Human Exposure Estimates and Oral Equivalents of *In Vitro* Bioactivity for Prioritizing, Monitoring, and Testing of Environmental Chemicals

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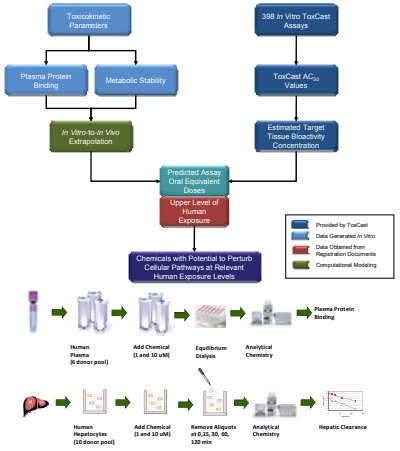
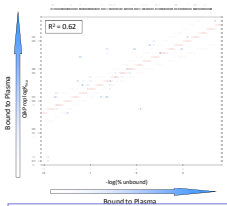
Abstract

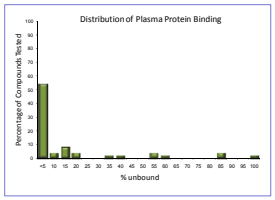
High-throughput, lower-cost, *in vitro* toxicity testing is currently being evaluated for use in prioritization and eventually for predicting *in vivo* toxicity. Interpreting *in vitro* data in the context of *in vivo* human relevance remains a formidable challenge. A key question in using *in vitro* data to predict *in vivo* toxicity is whether dosimetry is sufficient to establish dose-response relationships. In this study, hepatocyte clearance rates and plasma protein binding were experimentally measured for 39 ToxCast Phase I chemicals. The experimental data was modeled using the population-based kinetic simulation software Simcyp® to estimate human oral equivalent doses required to achieve steady-state plasma concentrations at the *in vitro* AC50 (50% activity concentration) for 39 chemicals in 467 ToxCast Phase I assays. Results were compared to published PBPK models to assess model performance. Human oral equivalents of ToxCast *in vitro* results were compared to EPA chronic aggregate human oral exposure estimates, chronic population adjusted doses (cPAD) for humans, no and lowest observed adverse effect levels (NOAEL and LOAEL) from animal toxicity studies. EPA estimates of human exposure and cPAD were available for 24 of the 39 chemicals. NOAEL and LOAEL were available for 26 of 39 chemicals. These data were used to develop prioritization methods applicable to environmental chemicals and based on doses associated with *in vitro* bioactivity, estimates of human exposure, doses considered acceptable for human populations, and doses in animal studies that do and do not cause adverse effects. *In vivo* bioactivity based on predicted oral equivalents and estimated human exposures could be interpreted as a higher priority for further testing and monitoring. *Approved for publication but does not necessarily reflect Agency policy.*

Methods



Results





- For four chemicals (etoxazole, MGK, parathion, and triclosan) there was no unbound chemical detected at either the 1 µM or 10 µM concentration
- Of the 12 chemicals that showed metabolic saturation or no metabolism at the 10 µM concentration, only four (acetamiprid, bromacil, emamectin, and bentazone) also showed saturation or no metabolism at the 1 µM concentration.

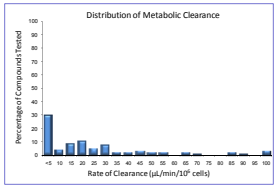
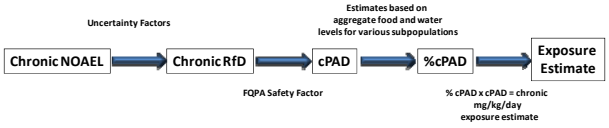
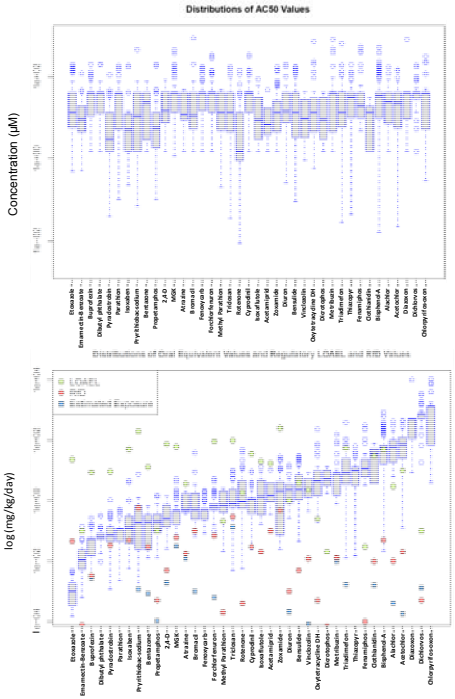


Table 1. Comparison of *in vitro*-to-*in vivo* extrapolation modeling results with published PK or PBPK models

Chemical	PK- or PBPK-Derived C_{ss}[a] (µM)	*In Vitro*-to-*In Vivo* Extrapolation C_{ss}[a] (µM)
2,4-Dichlorophenoxyacetic acid	9.05-90.05	40.77
Oxytetracycline-dihydrate	0.36	2.02[b]
Triclosan	2-10	3.68
Bisphenol-A	<0.13	0.42
Parathion	0.17	0.15

[a] C_{ss}, concentration at steady state
[b] Does not consider low oral bioavailability





Model Assumptions:

- GI absorption for all chemicals is 100%
- The varying of bodyweight, gender, and age does not alter specific enzyme expression
- Metabolizing enzyme variation is approximately 30%
- *In vitro* metabolic clearance and *in vitro* plasma protein binding assays accurately represent these dynamic processes *in vivo*.
- Kinetics at ToxCast AC50 values are linear and related to Css
- The concentration at 50% efficacy (AC50) is the point at which activity is biologically meaningful.

Important Considerations:

- Exposure estimates from Reregistration Eligibility Documents are based on aggregate exposure to maximum food residue and water levels for the highest exposed subpopulation
- Bioactivity in ToxCast assays do not represent adverse effects, but could represent pathways leading to toxicity
- Overlap in exposure and bioactivity could be used to prioritize further testing
- The oral equivalents displayed are based on the upper 95th percentile of steady-state concentrations, representing a "susceptible population"
- Species differences between rodents and humans explain many of the differences observed in predicted bioactivity and in vivo toxicity represented by LOAEL and RfD values. This emphasizes the need for a rodent dosimetry data, which is currently being collected as part of the ongoing EPA-Hamner Institutes collaboration

Table 2. *In vitro* assays with oral equivalent doses below that of the estimate human exposure

Chemical	Assay	Endpoint	AC_{50} (µM)	Oral Equivalent Dose (mg/kg/day)[a]	Human Exposure (mg/kg/day)[b]
Triclosan	CLZD_CYP2B6_24	CYP2B6 mRNA in Primary Human Hepatocytes (24 h) [1]	0.034	0.0048	0.13
Triclosan	ACEA_LOCdec	Cellular impedance measuring alterations in cell morphology and cell survival [2]	0.046	0.0065	0.13
Triclosan	NVS_TR_hNET	Competitive binding of the human norepinephrine transporter [3]	0.31	0.043	0.13
Pyrithiobac-sodium	CLZD_SLC01B1_48	SLCO1B1 mRNA in Primary Human Hepatocytes (48 h) [1]	0.067	0.0011	0.0012

[a] Oral equivalent dose for the lower 5th percentile of a cohort of 100 healthy individuals of both sexes from 20-50 years of age.

[b] Aggregate human exposure from food and drinking water sources for the most highly exposed group or subpopulation.

[1] Rotroff et al., Xenobiotic Metabolizing Enzyme and Transporter Gene Expression in Primary Cultures of Human Hepatocytes Modulated by ToxCast Chemicals. JTEH. 2010 *in press*

[2] Judson et al., In vitro screening of environmental chemicals for targeted testing prioritization - The ToxCast project. *Environ Health Perspect* 2009. **doi: 10.1289/ehp.0901392**.

[3] www.epa.gov/ncct/toxcast/

Conclusions

- Further efforts are being made to characterize dosimetry in rodents to improve understanding of dose-response and species extrapolation relative to adverse effect phenotypes
- Extrapolating from *in vitro* to *in vivo* effects using only AC_{50} values could underestimate or overestimate relationships and requires accounting for pharmacokinetic parameters.
- The integration of dosimetry and human exposure information with results from high throughput screening efforts is critical for informed decisions on chemical testing priorities

Building a scientific foundation for sound environmental decisions