Evaluating Several *in vitro* Disposition Models for use in High-throughput Toxicokinetic Research

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Introduction

- *In vitro* high-throughput screening assays are increasingly adopted as part of a tiered testing strategy for chemical hazard evaluation.
- It is important to understand chemical behavior within *in vitro* assay systems to accurately predict the bioavailable chemical concentration at a calculated nominal *in vitro* potency.
- Several *in vitro* distribution models have been developed to predict chemical partitioning using physiochemical properties along with assay-specific parameters (Figure 1).
- Final model outputs are then applied to the nominal point-of-departure (POD) to calculate a free chemical concentration, which will then be used for *in vitro-to-in vivo* extrapolation (IVIVE).

Modeled Materials

- Models were run with parameters from the Rainbow trout gill cell line (RTgill-W1) in the OECD test guideline 249 (OECD TG249) assay miniaturized to 384-well format.
- These exposures were conducted using 231 environmentally relevant chemicals (log K_{OW} Range -2.63 to 7.61, RTgill-W1 cell line, 384-well format) as part of a separate project.
- A subset of 12 chemicals will be empirically measured to compare with and validate model outputs. These chemicals span a range of physicochemical properties (log K_{OW} -1.31 – 5.76) and include 17 beta-estradiol, pyrene, malathion, imidacloprid, 4-nonylphenol, ethanolamine, bisphenol A, Fluoxetine hydrochloride, diethyl phthalate, benzaldehyde, methyoxychlor, and triazophos (Figure 2, black outlined points).

Model Predictions

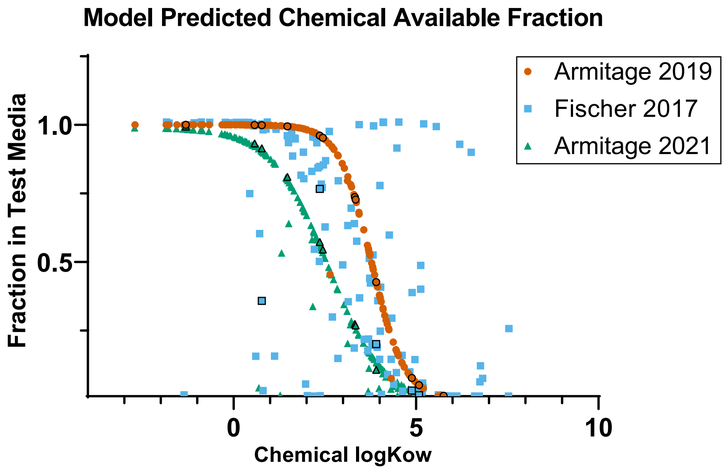

Figure 2 – Fraction of chemical in test media outputs from the 3 models run (Armitage 2019, Fischer 2017, and Armitage 2021). 12 test chemicals are represented with black outlines.

Discussion

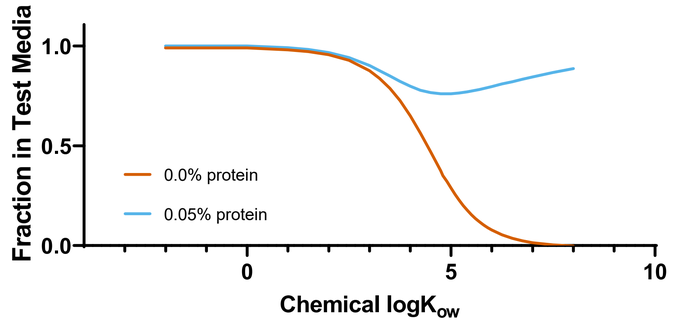

Figure 3 – Chemical fraction in Media vs. chemical log K_{OW} in two media of differing protein content using the model by Fischer et al. 2018

- Chemical sorption to the walls of multiwell plates was found to be potentially very high in experiments where the media lack protein in chemical with higher K_{OW}(Figure 3).
- The models only work for chemicals with known physiochemical traits. This data can be hard to come by, difficult to consolidate, and much of it modeled itself.
- Accurate parameters will often need to be identified and calculated/input manually into the models for optimal accuracy. Well plate measurements vary slightly from brand to brand.
- The Armitage vs. Fischer models predictions commonly showed discrepancies (Figure 2). This is likely due to different input parameters (i.e., K_{OW} vs. PP-LFERs)used in calculating distribution.
- The three models mostly agreed on low log K_{OW} chemical fraction in the media. As log K_{OW} increases model agreement decreases, potentially due to additional factors quantified by PP-LFERs (i.e. dipolarity/polarizability, H-bond donor properties, molar volume, etc.).

Future Work

- Calculate outputs from additional models (e.g., Fisher 2019) to compare with our current model outputs and future experimental data.
- Gather measurement from well plate studies for a subset of 12 chemicals (K_{OW} = -1.31 – 5.76) and qualify model outputs with those experimental measurements. These exposures will be run using RTgill-W1 and the OECD TG249 assay miniaturized to the 384-well format over 24 hours.
- Determine if certain chemical groups' (from our 231 test chemicals, e.g. organophosphates, chemicals with a log K_{OW} > 1, pharmaceuticals, etc.) distributions are better predicted by any one model using results of modeling and empirical measurements.
- Final outputs of the selected model will be used to adjust *in vitro* PODs and compare IVIVE estimates with known *in vivo* PODs.

Models

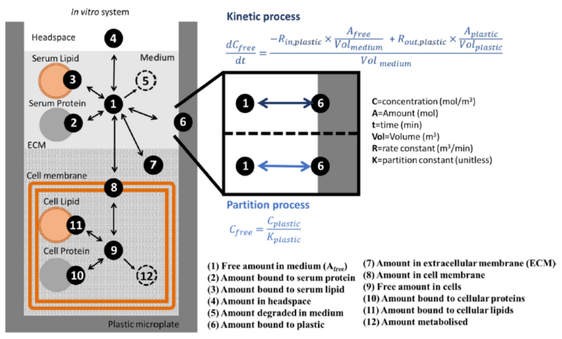

Figure 1 – Basic diagram of in vitro disposition and modeling

Proença, S., Escher, B. I., Fischer, F. C., Fisher, C., Grégoire, S., Hewitt, N. J., ... & Kramer, N. I. (2021). Effective exposure of chemicals in *in vitro* cell systems: A review of chemical distribution models. *Toxicology in Vitro*, *73*, 105133.

Primary Information Needed for Models

- Well plate parameters
  - — Volume, well bottom area, fill, time, material
  - > 145 µL, 8.35 mm², 120 µL, 24 h, polypropylene
- Cell/biological/serum parameters
  - — Protein, lipid, and DOM content, pH, Ionic strength, temp, cell number
  - > 0.05, 0.05, 0, 7.4, 0.104 M, 19°C, 22500
- Chemical Traits
  - — Log K_{OW}, Log K_{AW}, water solubility, MW
  - > Generated using **CompTox dashboard**
  - — Polyparameter linear free energy relationships(PP-LFERs)
  - > Generated using **UFZ-LSER database**

Displayed Models

Armitage 2019 (Figure 2)-update of the 2014 Armitage model-

- K_{OW} Focused

Honda GS, Pearce RG, Pham LL, Setzer RW, Wetmore BA, Sipes NS, et al. (2019) Using the concordance of *in vitro* and *in vivo* data to evaluate extrapolation assumptions. PLoS ONE 14(5): e0217564.

Fischer 2017 (Figure 2)

- Polyparameter linear free energy relationships (PP-LFERs) focused

Fischer, F. C., Henneberger, L., Schlichting, R., & Escher, B. I. (2019). How to improve the dosing of chemicals in high-throughput *in vitro* mammalian cell assays. Chemical research in toxicology, 32(8), 1462-1468.

Armitage 2021 (Figure 2)

- K_{OW} Focused

Armitage JM, Sangion A, Parmar R, Looky AB, Arnot JA. Update and Evaluation of a High-Throughput *In Vitro* Mass Balance Distribution Model: IV-MBM EQP v2.0. Toxics. 2021; 9(11):315.

Fischer 2018 (Figure 3)

- Media Protein vs. K_{OW}

Fischer, F. C., Cirpka, O. A., Goss, K. U., Henneberger, L., & Escher, B. I. (2018). Application of experimental polystyrene partition constants and diffusion coefficients to predict the sorption of neutral organic chemicals to multiwell plates in *in vivo* and *in vitro* bioassays. Environmental science & technology, 52(22), 13511-13522

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