Hepatobiliary Disposition of 15 Non-Therapeutic Chemicals in Sandwich-Culture Rat Hepatocytes using B-CLEAR® Technology

J. F. Wambaugh[1], J. P. Jackson[2], K. R. Brouwer[2], A. Herman[2], G. S. Honda[1,3], R. G. Pearce[1,3], and R. S. Thomas[1]

[1]US EPA, Research Triangle Park, NC; [2]Qualyst Transporter Solutions, a BioIVT company, Durham, NC; and [3]Oak Ridge Institute for Science and Education, Oak Ridge, TN

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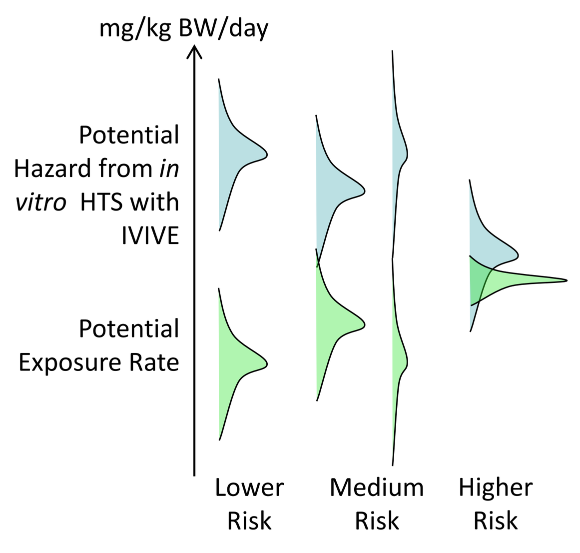
John Wambaugh I orcid.org/0000-0002-4024-534X I wambaugh.john@epa.gov I 919-541-7641

Background: Two Problems with *In Vitro-In Vivo* Extrapolation

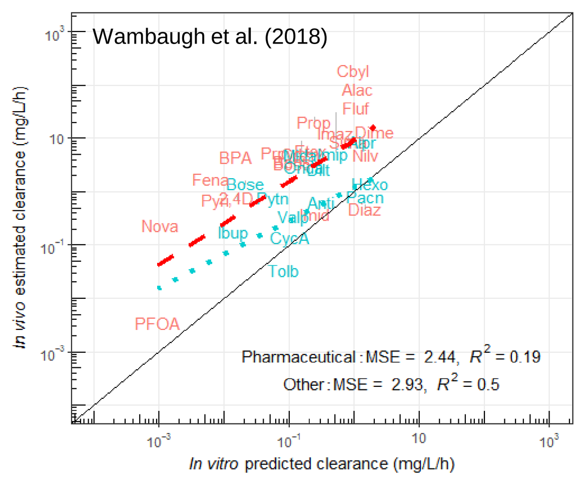
High throughput in vitro screening provides surrogate toxicity data for thousands of chemicals occurring in commerce and the environment without traditional toxicity testing data

In vitro-in vivo extrapolation (IVIVE) via high throughput toxicokinetics (HTTK) allows screening data to be placed in a risk prioritization context



Risk-based prioritization

In vitro TK tools underestimate toxicokinetic clearance (L/h/kg BW) when comparing with *in vivo* data



HTTK currently calculates clearance based upon elimination (disappearance) observed in hepatocyte suspension over 4 hrs, and estimated passive glomerular filtration

Possible Reasons:

- Hepatocytes in suspension
  - Drug metabolism activity/cell viability rapidly lost
  - Less accurate with low turnover
- Incubation time may miss slow clearance
- Extra-hepatic metabolism
- Active transport in kidney
- **Biliary excretion**

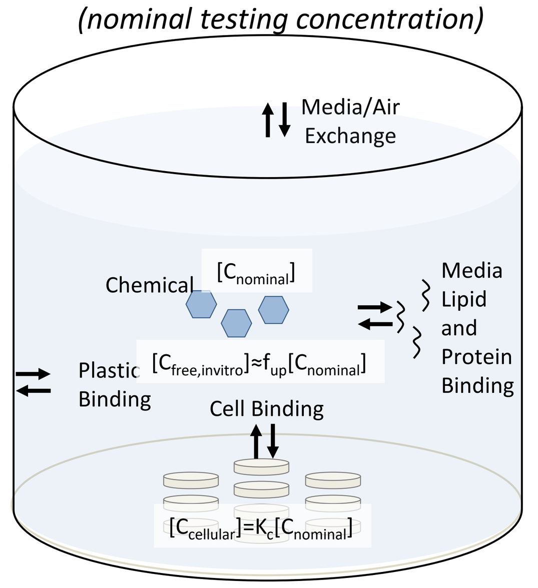
We typically do not know how a chemical partitions *in vitro*

We expect that the free and cellular concentrations of chemical *in vitro* will differ from the nominal (tested) concentration due to (at least) binding to plastic, lipids proteins, and gas exchange

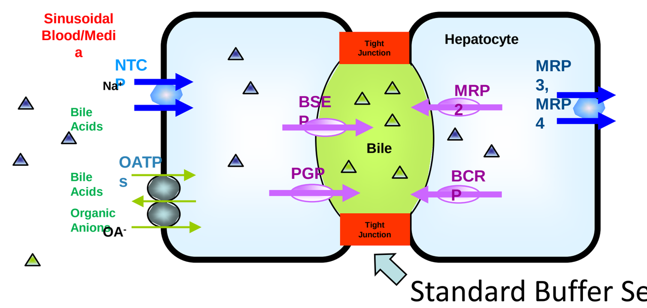
Mathematical chemical partitioning models exist that predict *in vitro* distribution such as the Armitage et al. (2014) for neutral compounds, which was extended by Fischer et al. (2017) for ionized compounds

However, there is limited evaluation data for cellular partitioning for any chemical (six chemicals reviewed by Kramer et al. (2015)

in vitro
(nominal testing concentration)

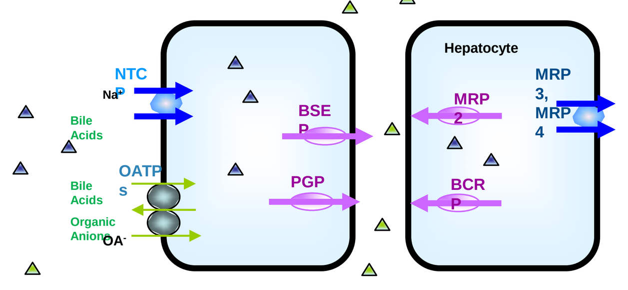


Methods



Standard Buffer Sequesters Bile

Ca2+-free Buffer Allows Bile to Mix with Media



- B-CLEAR® Technology utilizes tight junction modulation in sandwich-cultured hepatocytes (SCH) to quantify biliary efflux of test article (Figure, left). The presence of calcium [Plus (+) Buffer] maintains the integrity of tight junctions and formation of the bile pockets. Biliary clearance of a compound requires uptake into the hepatocytes and excretion into the bile pockets. In the absence of calcium [Minus (-) Buffer], the tight junctions open and the contents of the bile pockets are released. The mass of the test article excreted into bile (e.g. bile accumulation) is the difference between the two conditions. Quantitation of test articles in cell lysates and dosing solutions was determined using LC-MS/MS equipped with an ESI interface.
- Biliary efflux was assessed for 15 compounds where HTTK has previously underestimated clearance
- All compounds assessed at two concentrations (10 and 30 µM) and two time points (10 and 30 minutes) in rat SCH

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Results

15 Test Chemicals

Metabolism rate and fraction unbound in plasma (f_{up}) measured *in vitro* by Wetmore *et al.* (2012, 2015)

Wambaugh et al. (2015) TK triage predictor estimated error of in vivo clearance relative to HTTK estimated clearance

Chemicals were selected such that they are likely to be underestimated by standard HTTK

Compound	logP	MW	Charge at pH 7.4	Metabolism	F_{up}	Predicted Cellular Concentration vs. Nominal	Predicted Clearance Error
Diclosulam	3.5	406.2	Neutral	Moderate	Low	>3.2x	>10x Underestimated
Diniconazole	4.3	326.2	Zwitterionic	Moderate	Low	>3.2x	>10x Underestimated
Ethametsulfuron methyl	1.6	410.4	Zwitterionic	Moderate	Low	NA	>10x Underestimated
Flumetsulam	1.5	325.3	Neutral	Moderate	Low	NA	>10x Underestimated
Fulvestrant	9.4	606.8	Neutral	Fast	Low	>100x	NA
Iodosulfuron-methyl-sodium	3.2	529.2	Anionic	Moderate	Low	>3.2x	>10x Underestimated
Mesotrione	1.5	339.3	Neutral	Slow	Moderate	NA	>10x Underestimated
Monobutyl phthalate	2.8	222.2	Anionic	Slow	Moderate	>3.2x	On the Order
Oxytetracycline dihydrate	-4.0	496.5	Neutral	None	Moderate	<100x	>10x Underestimated
Penoxsulam	3.0	483.4	Anionic	None	Low	>3.2x	>10x Underestimated
Perfluorooctanoic acid	5.1	414.1	Anionic	Moderate	Low	NA	Does Not Reach Steady State
Pyrithiobac-sodium	0.6	348.7	Anionic	None	Low	<3.2x	>10x Underestimated
Quinclorac	3.0	242.1	Neutral	None	Low	>3.2x	>10x Underestimated
Thidiazuron	1.9	220.3	Neutral	Moderate	Low	NA	>10x Underestimated
Triflumizole	1.4	345.7	Anionic	Moderate	Low	NA	>10x Underestimated

Biliary Clearance: Only four compounds (any time, any concentration) demonstrated bile accumulation

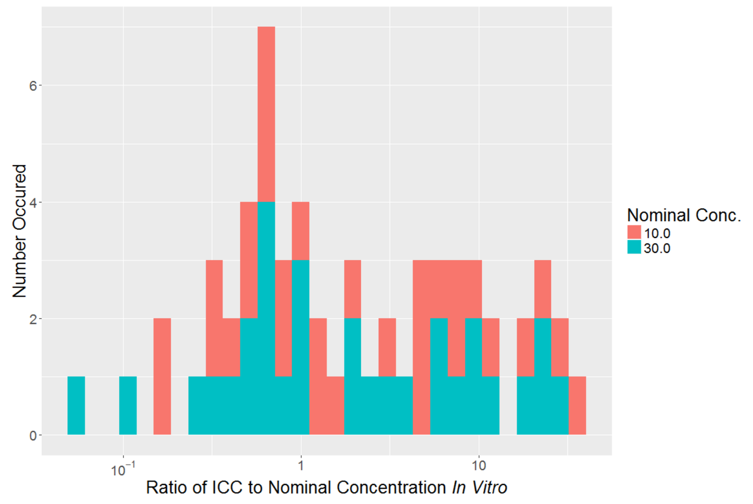
Compound	logP	MW	Charge at pH 7.4	Metabolism	F_{up}	Time (min)	Conc. (µM)	$Cl_{biliary}$ (mL/min/kg)
Flumetsulam	1.5	325.3	Neutral	Moderate	Low	10	30	0.273 (0.18)
Iodosulfuron-methyl-sodium	3.2	529.2	Anionic	Moderate	Low	30	30	0.230 (0.022)
Mesotrione	1.5	339.3	Neutral	Slow	Moderate	30	30	0.119 (0.063)
Oxytetracycline dihydrate	-4.0	496.5	Neutral	None	Moderate	30	30	0.252 (0.087)

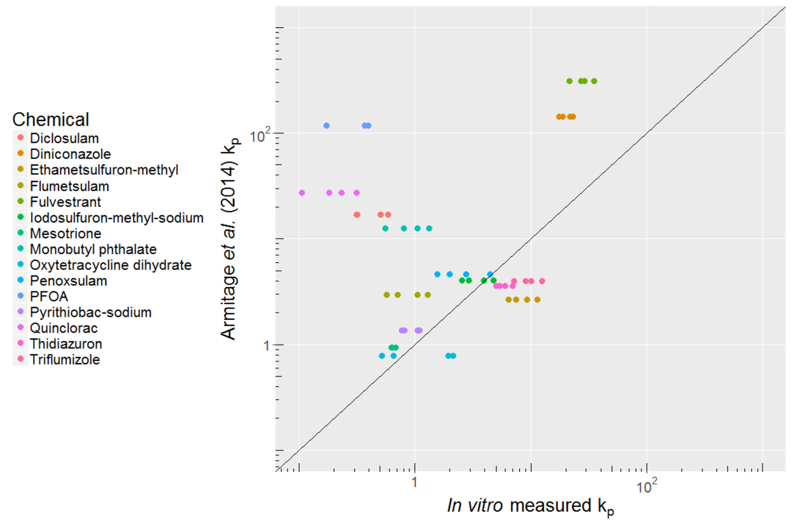
- These chemicals span a range of hydrophobicity (logP) and have no obvious distinctions from other chemicals

in vitro Disposition: Intracellular Concentration (ICC) Varied By Chemical

We compared the ratio (k_p) of the measured ICC to nominal concentration (either 10 or 30 µM)

Median ICC was 1.4x higher than nominal, low of 0.05x, max of 35x, with 95% of values within 0.13x to 28x)





ICC is predicted by the Armitage et al. (2014) model

Model tends to overestimate accumulation

Conclusion and Future Direction

- Biliary efflux (BEI) results of < 20% for all compounds evaluated suggested biliary excretion of all compounds studied was low or slow. Although no biliary efflux is sometimes observed in rat SCH, a compound with high accumulation potential may still be extensively excreted into the bile a result of a slow excretion process.
  - Biliary clearance does not seem to explain underestimation of clearance by HTTK in general, pointing to a potential role for extra-hepatic metabolism.
- The results indicate the importance of accounting for hepatic accumulation
  - Ratio of ICC to nominal concentration for four compounds (Diniconazole, Ethametsulfuron-methyl, Fulvestrant, Triflumizole) was greater than ten times
  - Ratio of ICC to nominal concentration less than ten times for only PFOA, and only at 30 minutes and 30 µM (0.05x nominal)
- Accumulation of three chemicals (Diclosulam, Quinclorac, and Monobutyl phthalate) was significantly over-predicted by the Armitage et al. (2014) partitioning model
  - SCH (rat) data suggested these compounds have low accumulation potential resulting from either low hepatic uptake potential or a possible role for efflux transporters (basolateral/canalicular) reducing accumulation potential
- Difference between cellular concentration and nominal concentrations exist, but there was no pronounced bias (median cellular concentration was 1.4x higher than nominal).

Recommendations for future testing of non-therapeutic chemicals in B-CLEAR® :

- **Longer incubation time may allow for greater accumulation of compounds with slow, but non-zero biliary clearance**
- **The maximum tested concentration was 30 µM – testing at higher chemical concentrations should make compound in bile easier to detect, but higher concentrations may cause cytotoxicity**
- **Current techniques rely on liquid chromatography mass spectrometry, could eventually expand chemical space using gas chromatography**

References

Armitage, James M., et al. "Application of mass balance models and the chemical activity concept to facilitate the use of in vitro toxicity data for risk assessment." Environmental science & technology 48.16 (2014): 9770-9779.
Fischer, Fabian C., et al. "Modeling exposure in the Tox21 in vitro bioassays." Chemical research in toxicology 30.5 (2017): 1197-1208.
Kramer, Nynke I., et al. "Biokinetics in repeated-dosing in vitro drug toxicity studies." Toxicology in Vitro 30.1 (2015): 217-224.
Wambaugh, John F., et al. "Toxicokinetic triage for environmental chemicals." Toxicological Sciences 147.1 (2015): 55-67.
Wambaugh, John F., et al. "Evaluating In Vitro-In Vivo Extrapolation" accepted at Toxicological Sciences,.
Wetmore, Barbara A., et al. "Integration of dosimetry, exposure and high-throughput screening data in chemical toxicity assessment." Toxicological Sciences (2012): kfr254.
Wetmore, Barbara A., et al. "Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted In Vitro Bioactivity to Inform Chemical Toxicity Testing." Toxicological Sciences 148.1 (2015): 121-136.