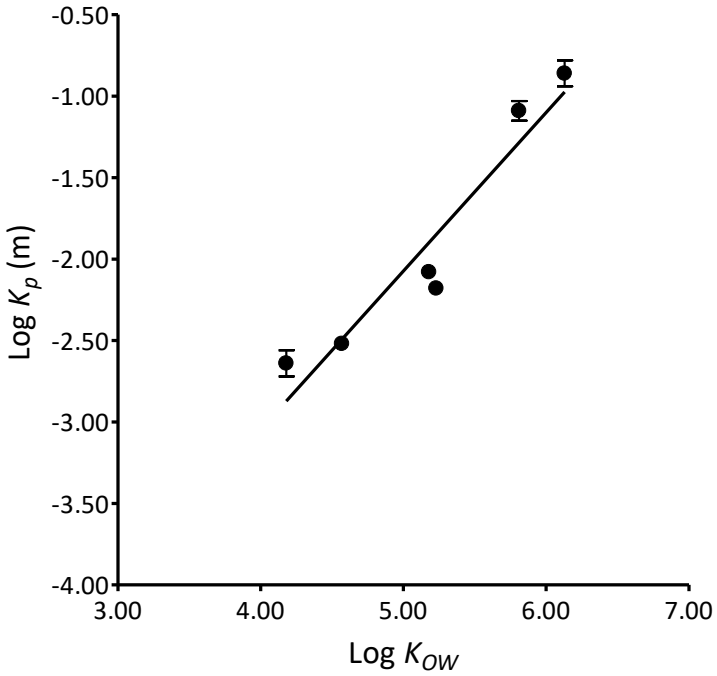




Kramer et al. (2012) Chem. Res. Toxicol. 25, 436
Schaap et al., manuscript in preparation

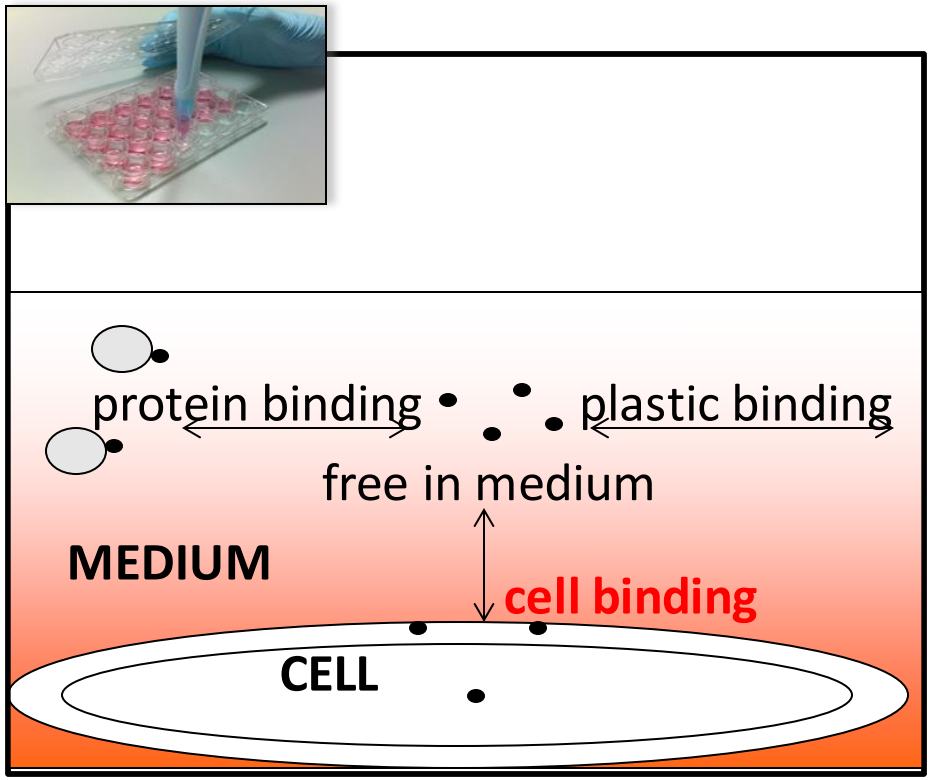
Well Plate Plastic Binding

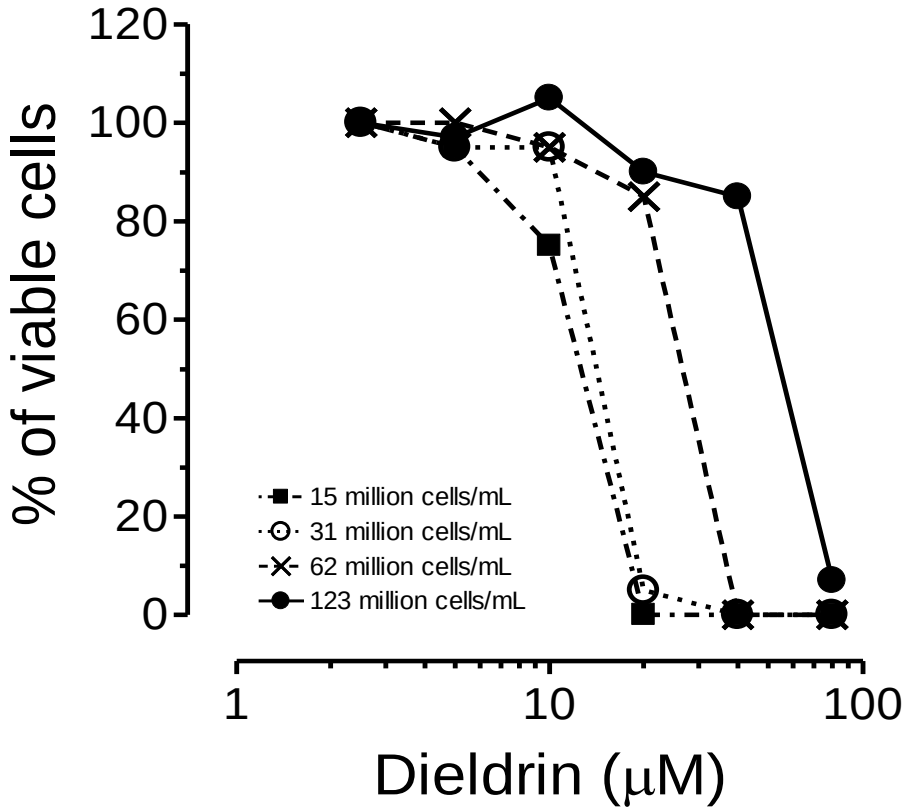




Groothuis et al., manuscript in preparation
Schaap et al., manuscript in preparation

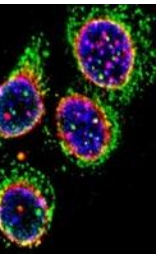
Cell Association

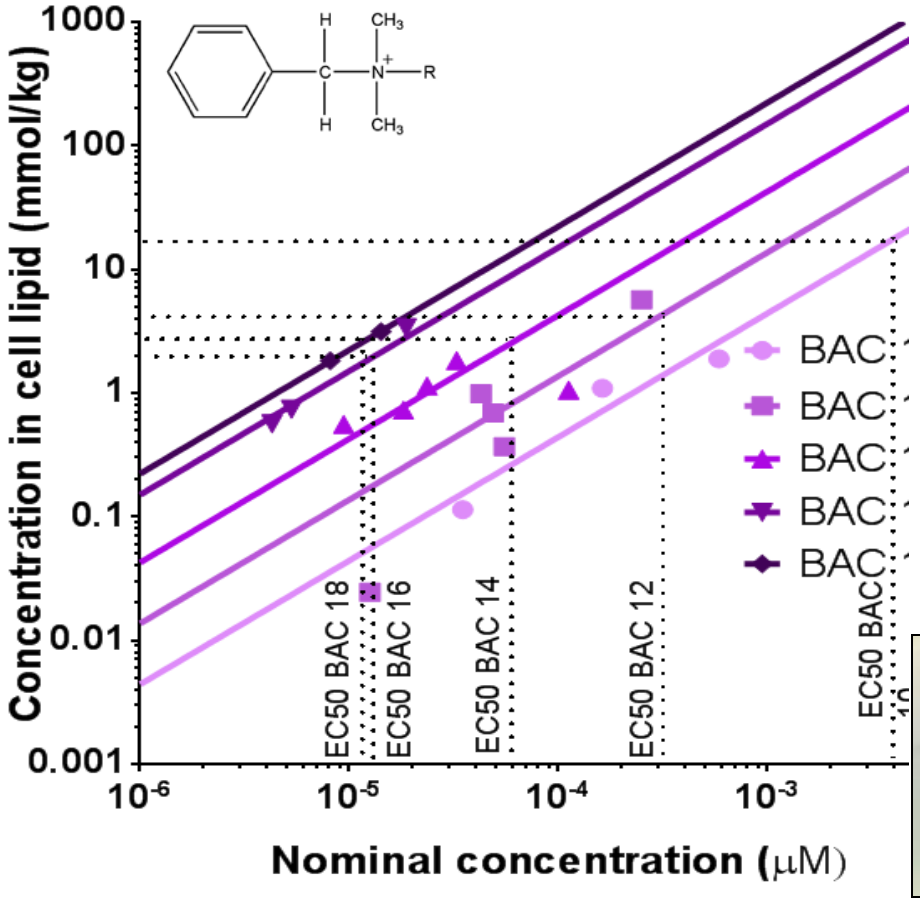


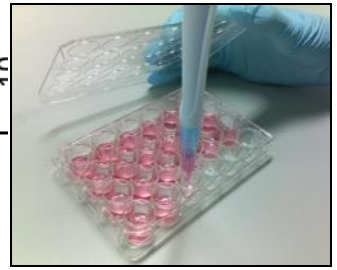


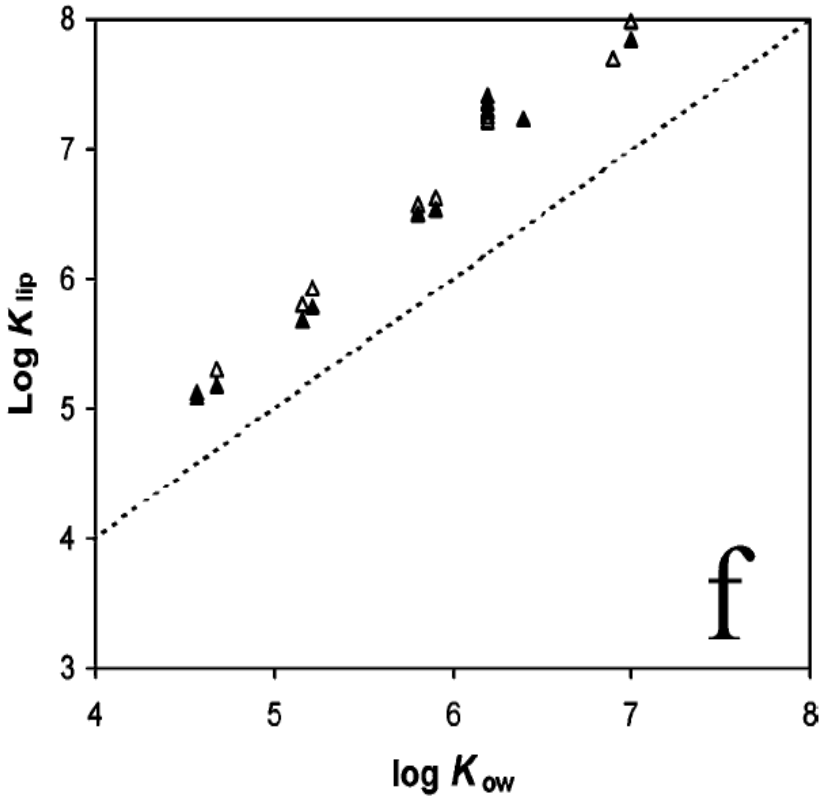
Gülden et al (2001) TIV 15: 233-243

Cell Binding Affinity vs. QIVIVE



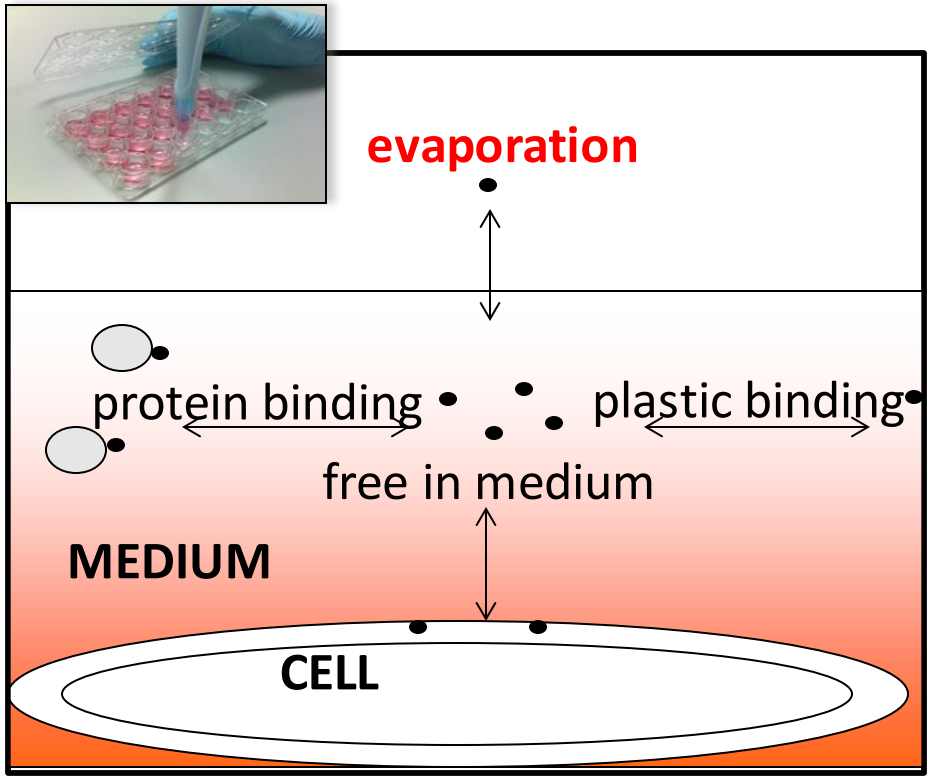


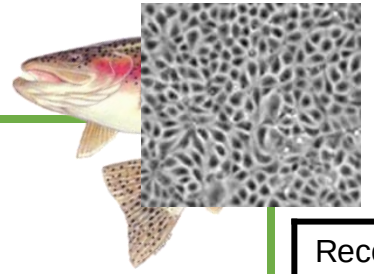


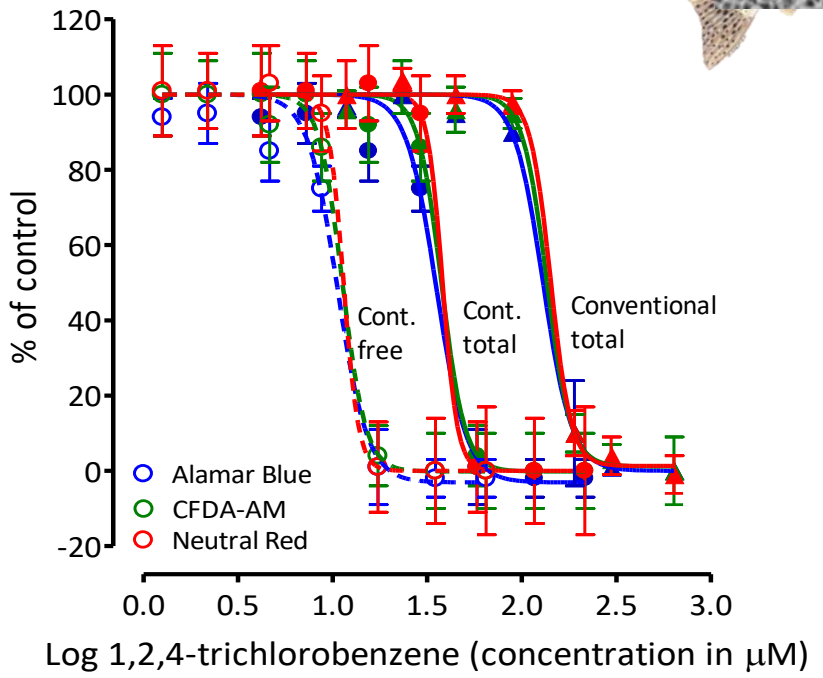


Groothuis et al. (2019) CRT

Evaporation



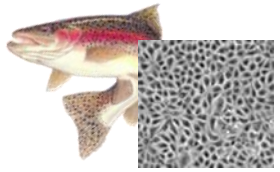


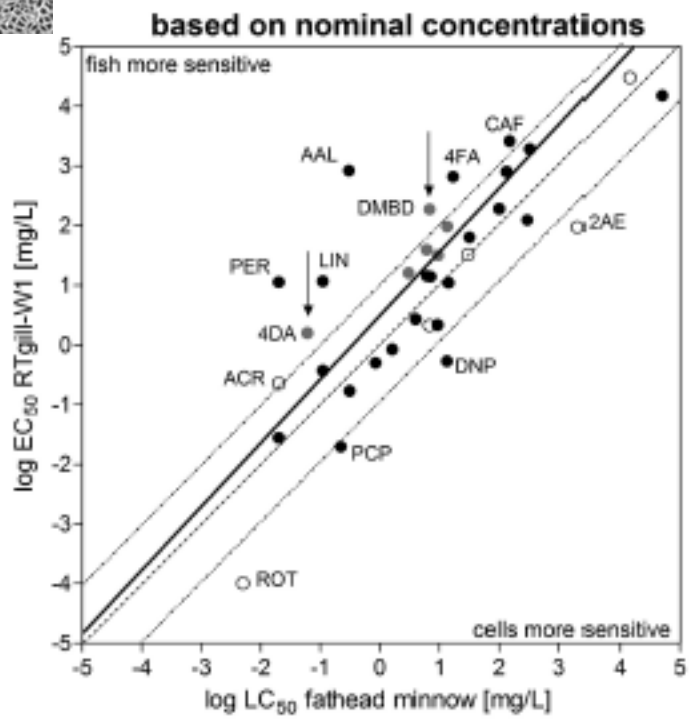


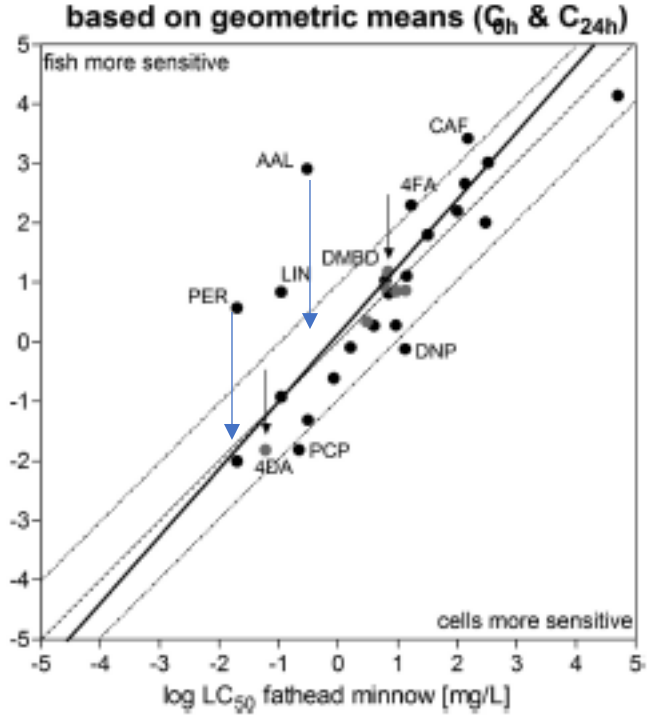
Recovery from medium after 48h	Conventional dosing: 11% Continuous dosing: 105%
EC_{50} (µM)	Conventional: 135 Continuous total: 38 Continuous free: 11 Fathead Minnow: 16
Bound to Serum Consti-tuents	70%

Kramer et al. (2010) Chem. Res. Toxicol. 23, 1806.

Importance of *In Vitro* Distribution Kinetics in QIVIVE







Halle 2003

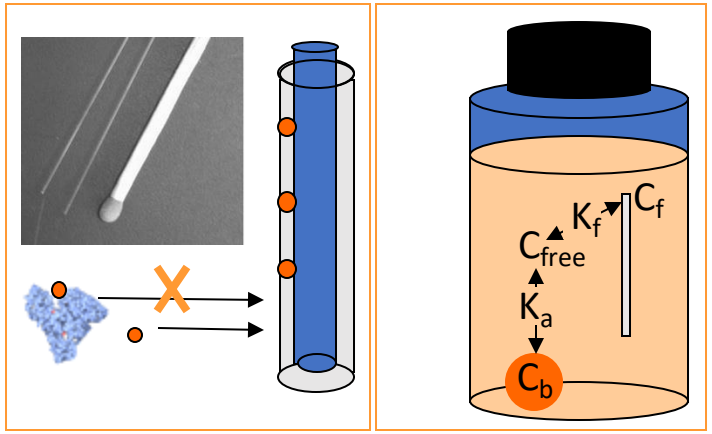
Basker & Murthy 2018

Tanneberger et al. 2013 EST 47 1110-1119

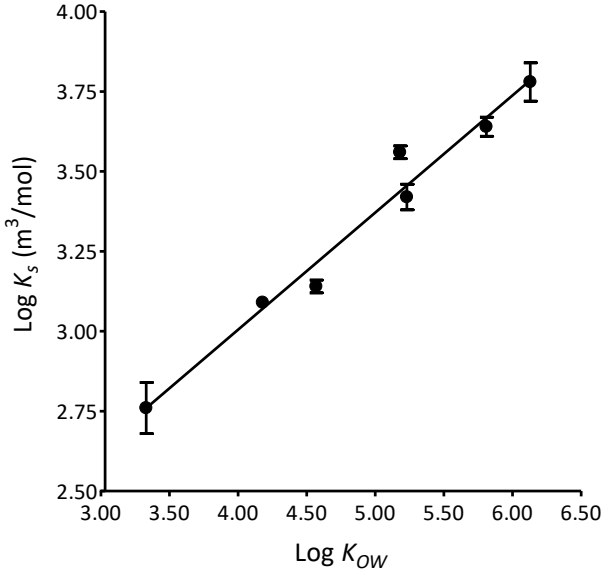
Analytical Methods for Assessing *In Vitro* Distribution Kinetics

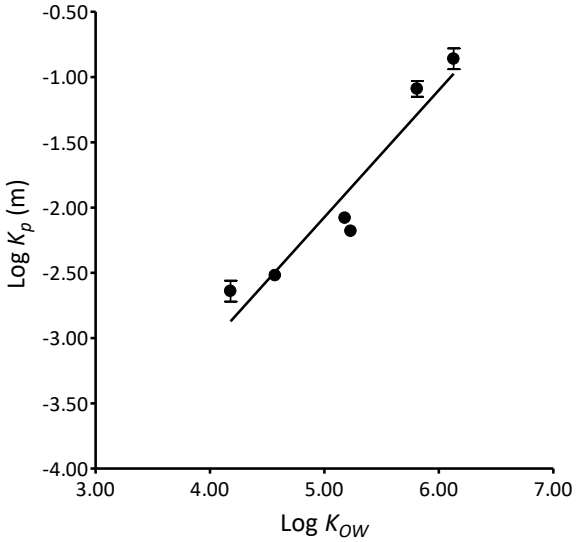
- Rapid Equilibrium Dialysis (RED)
- Ultrafiltration
- Ultracentrifugation
- Column Chromatography
- Solid Phase (Micro)extraction (SPME)

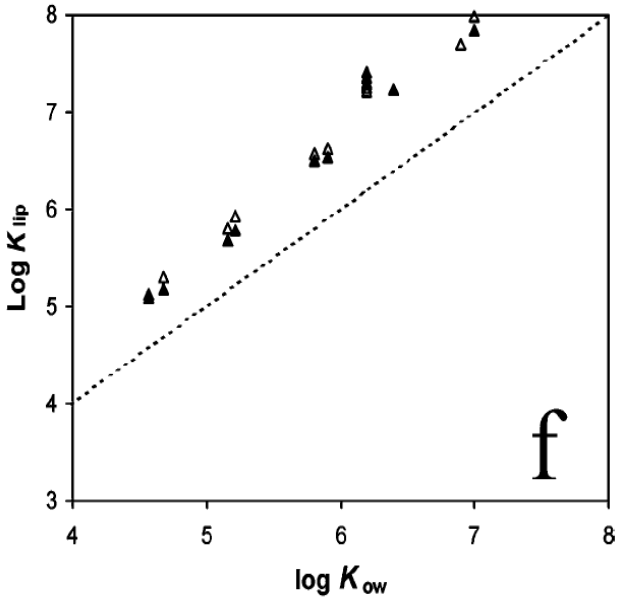
- Methods discussed in Groothuis et al. (2015) Toxicology *In Vitro* 332, 30-40.



Modelling *In Vitro* Distribution Kinetics



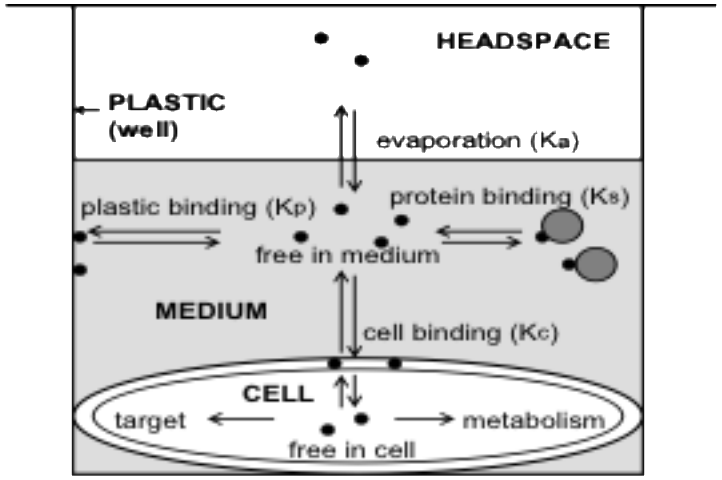




$$F = \frac{1}{1 + K_s \cdot [S] + K_p \cdot [P] + K_c \cdot [C] + K_a \cdot \frac{V_a}{V_m}}$$

Schaap et al. (in preparation).
Jonker et al (2007) Environ. Sci. Technol. 41, 7363

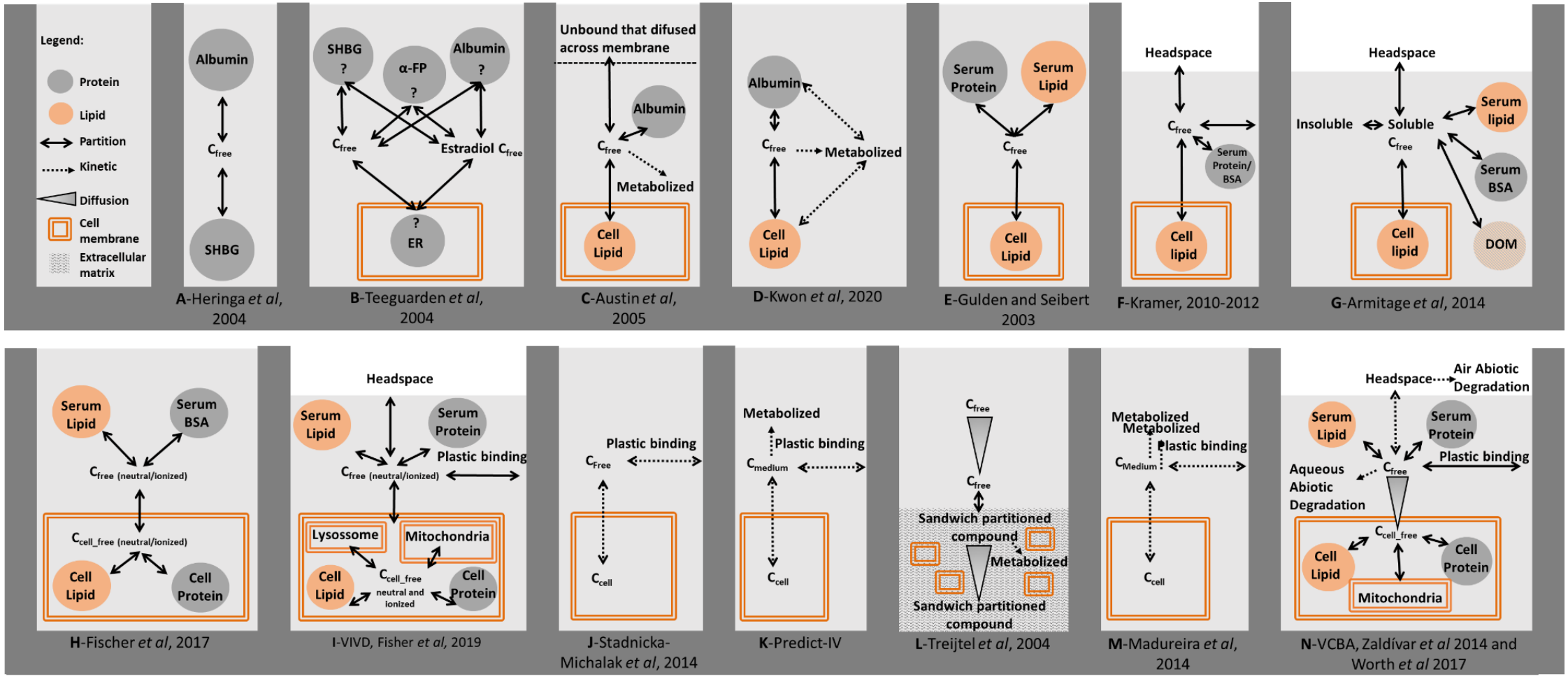
Modelling *In Vitro* Distribution Kinetics



$$F = \frac{1}{1 + K_s [S] + K_p [P] + K_c [C] + K_a [A]}$$

$$F = \frac{1}{1 + 10^{0.37 \log Kow - 0.29}[S] + 10^{0.97 \log Kow - 6.94}[P] + 10^{1.25 \log Kow - 3.70}[C] + \frac{H}{8.3144T} \cdot \frac{V_o}{V_m}}$$

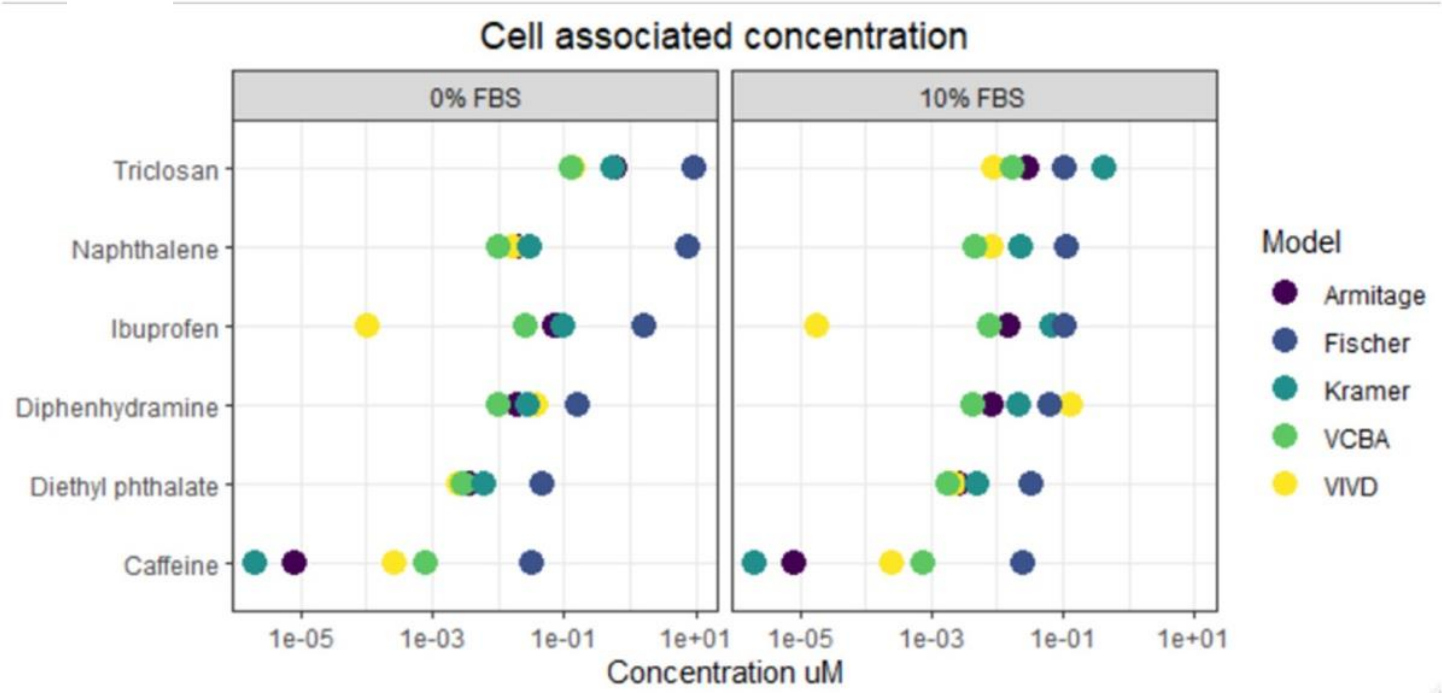
Modeling *In Vitro* Distribution Kinetics



Proenca et al., Accepted in *Toxicol. In Vitro*

Comparing Distribution Predictions

Simulating distribution kinetics of test chemicals at 100 µM nominal concentrations in *in vitro* assays with HepaRG (7,600 cell/cm^2)

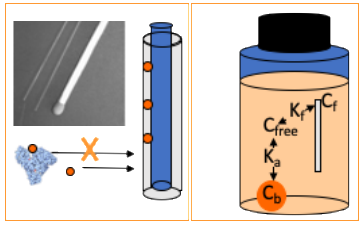


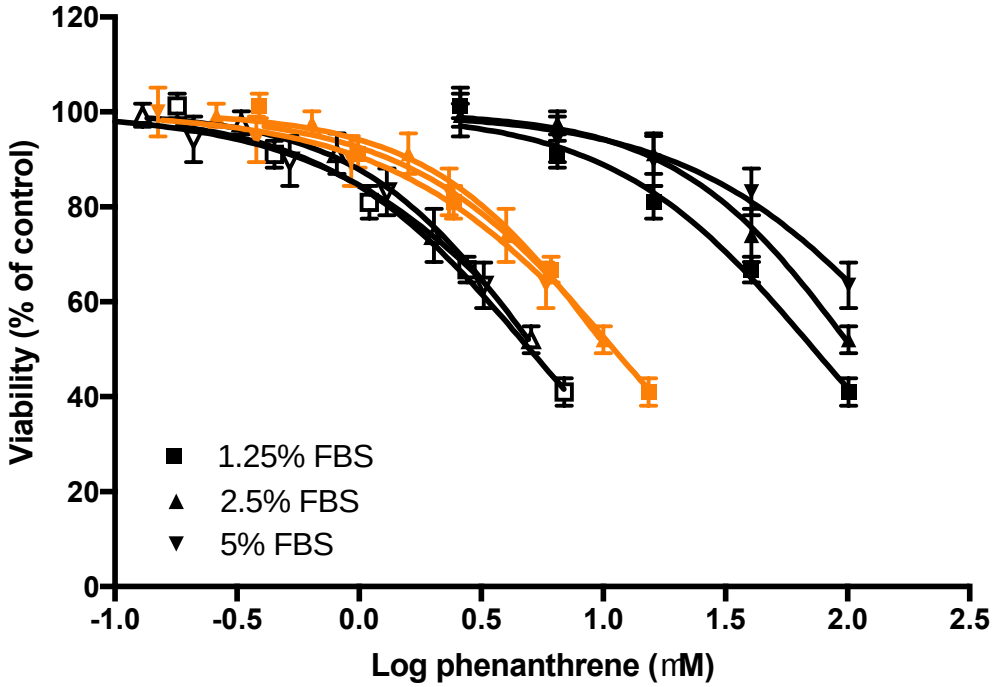
Proenca et al. (2021) *Toxicol. In Vitro*

Applying *In Vitro* Chemical Distribution Model

Polycyclic aromatic hydrocarbons: phenanthrene

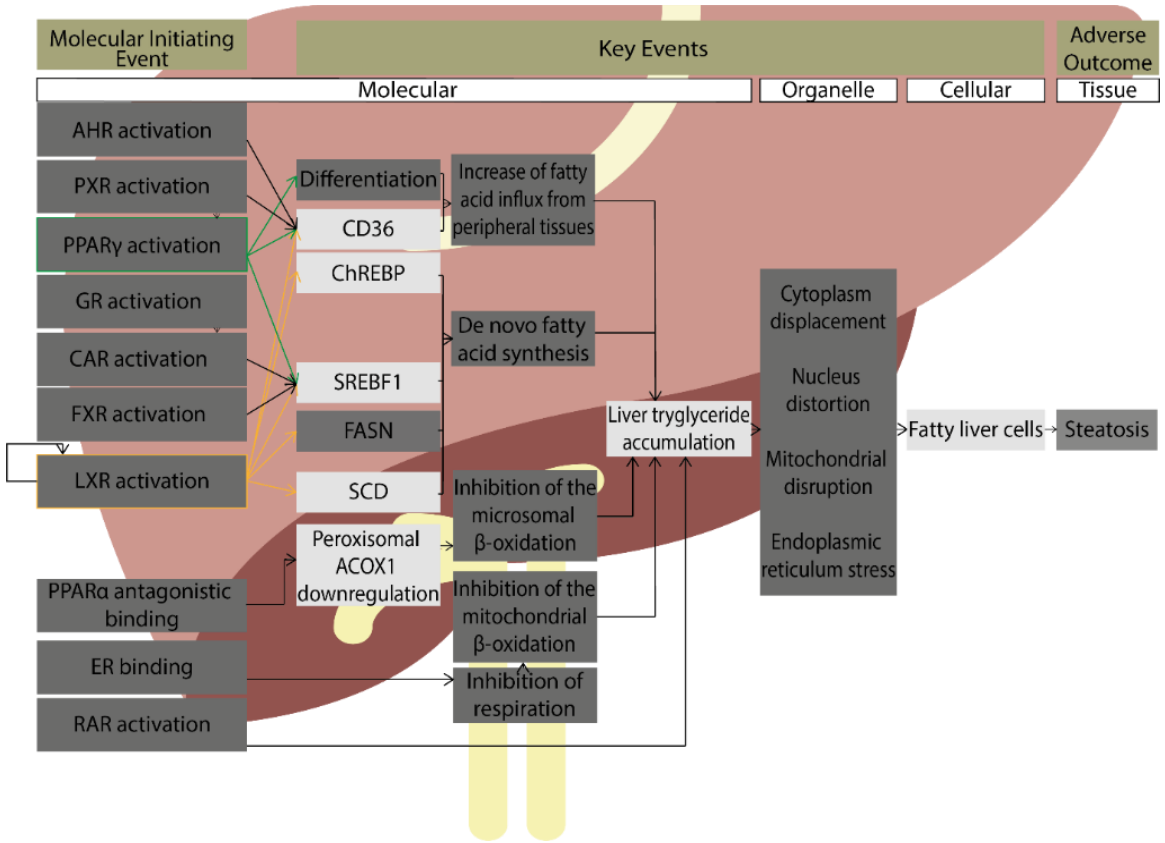
Serum	0%	2%	5%
Measured free	21%	8%	5%
Modeled free	32%	9%	5%
Measured in cells	10%	4%	2%
Modeled in cells	14%	5%	3%

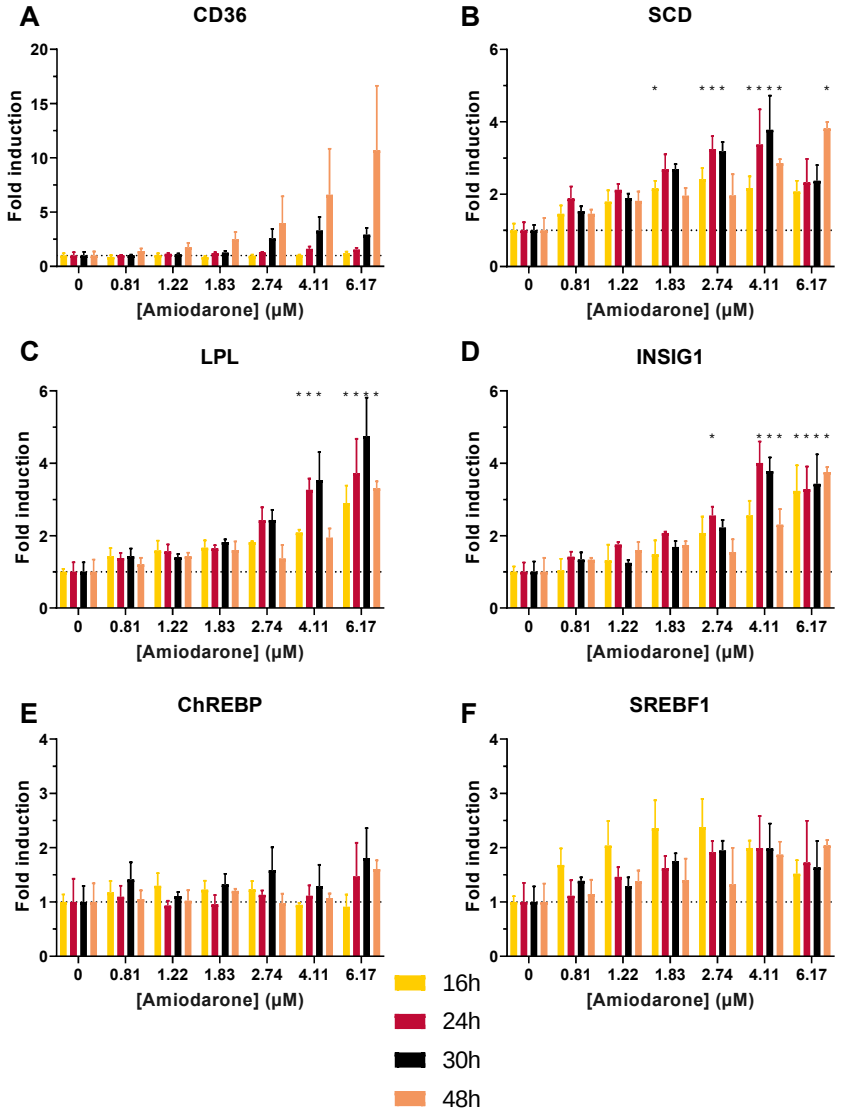




Kramer et al. 2012, Chem Res Toxicol, Schaap et al., manuscript in preparation

In vitro test battery for KE perturbation





Kasteel et al., manuscript submitted

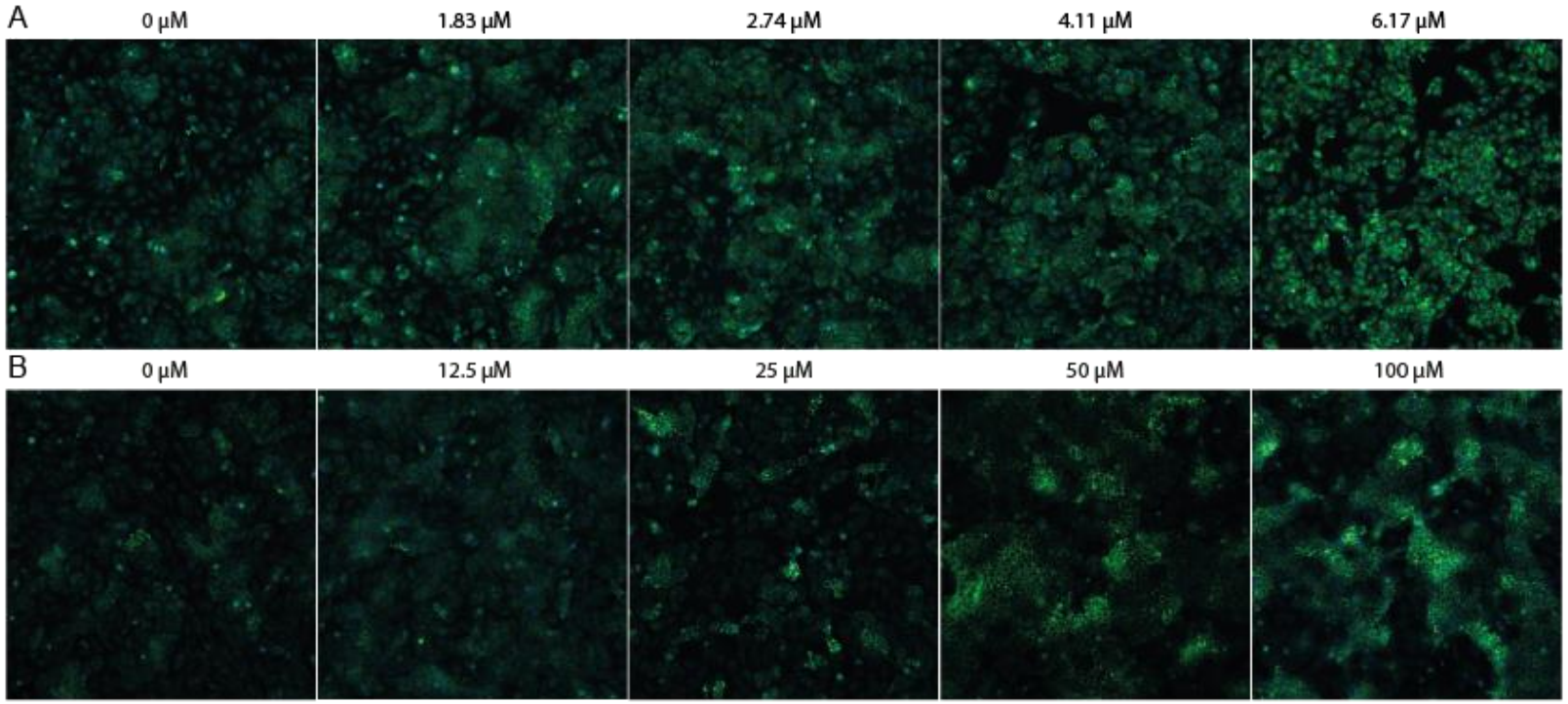
T_{max} is KE dependent

T_{max} is chemical dependent…

- Amiodarone (A) vs tetracycline (B)

After 72h

After 48h



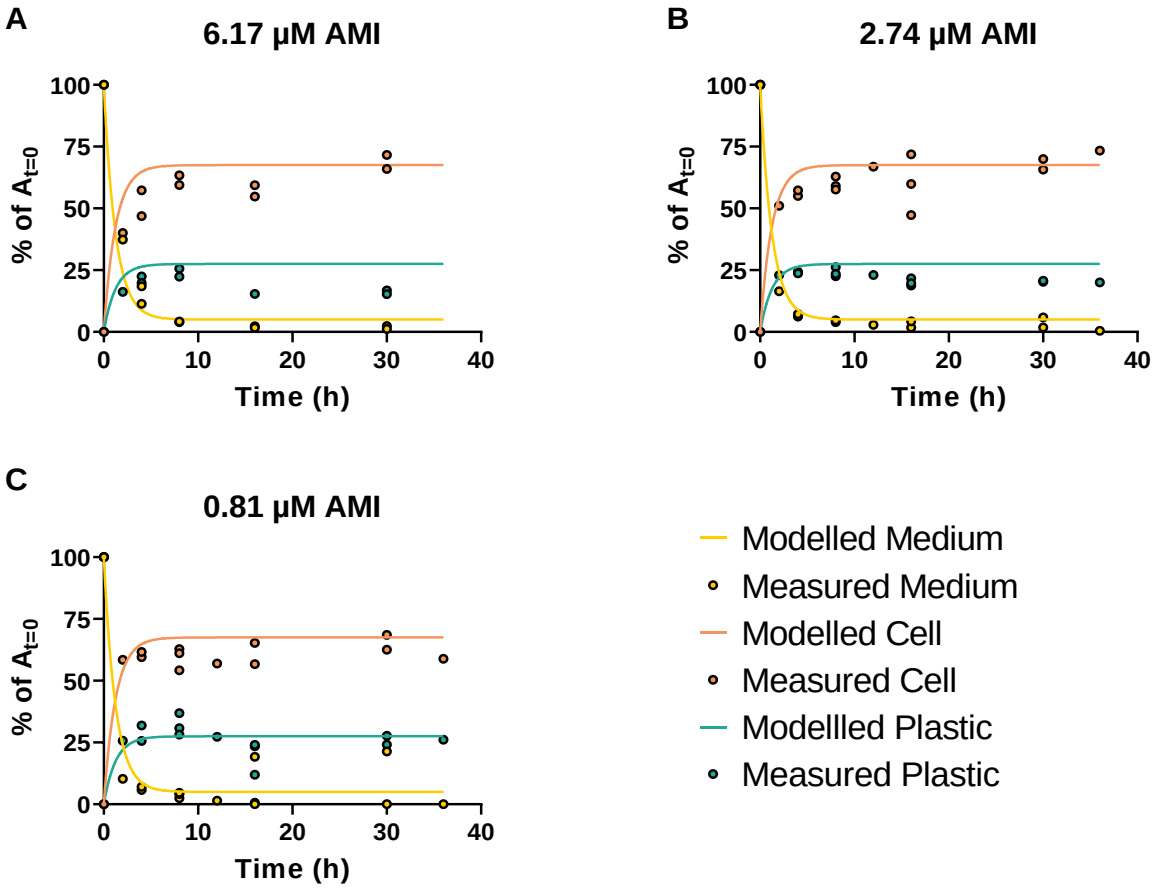
Sequence of KE may be misrepresented when using concentration response relationships of different chemicals

Kasteel et al., manuscript submitted

In vitro kinetics needed for response-response modelling…

Use concentration-effect relationships …

- at exposure time point leading to lowest EC
- based on internal cell concentrations



Kasteel et al., manuscript submitted

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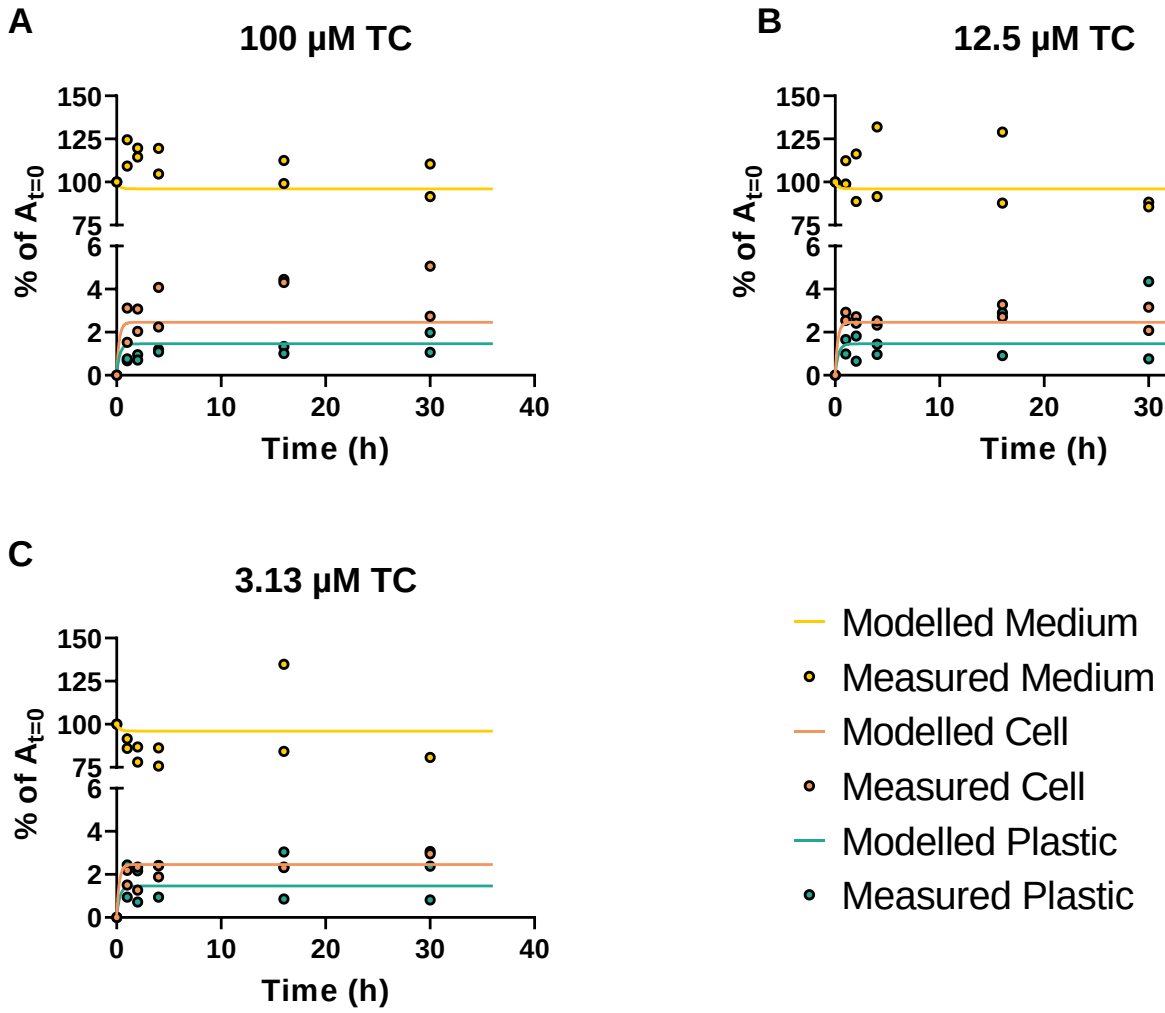

Kasteel et al., manuscript submitted

In vitro kinetics needed for response-response modelling…

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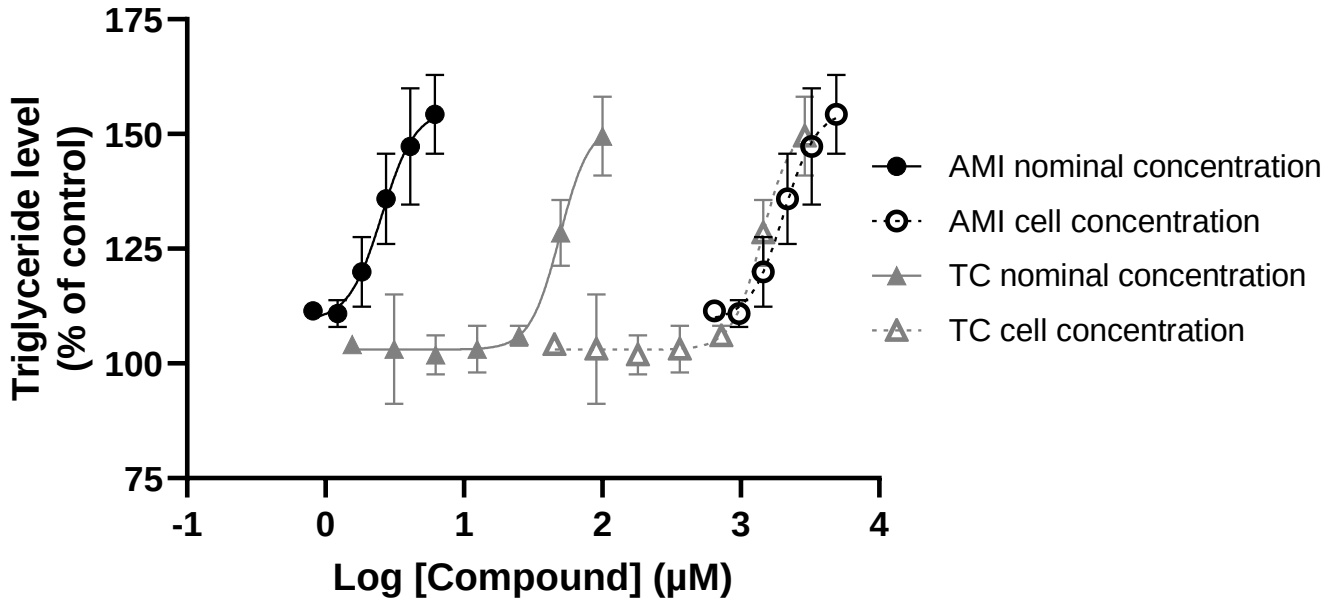
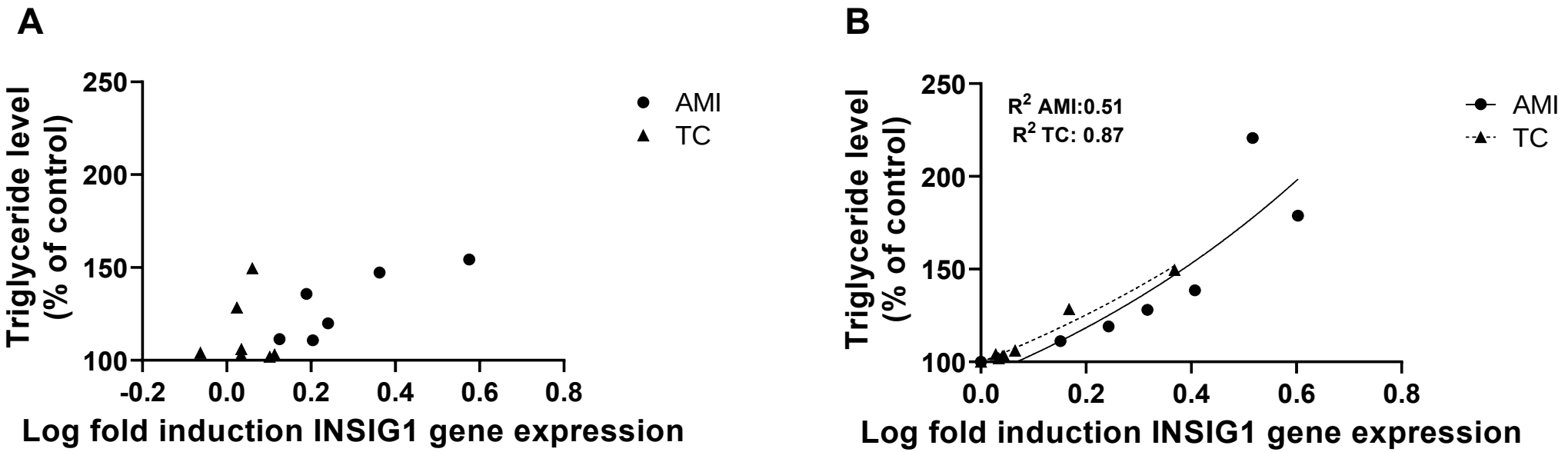


Kasteel et al., manuscript submitted

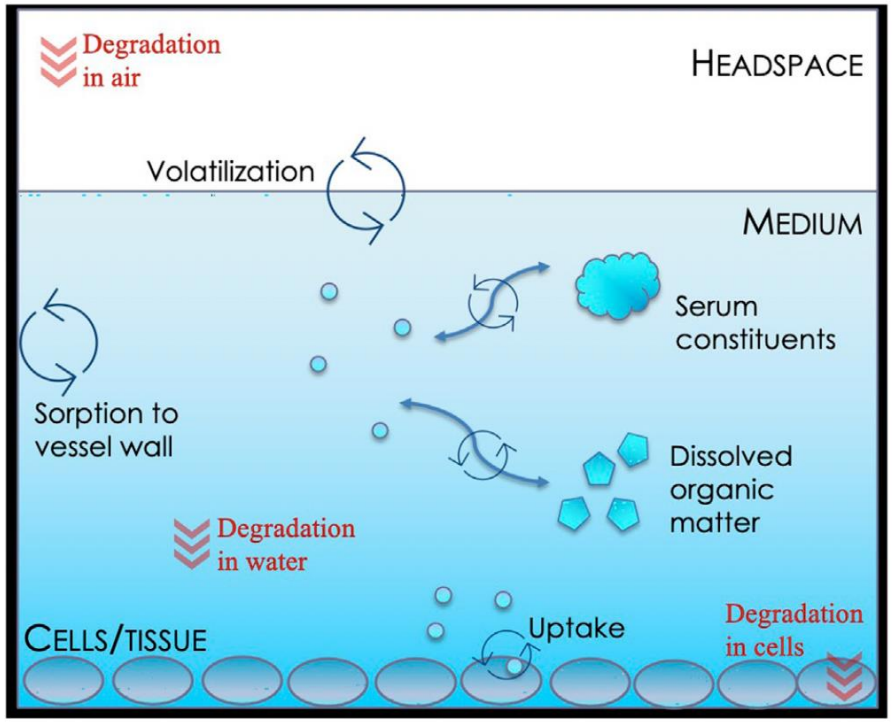
In vitro kinetics needed for response-response modelling…

- Standard: readout @24h exposure *vs* @exposure at Tmax & using C_{cell}



Kasteel et al., manuscript submitted

Dynamic *In Vitro* Distribution Kinetics Modelling



frontiers | Frontiers in Toxicology

ORIGINAL RESEARCH
published: 22 August 2022
doi: 10.3389/ftox.2022.911128

Check for updates

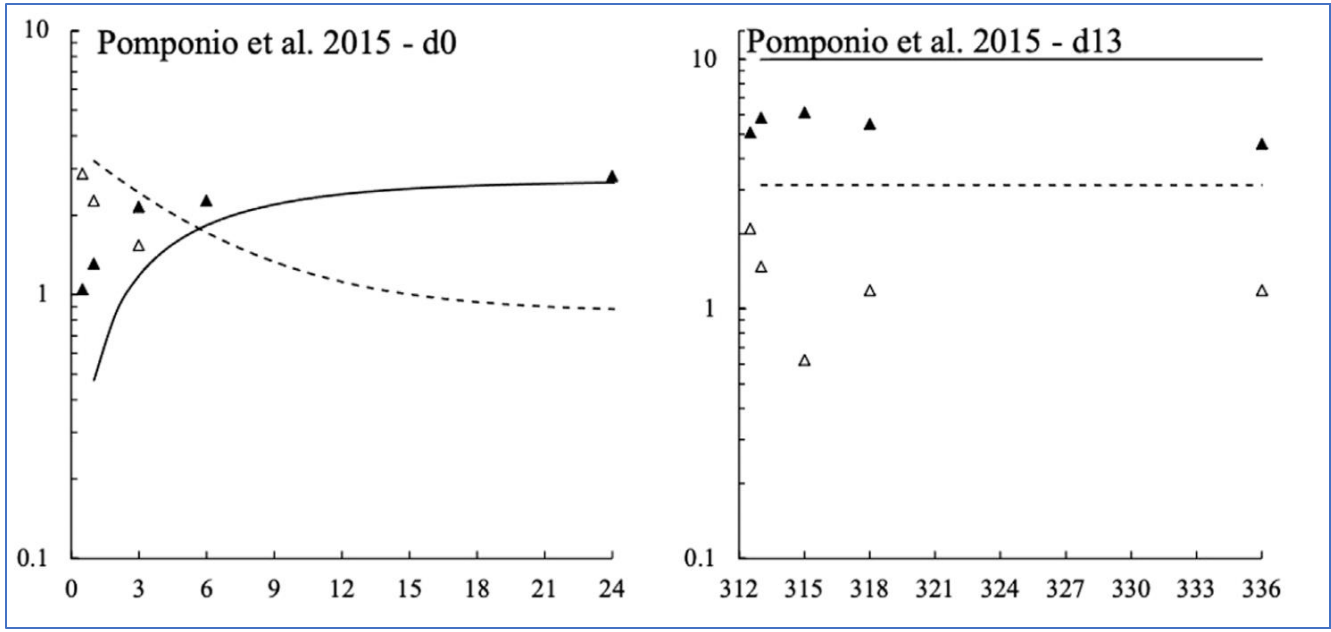
Dynamic Mass Balance Modeling for Chemical Distribution Over Time in *In Vitro* Systems With Repeated Dosing

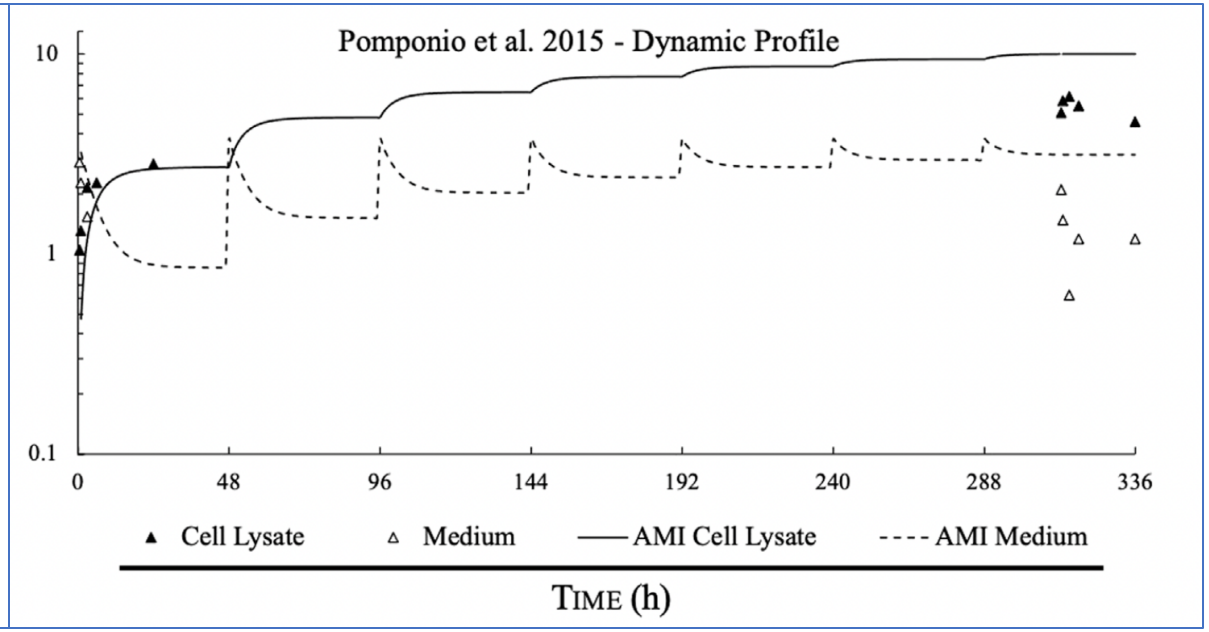
Sherri Bloch[1,2], *Jon A. Arnot*[3,4], *Nynke I. Kramer*[5], *James M. Armitage*[6] and *Marc-André Verner*[1,2]*

[1]Department of Occupational and Environmental Health, School of Public Health, Université de Montréal, Montreal, QC, Canada, [2]Centre de Recherche en Santé Publique, Université de Montréal et CIUSSS du Centre-Sud-de-l'Île-de-Montréal, Montreal, QC, Canada, [3]Department of Physical and Environmental Sciences, University of Toronto Scarborough, Scarborough, ON, Canada, [4]ARC Arnot Consulting and Research, Inc., Toronto, ON, Canada, [5]Division of Toxicology, Wageningen University, Wageningen, Netherlands, [6]AES Armitage Environmental Sciences, Inc., Ottawa, ON, Canada

Dynamic *In Vitro* Distribution Kinetics Modelling

Amiodarone (nmol/well)

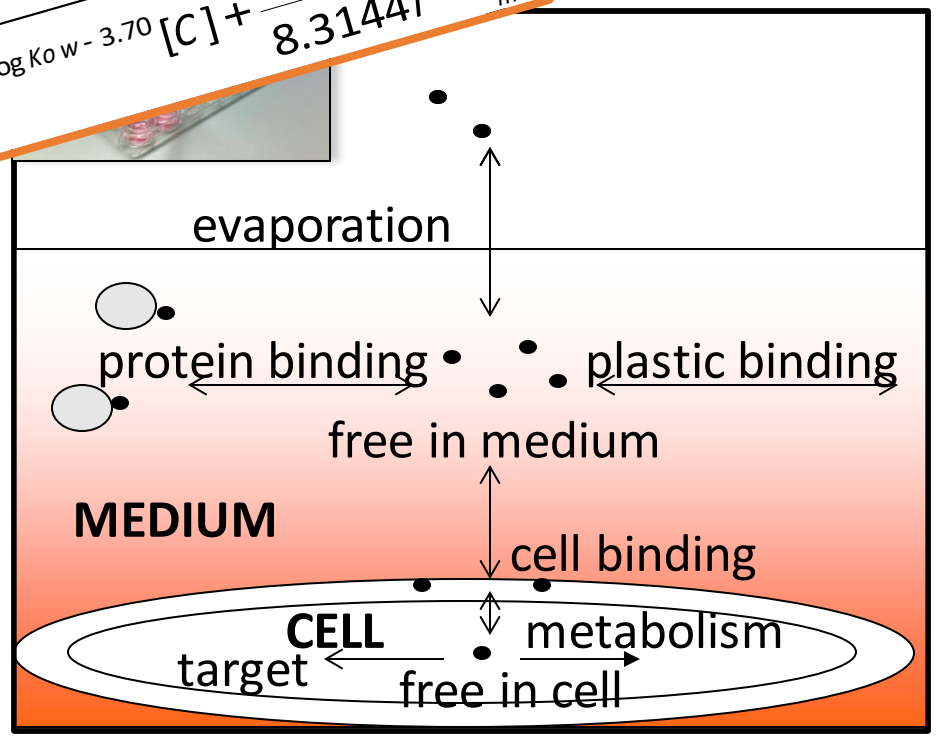




Bloch et al. (2022) Frontiers in Toxicology, in press

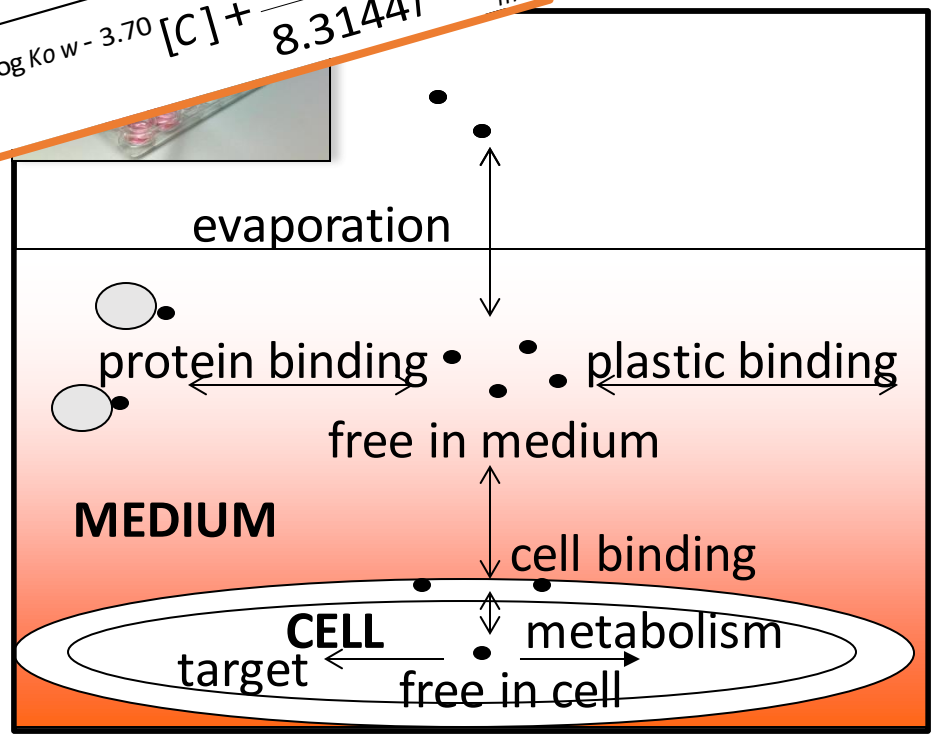
Modelling Evaporation

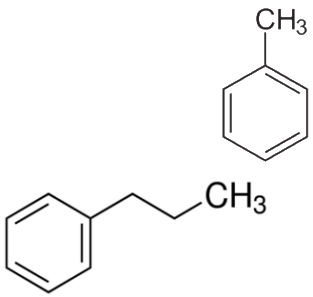
$$F = \frac{1}{1 + 10^{0.37 \log K_{ow} - 0.29}[S] + 10^{0.97 \log K_{ow} - 6.94}[P] + 10^{1.25 \log K_{ow} - 3.70}[C] + \frac{H}{8.3144T} \times \frac{V_a}{V_m}}$$



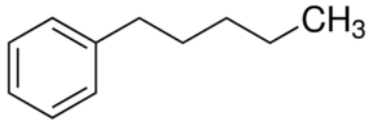
Modelling Evaporation of Alkylbenzenes

$$F = \frac{1}{1 + 10^{0.37 \log K_{o/w} - 0.29}[S] + 10^{0.97 \log K_{o/w} - 6.94}[P] + 10^{1.25 \log K_{o/w} - 3.70}[C] + \frac{H}{8.3144T} \times \frac{V_a}{V_m}}$$





Alkylbenzene	Modelled free
Toluene	43%
Propylbenzene	29%
Pentylbenzene	15%

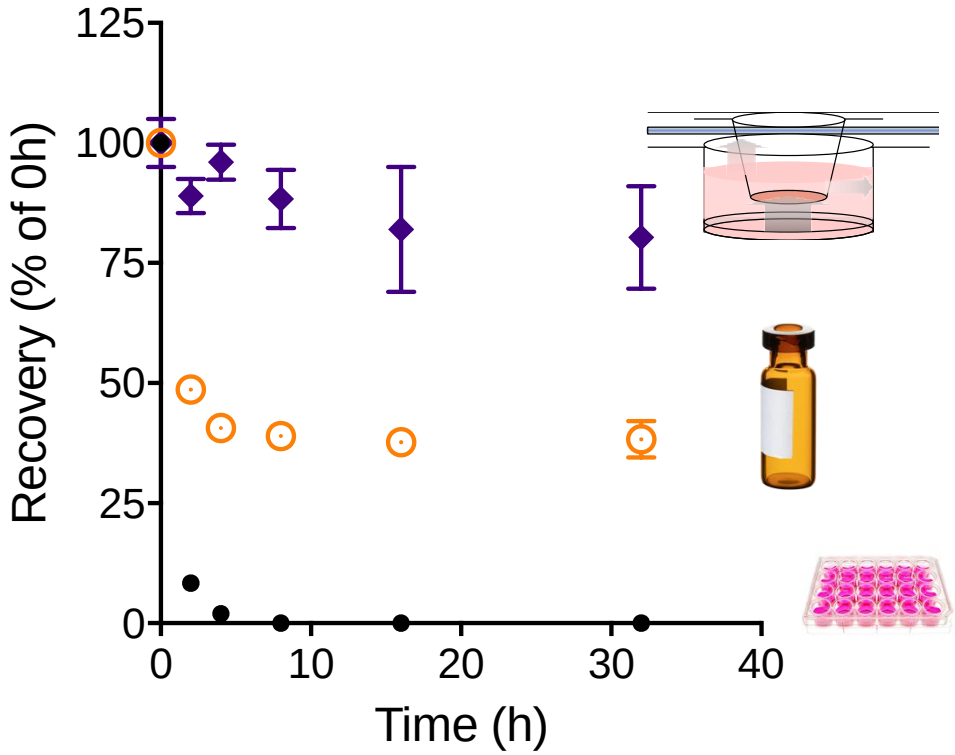


Kramer et al., manuscript in preparation

Alternative Dosing Methods

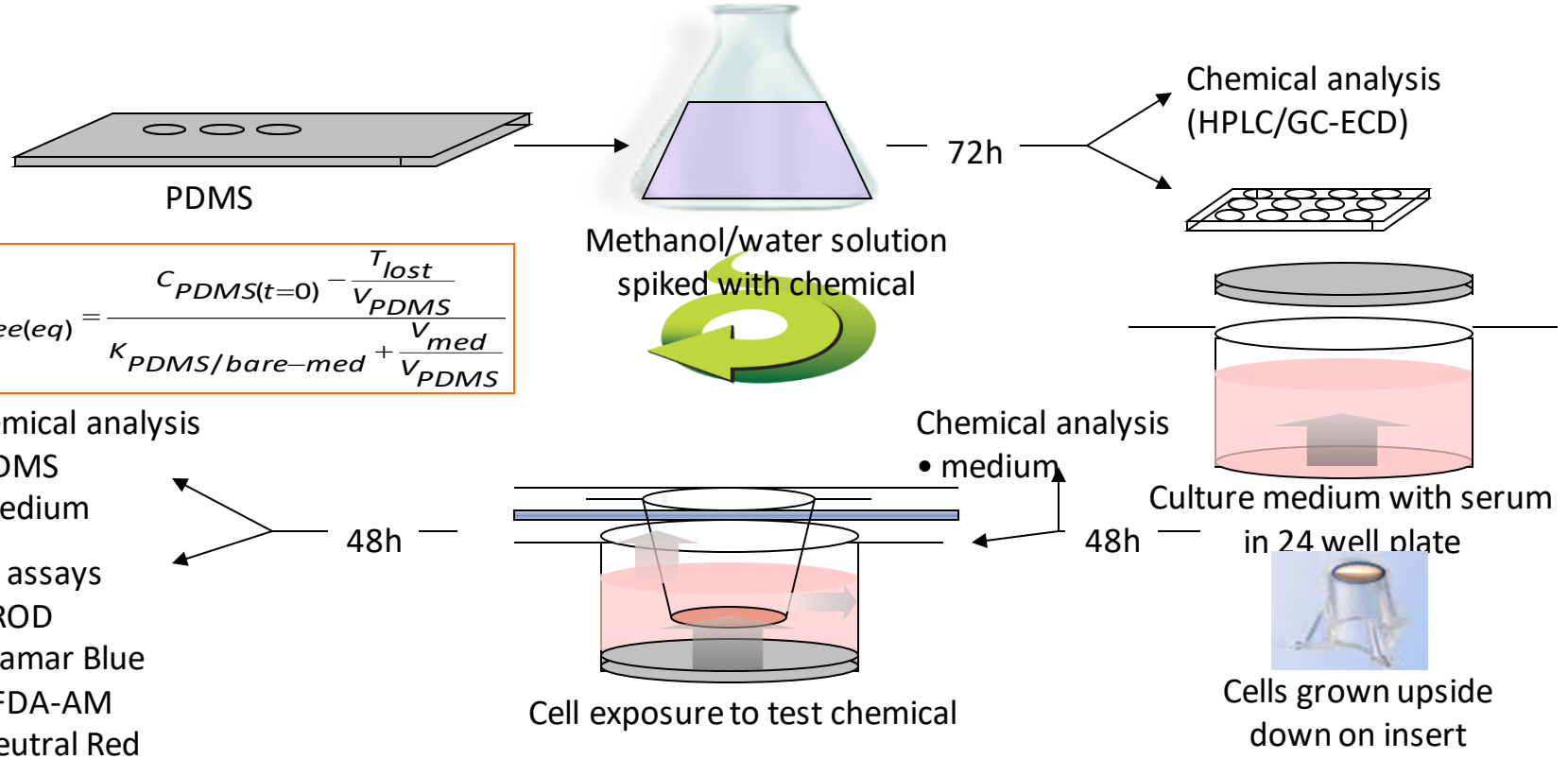
$$F = \frac{1}{1 + 10^{0.37 \log K_{o\,w} - 0.29}[S] + 10^{0.97 \log K_{o\,w} - 6.94}[P] + 10^{1.25 \log K_{o\,w} - 3.70}[C] + \frac{H}{8.3144T} \times \frac{V_a}{V_m}}$$

Alkylbenzene	Modelled free	Measured free	Measured free
Toluene	43%	0%	40±6%
Propylbenzene	29%	0%	27±3%
Pentylbenzene	15%	0%	17±1%



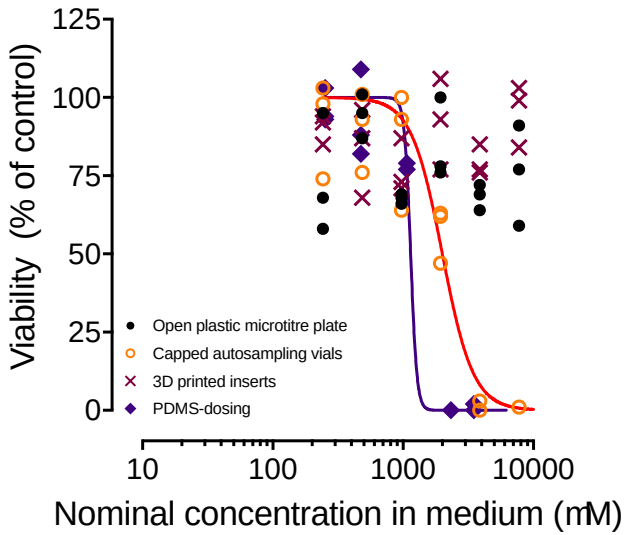
Kramer et al., manuscript in preparation

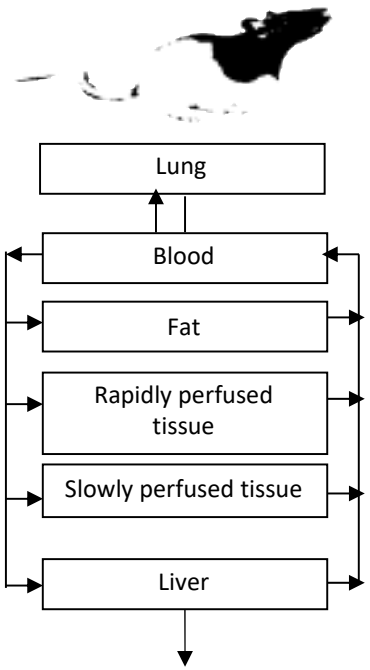
Partition Controlled Dosing

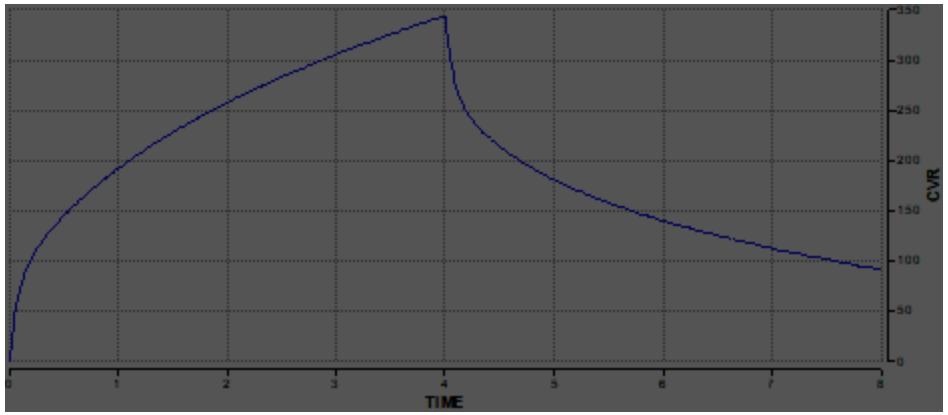


Kramer et al. (2010) Chem. Res. Toxicol. 23, 1806.

Alternative Dosing Methods and QIVIVE







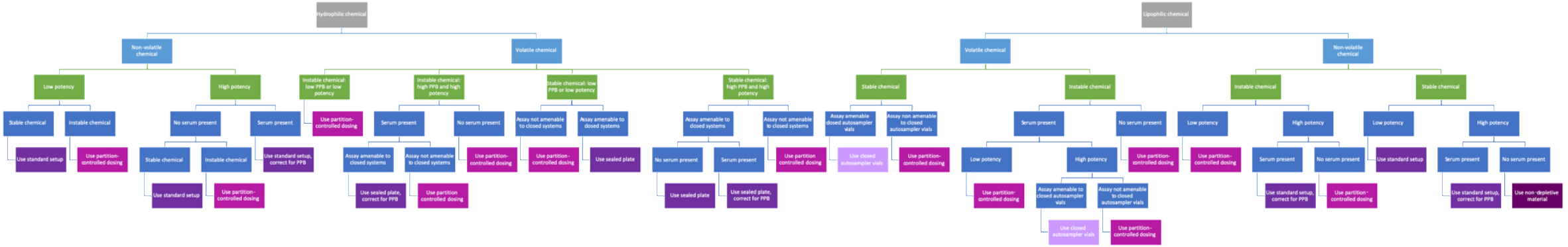
Est. 1230 ppm vs measured 1600 ppm
LD50 1h inhalation in open chamber

EC50 vials free (est.): 760 µM
$LC50_{acute}$ fish: 400 µM

Kramer et al., manuscript in preparation

Decision Tree

So when should you worry about nominal concentrations *in vitro*?



Kramer et al., manuscript in preparation

When is Exposure ‘Out of Control’?

Conclusion

Choose your *in vitro* dose carefully!