Exposure and Dosimetry Considerations for Adverse Outcome Pathways

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Adverse Outcome Pathways: From Research to Regulation
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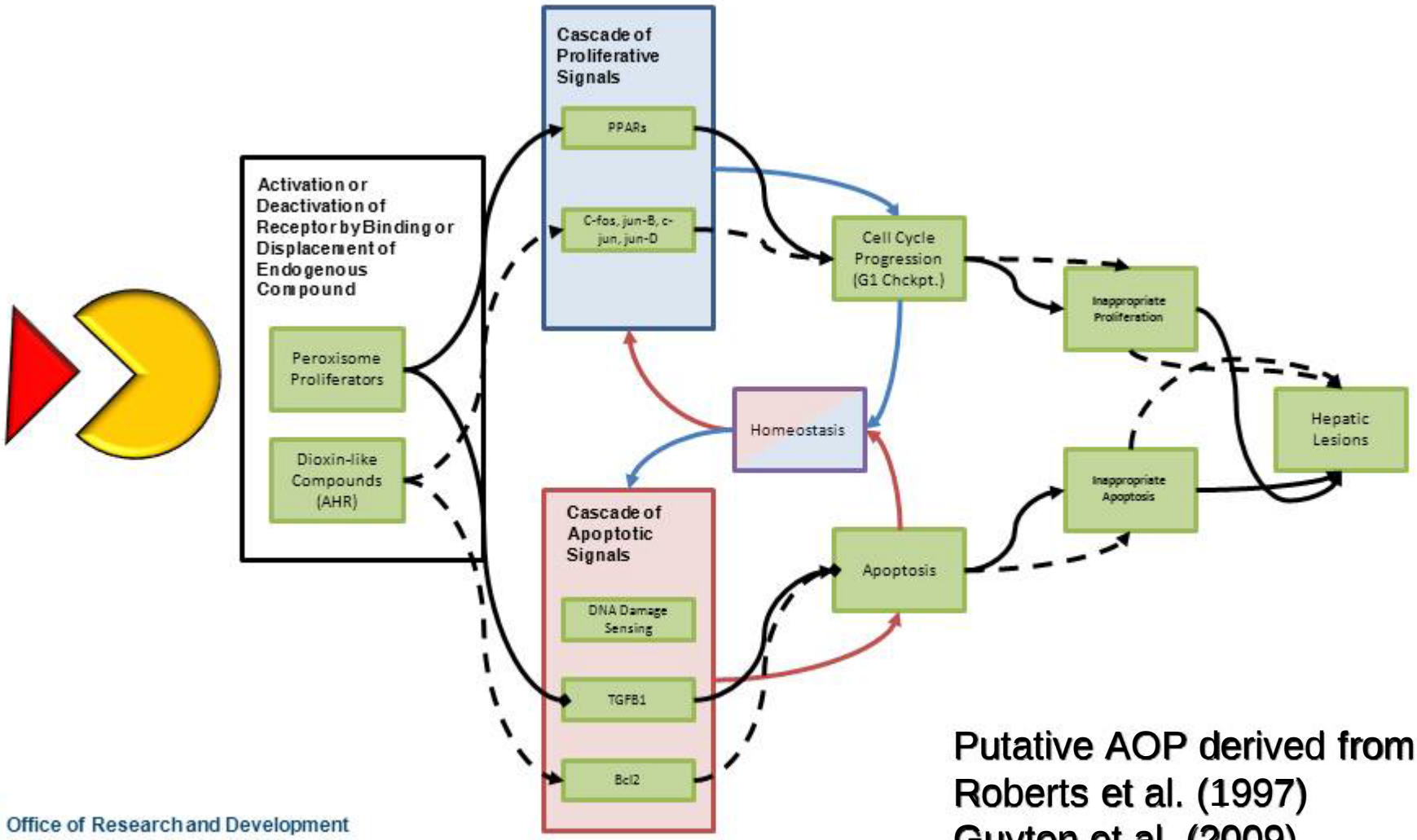
Introduction

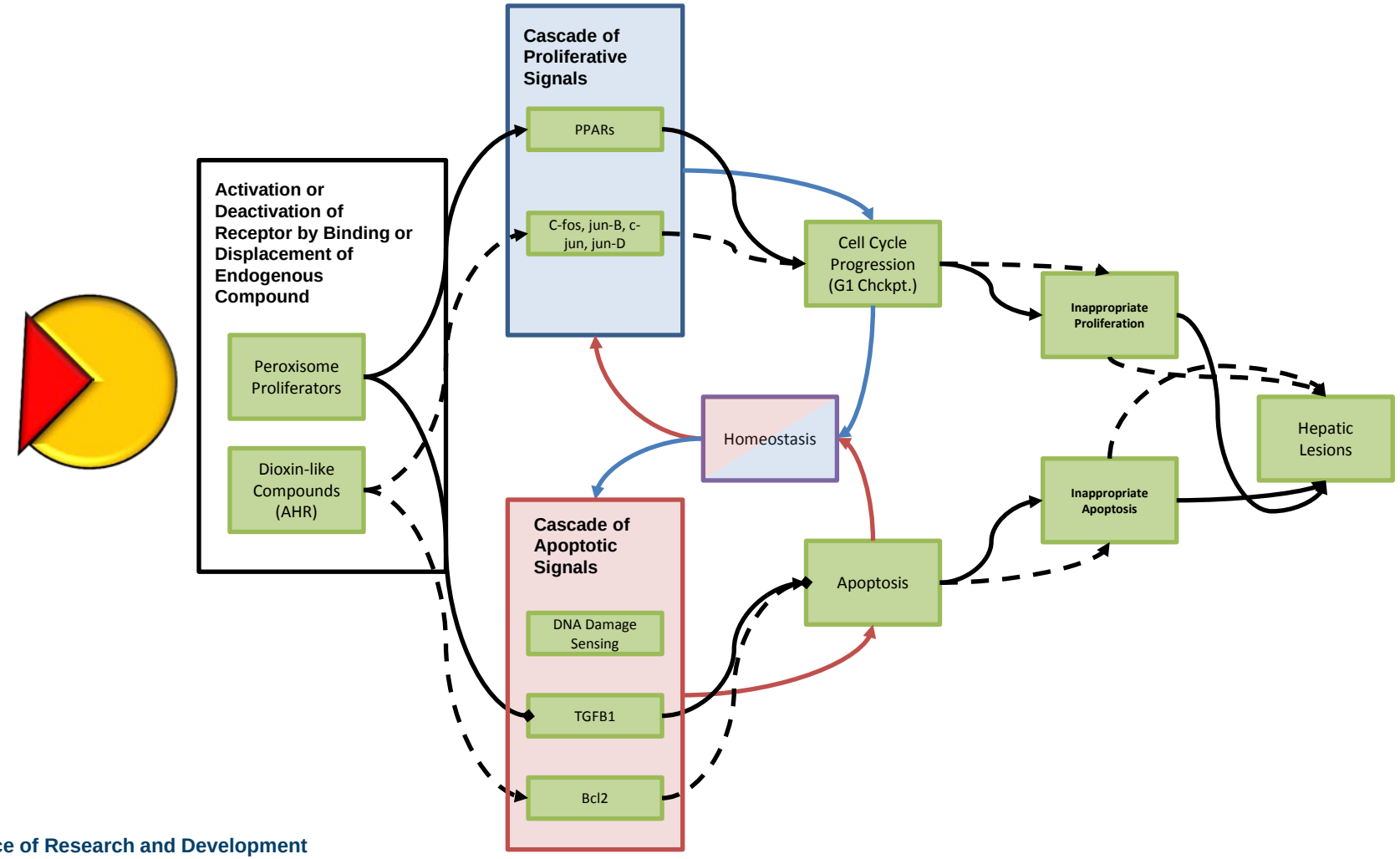
- Risk is a function of both of hazard and exposure
- Toxicokinetic (TK) models can determine whether chemical exposures produce potentially hazardous tissue concentrations
- Whether or not an AOP initial molecular event (MIE) occurs depends on both exposure and TK
- As high throughput screening (HTS) identifies putative MIEs and key events, chemical-specific TK and exposure data will be needed to make prioritizations based on risk

AOP Context

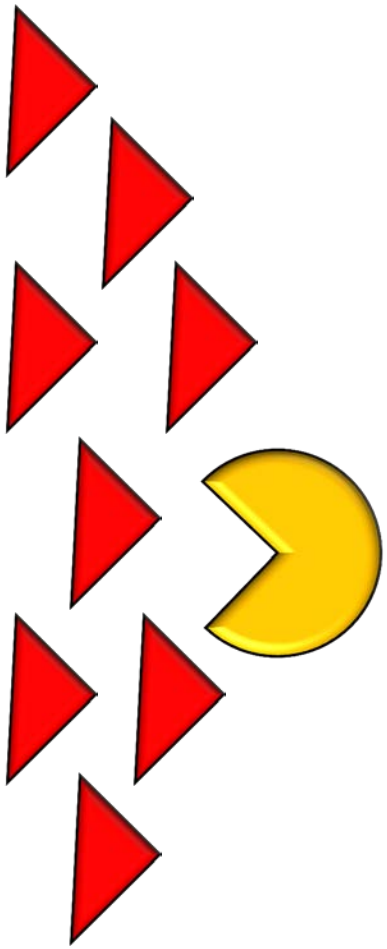


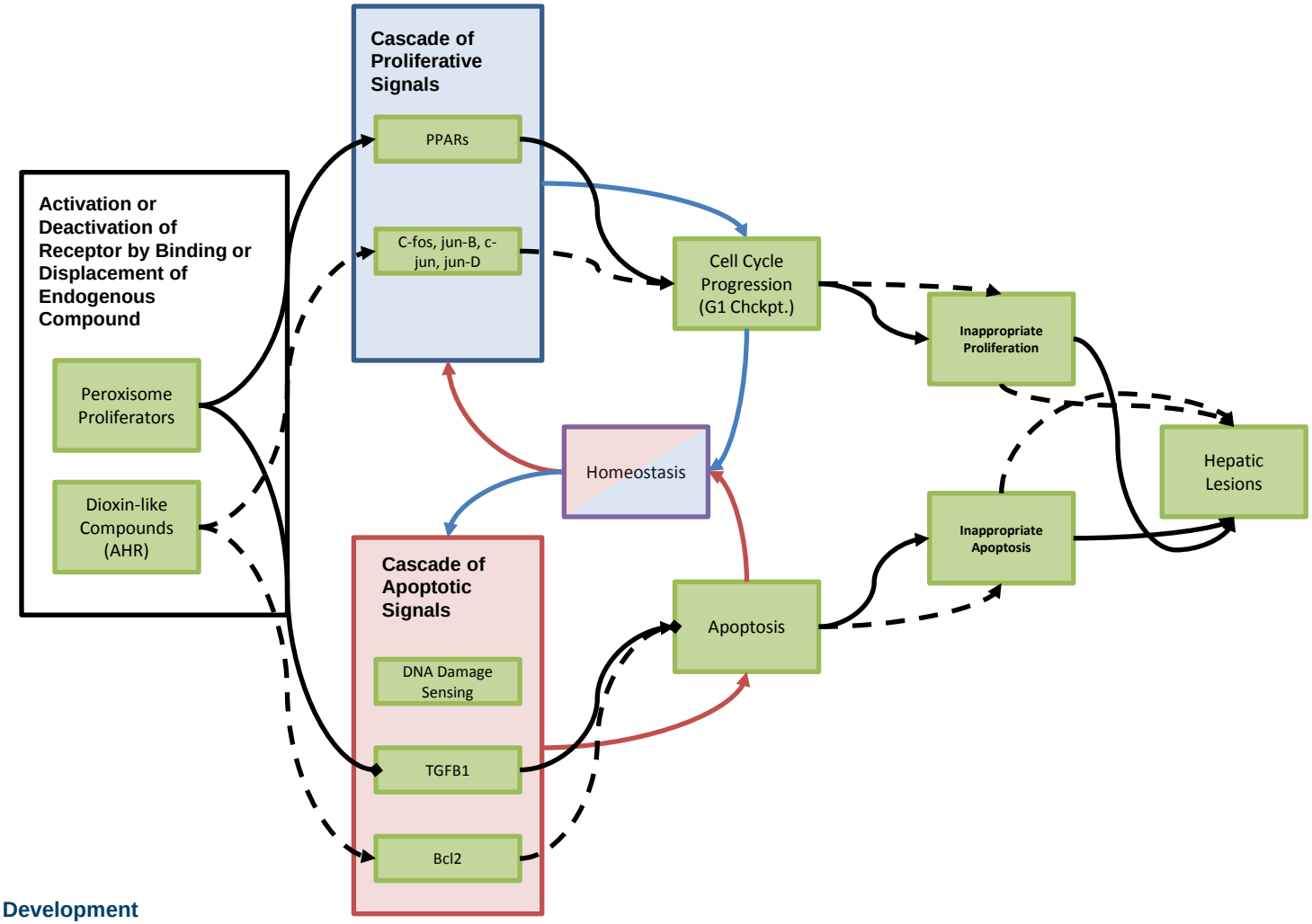
Putative AOP derived from:
Roberts et al. (1997)
Guyton et al. (2009)

Initial Molecular Event

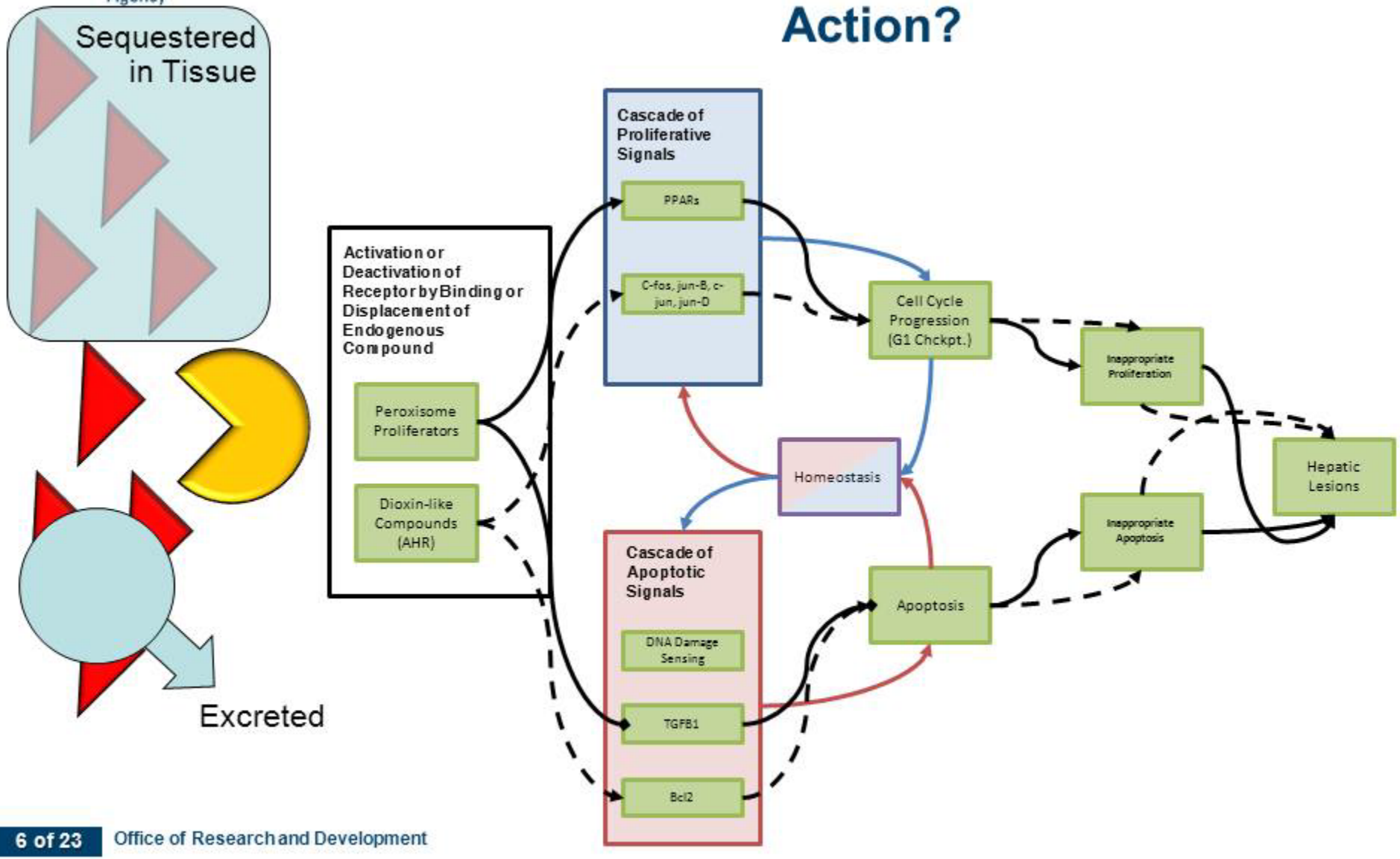


Exposure: How Many Molecules Are There?

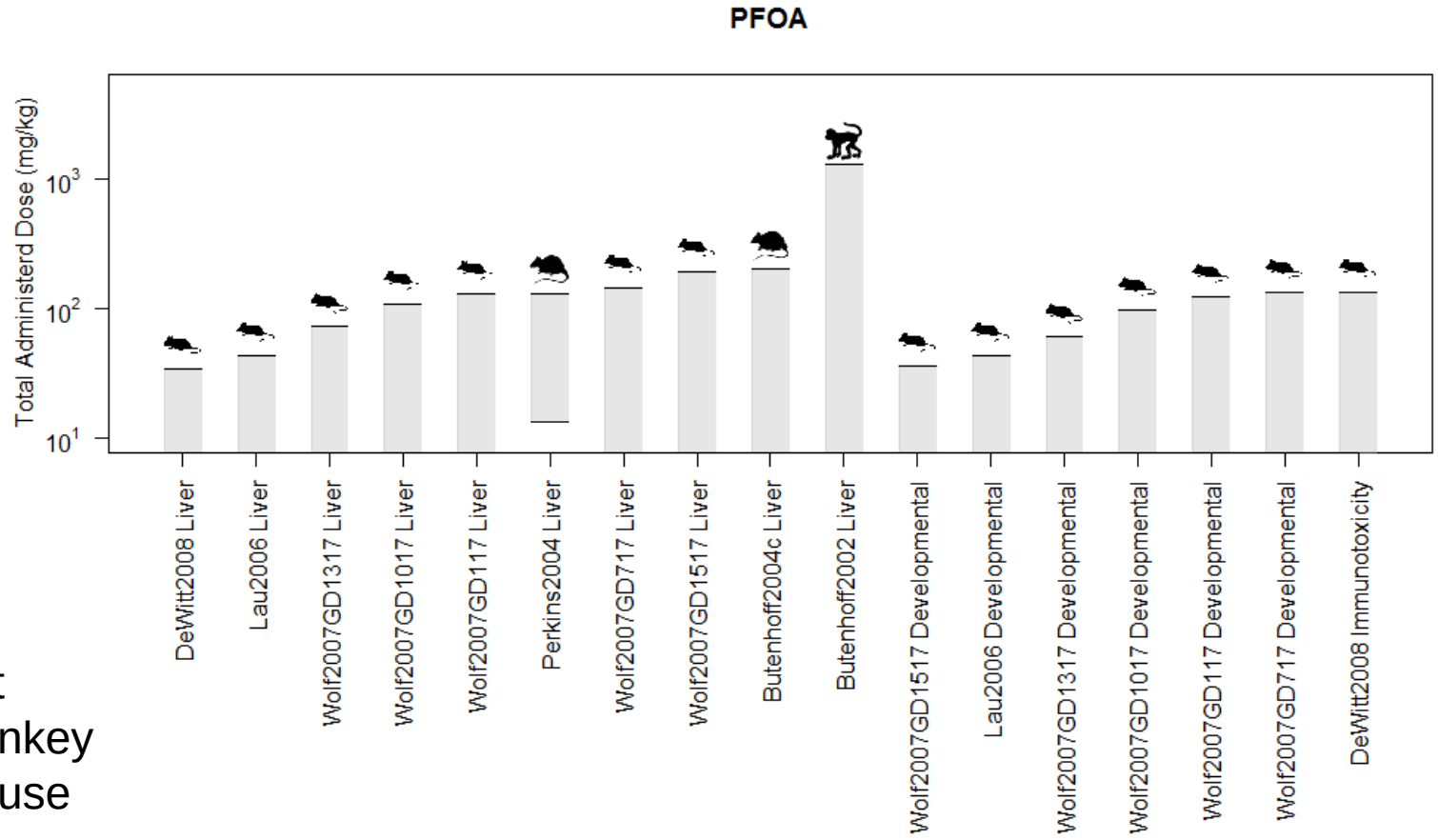




Toxicokinetics: How Many Molecules Get to Site of Action?



Dosimetry Matters

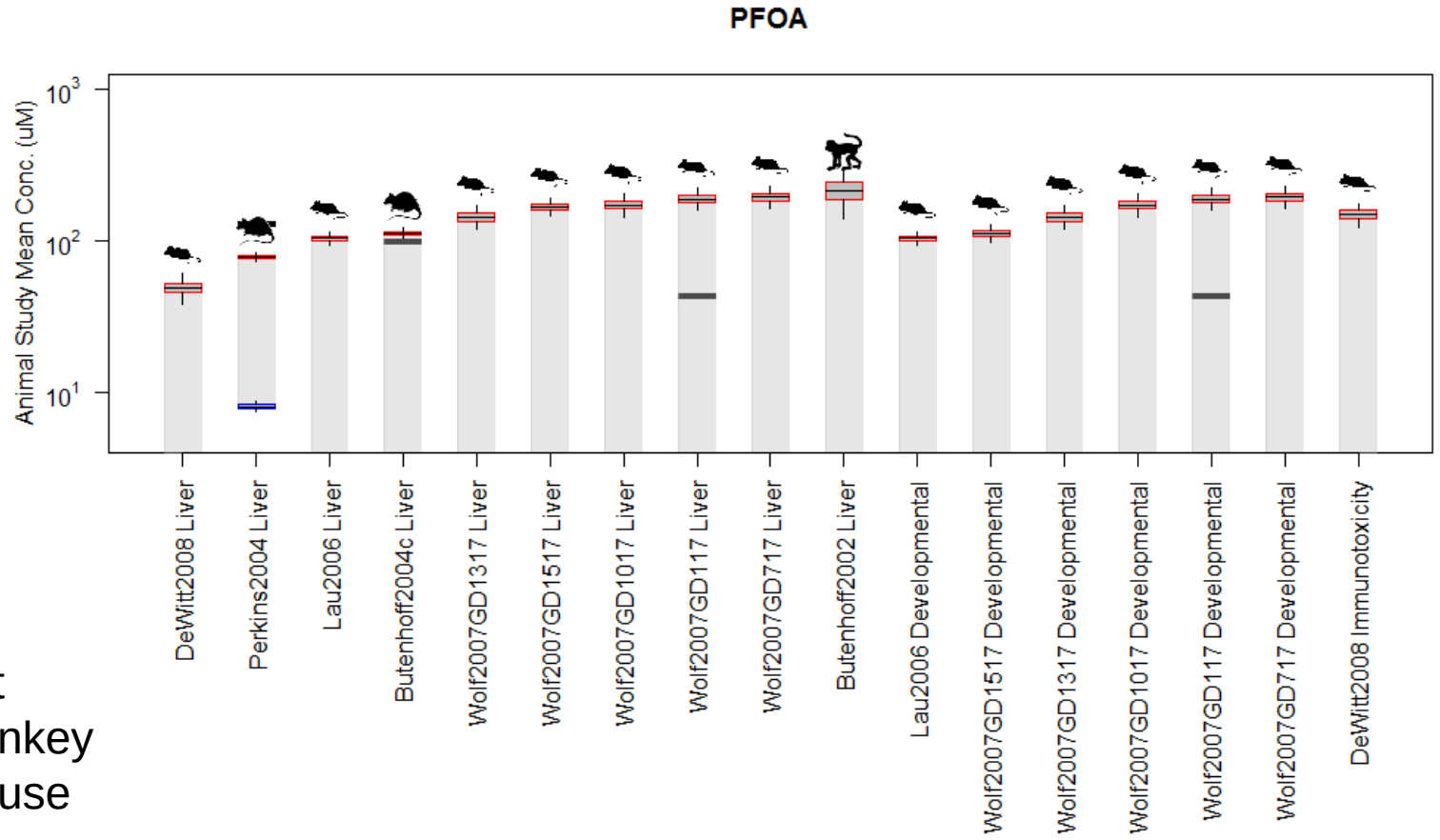


Rat
Monkey
Mouse

Differences in species and dosing regimen can create apparent differences in doses needed to produce adverse effects.

Figure from Wambaugh *et al*. Tox. Sci. (2013)

Dosimetry Matters



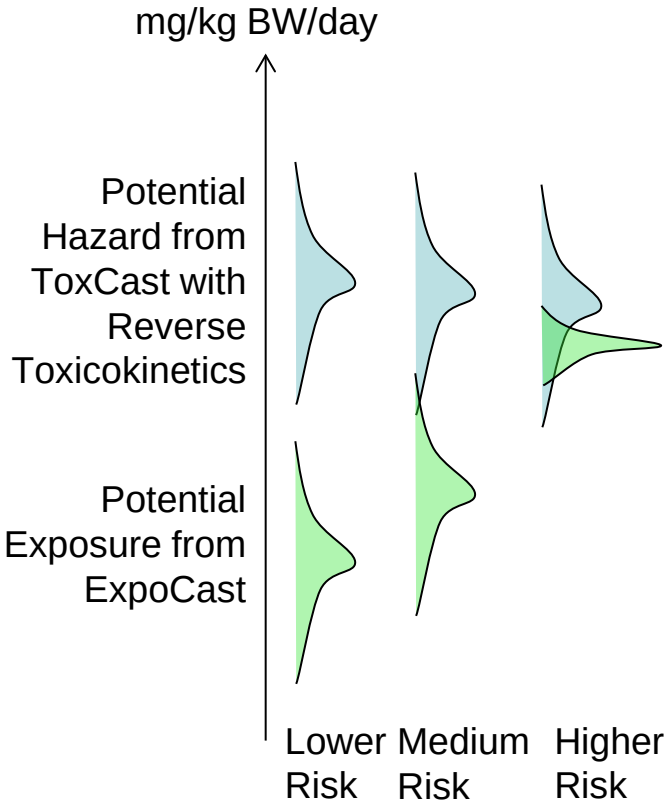
Rat
Monkey
Mouse

PK Modeling of tissue concentrations can reconcile these differences.

Figure from Wambaugh *et al*. Tox. Sci. (2013)

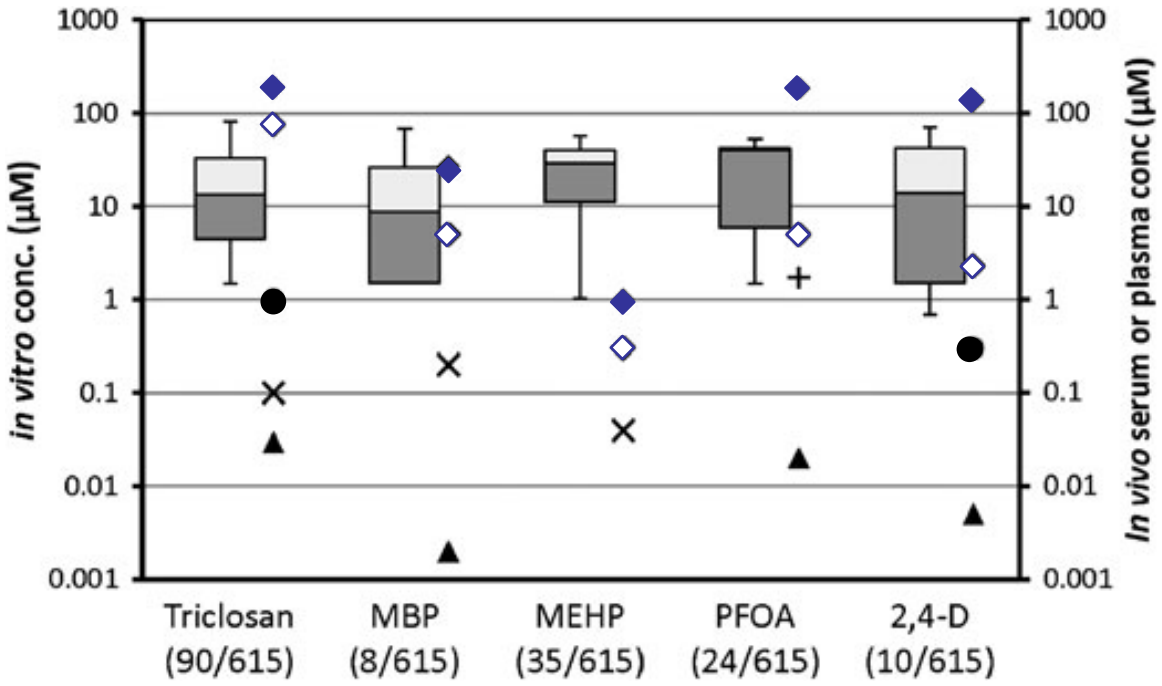
The Risk Context

- There are thousands of chemicals, most without enough data for evaluation
- High throughput *in vitro* methods (e.g., ToxCast) beginning to bear fruit on potential hazard for many of these chemicals
- High throughput toxicokinetic methods (HTTK) approximately convert these *in vitro* results to daily doses needed to produce similar levels in a human (IVIVE)
- High throughput exposure forecasting (ExpoCast) can bound mean human exposures for key populations



e.g. Judson *et al.*, (2011)

Concordance of *In Vitro* Bioactivity, *In Vivo* Toxicity, and Exposure



Aylward and Hays (2011)
Journal of Applied Toxicology **31** 741-751

Estimated or measured average serum or plasma concentrations associated with the

- ◆ **LOAEL** (solid) or
- ◇ **NOAEL** (open)

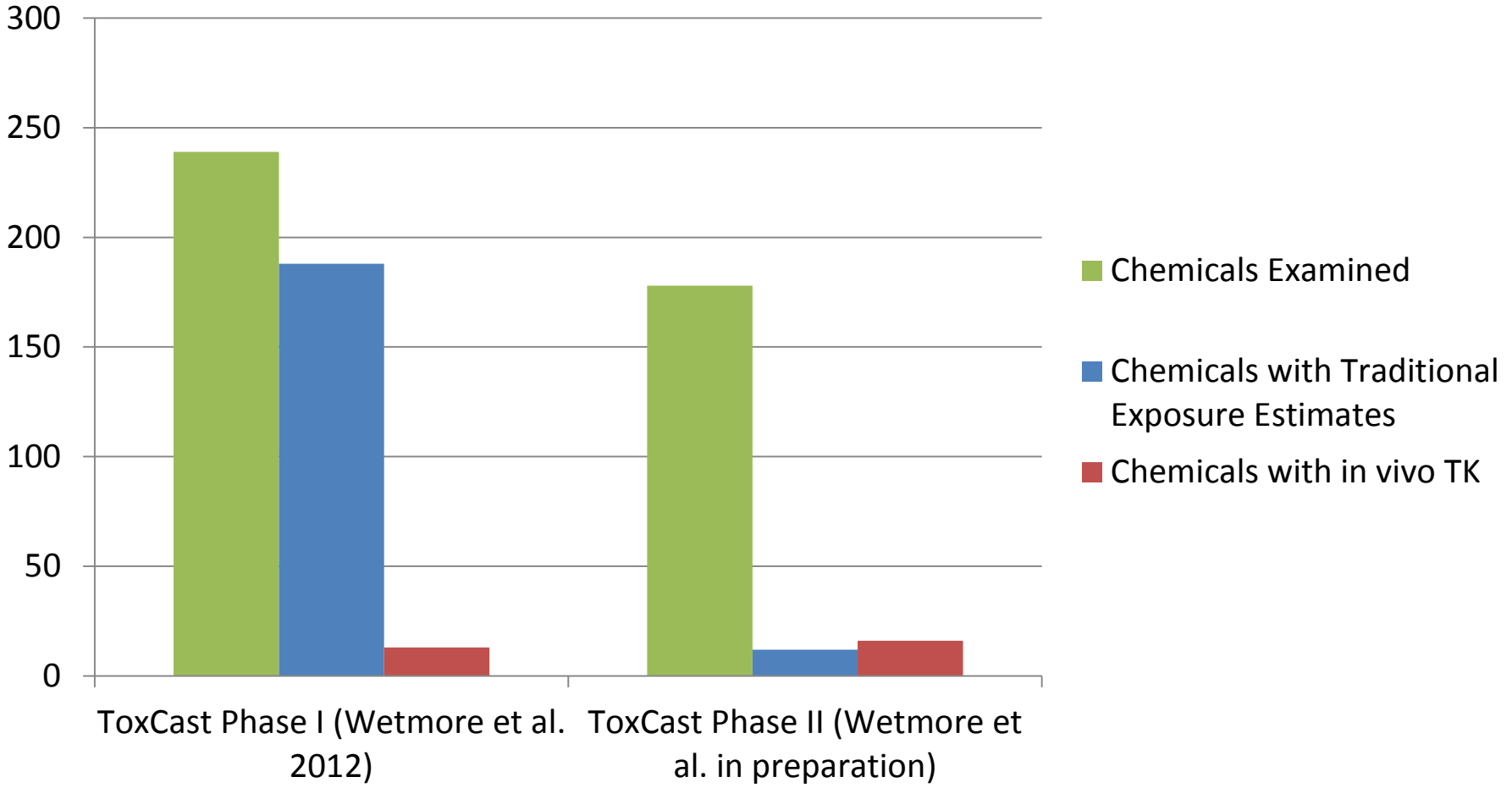
dose rates in animal studies underlying existing toxicity reference values

Estimated average serum or
- ● plasma concentrations in humans consistent with chronic exposure reference values

Biomonitored serum or plasma concentrations in:

- \+ occupational populations
- × in volunteers using products containing the chemical
- ▲ the general population

Data Availability for *In Vitro* Bioactivity, *In Vivo* Toxicity, and Exposure



- As in Egeghy et al. (2012), there is a paucity of data for providing context to HTS data

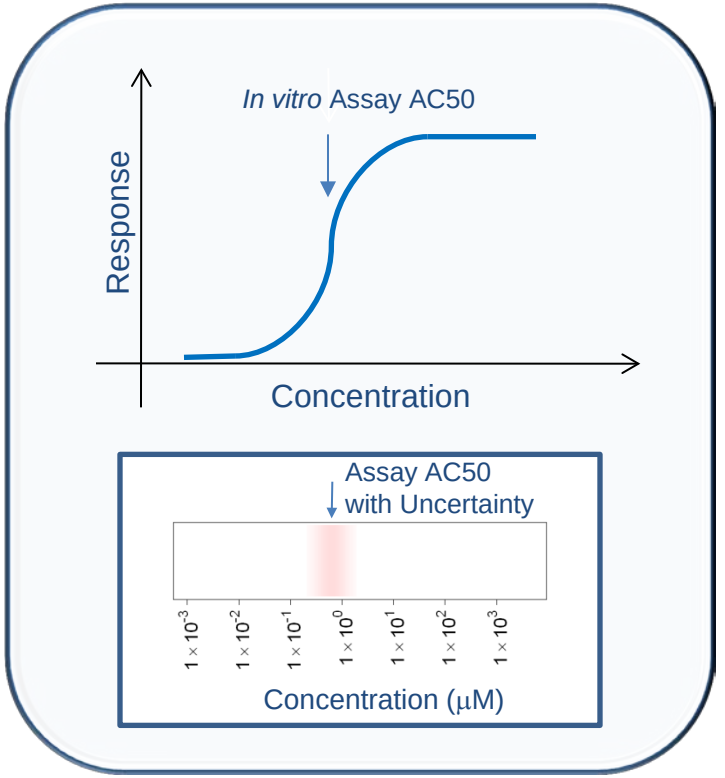
High-Throughput Toxicity Testing

Tox21: Examining >10,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)

ToxCast: For a subset (>1000) of Tox21 chemicals ran >500 additional assays (Judson *et al.*, 2010)

Most assays conducted in dose-response format (identify 50% activity concentration – AC50)

All data is public: http://actor.epa.gov/

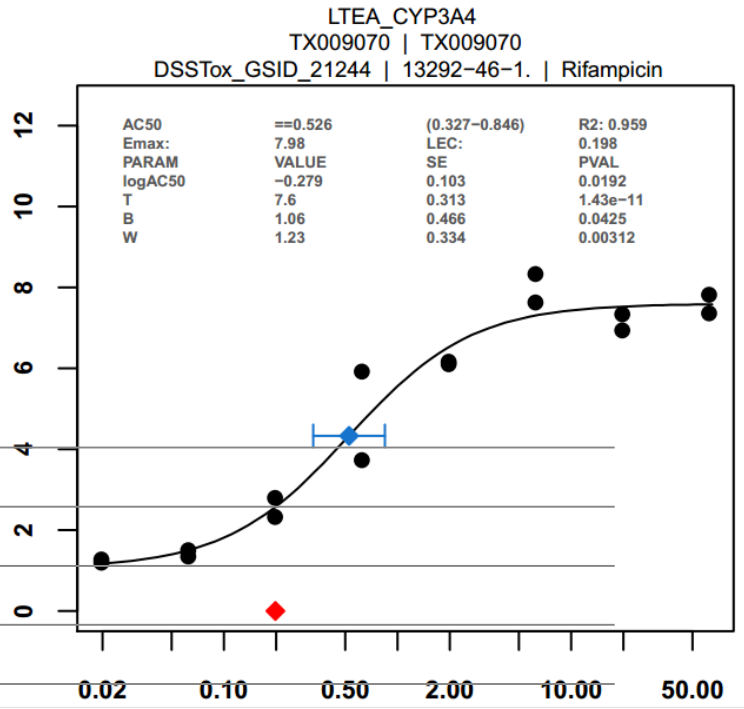


Putative Molecular Initiating Events

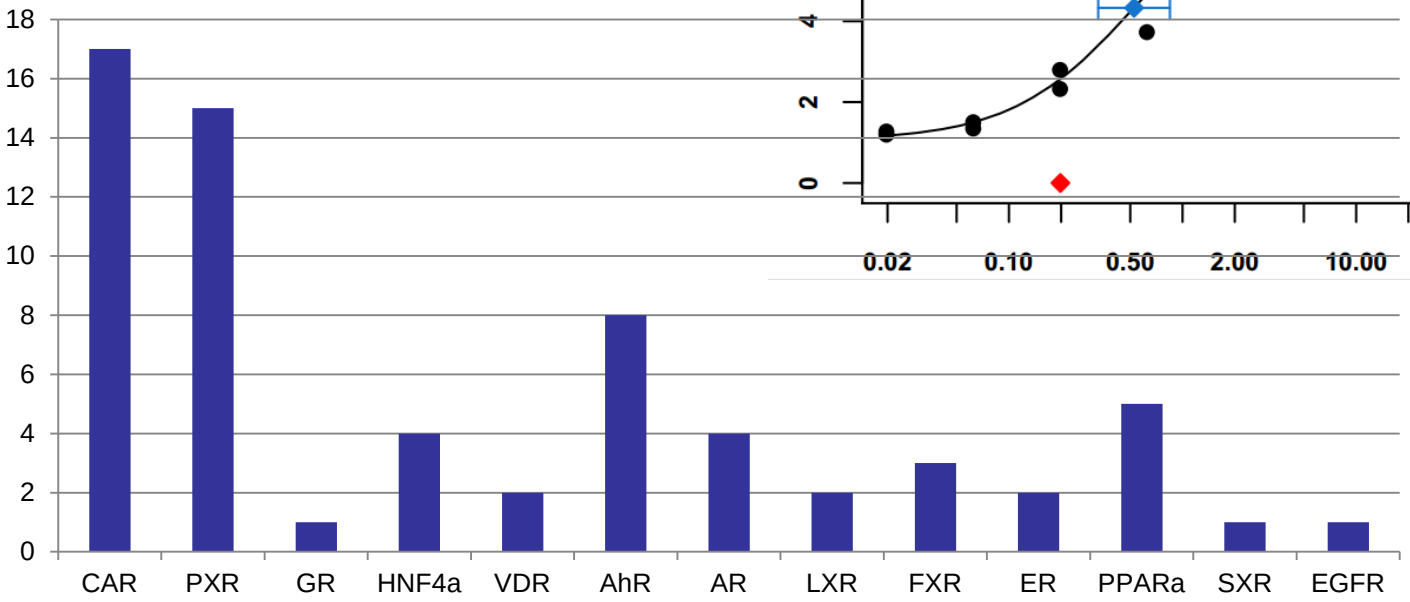
HepaRG cells treated by ThermoFisher (formerly Cellzdirect)

Gene expression conducted by Expression Analysis

93 assay genes + 3 house keeping genes (for normalization) on a Fluidign Chip

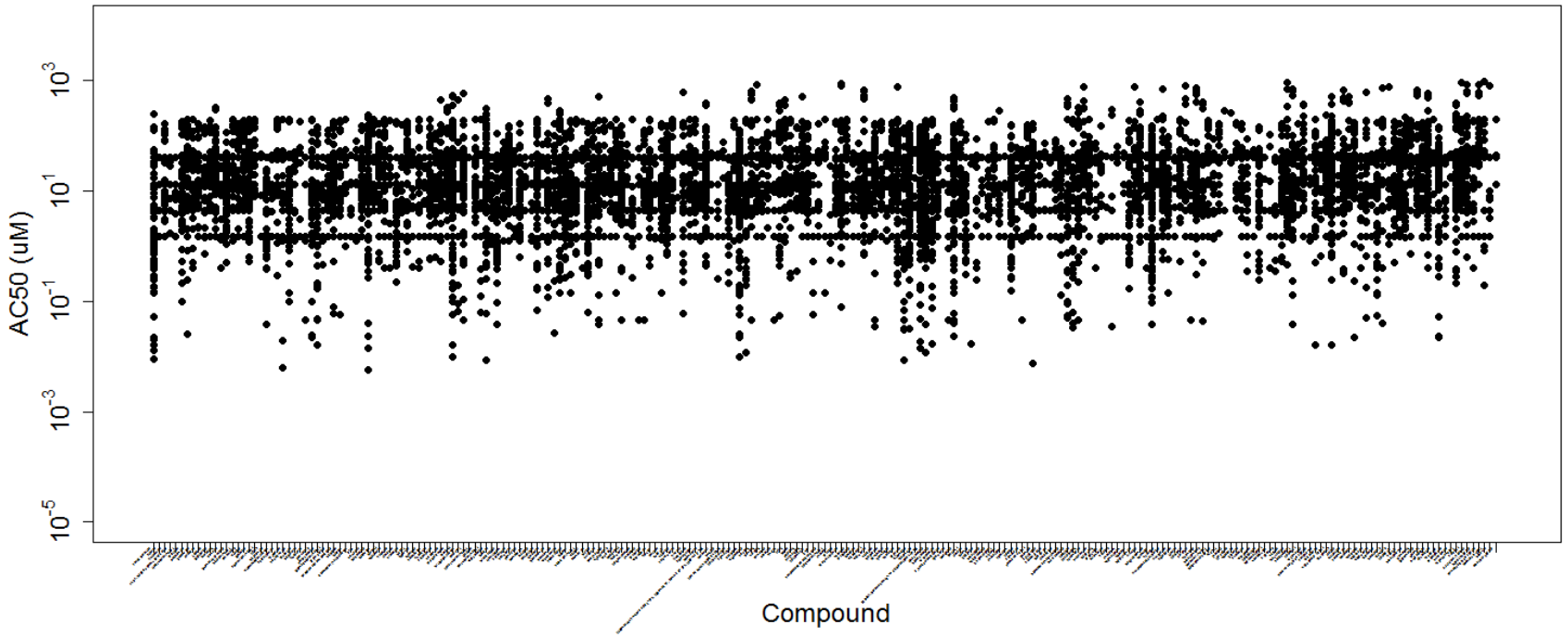


Number of Assayed Genes Downstream of Nuclear Receptor



ToxCast HepaRG analysis not yet complete

ToxCast *in vitro* AC50s

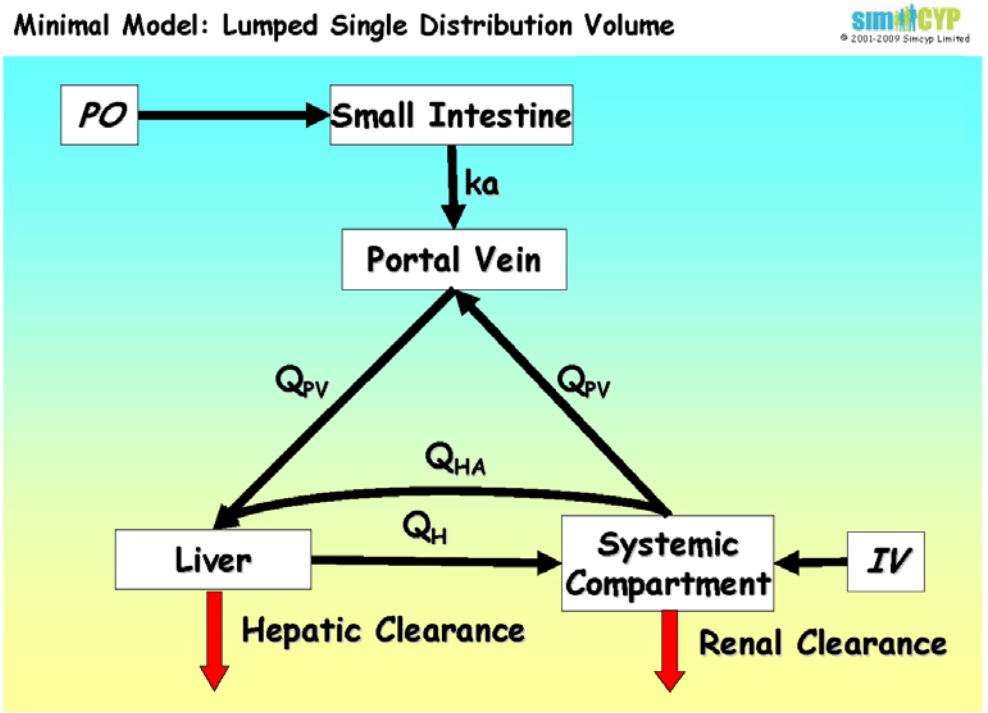


- One point for each chemical-*in vitro* assay combination with a systematic (Hill function) concentration response curve

Results from Wetmore et al. *(2012)*

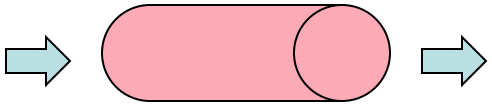
Steady-State Plasma Concentration

- Successful methods have been developed for pharmaceutical compounds to determine high throughput TK (HTTK) from limited in vitro measurements and chemical structure-derived property predictions
- *In vitro* plasma protein binding and metabolic clearance assays allow approximate hepatic and renal clearances to be calculated
- At steady state this allows conversion from concentration to administered dose
- No oral absorption/ bioavailability included



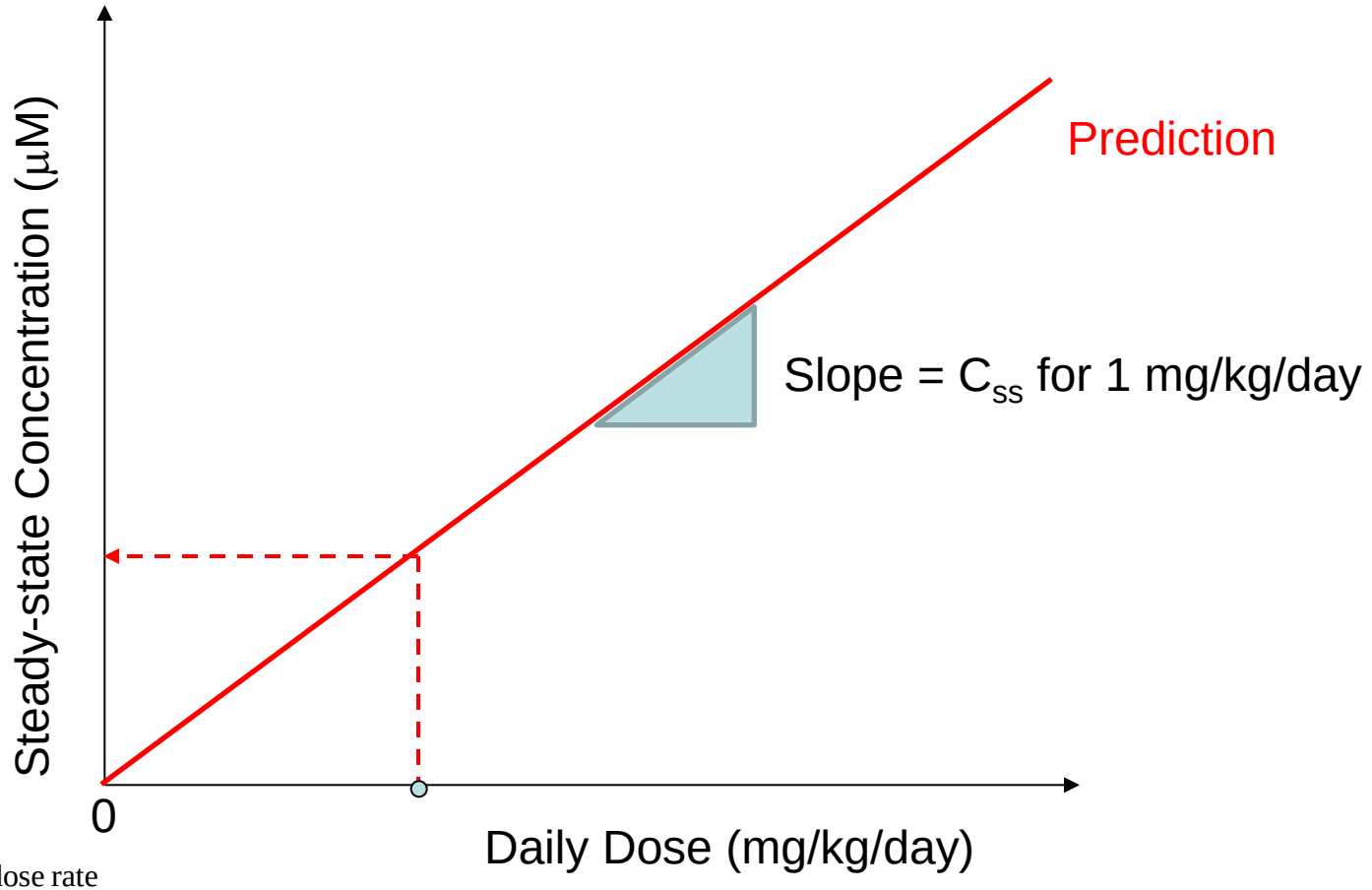
$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day)



Sum of hepatic and renal clearance (mg/kg/day)

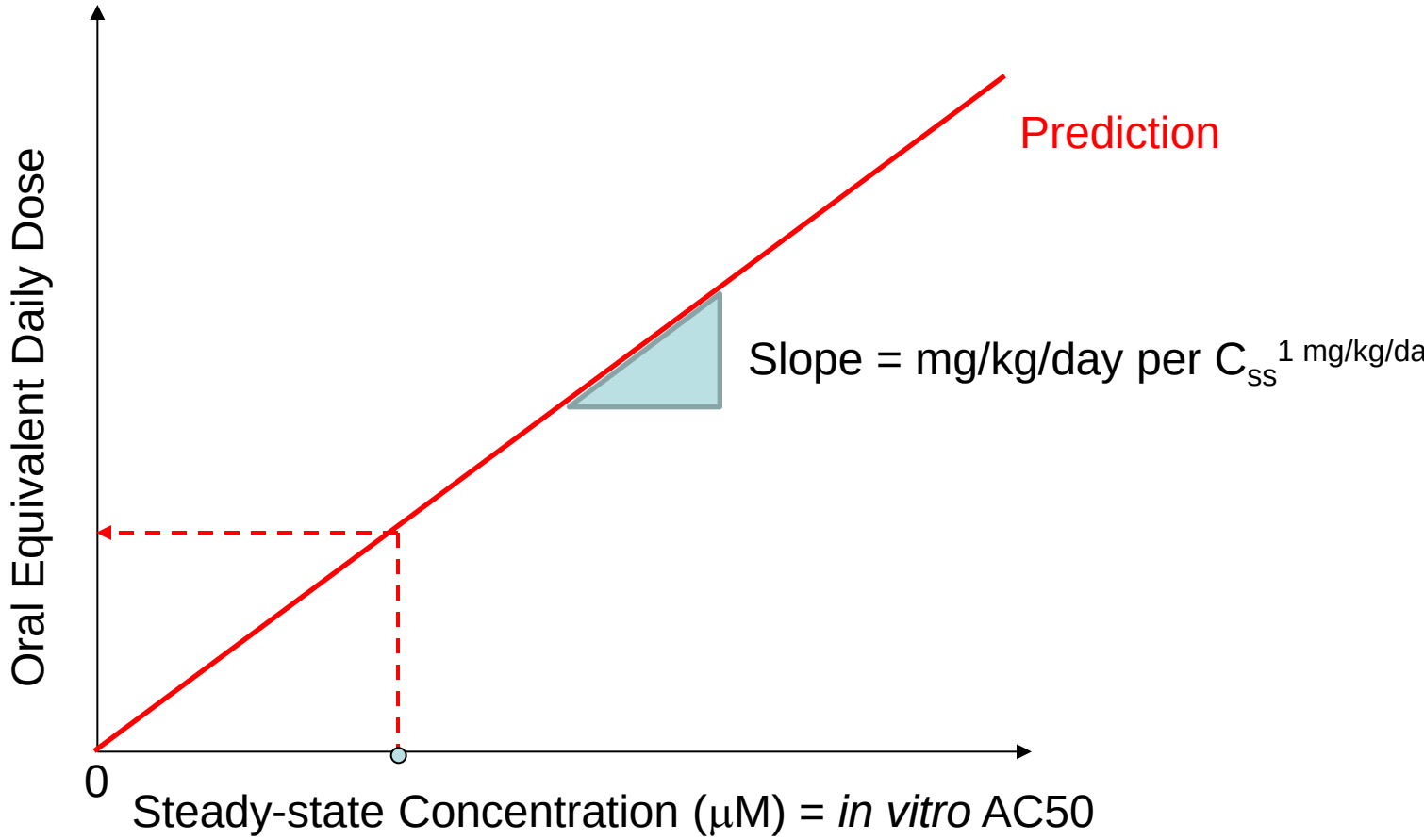
Steady-State Model is Linear



$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

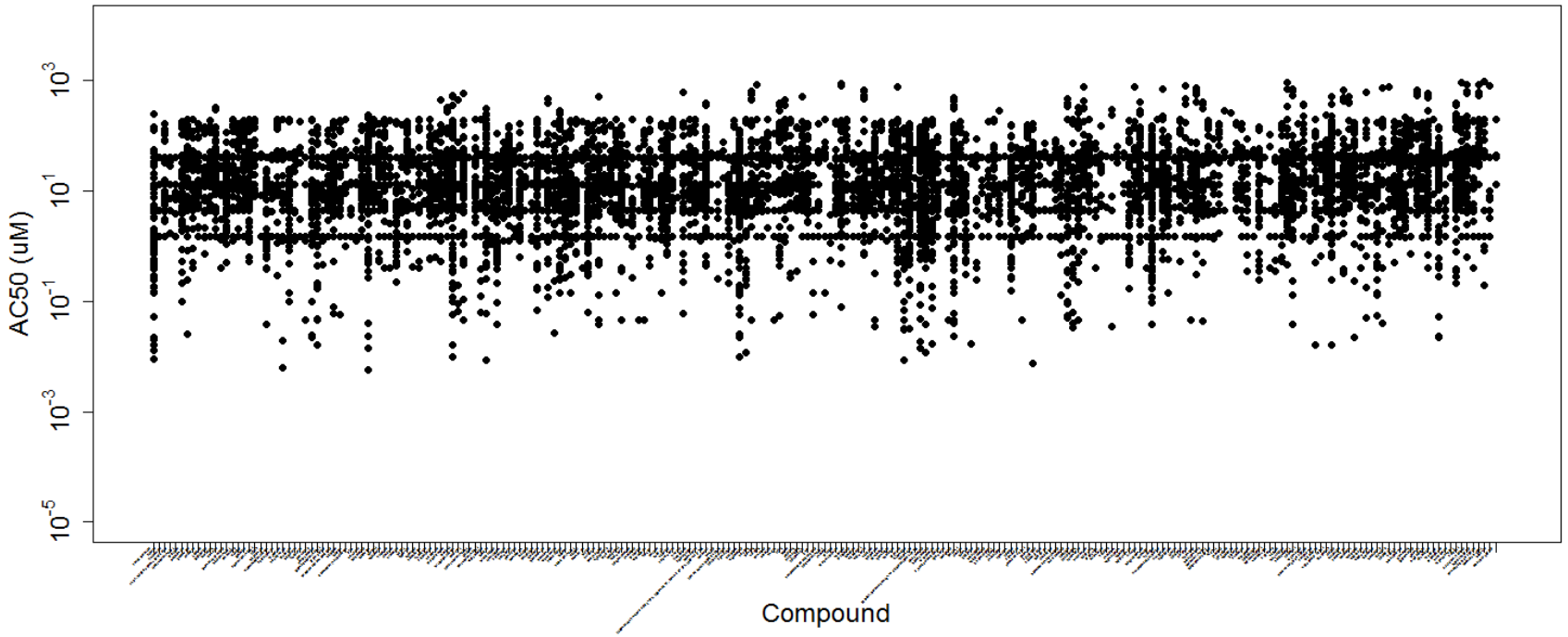
- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



- Swap the axes
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose

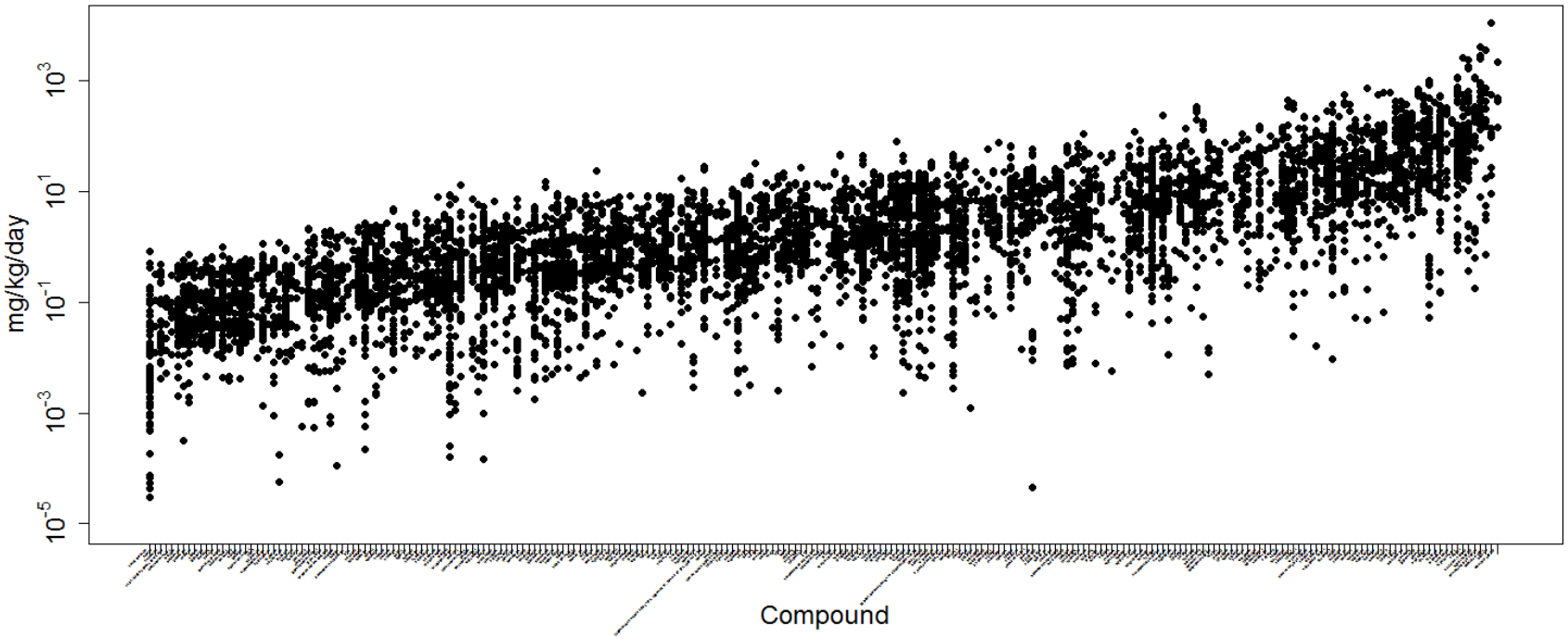
ToxCast *in vitro* AC50s



- It appears harder to prioritize on bioactive *in vitro* concentration without *in vivo* context

Results from Wetmore et al. *(2012)*

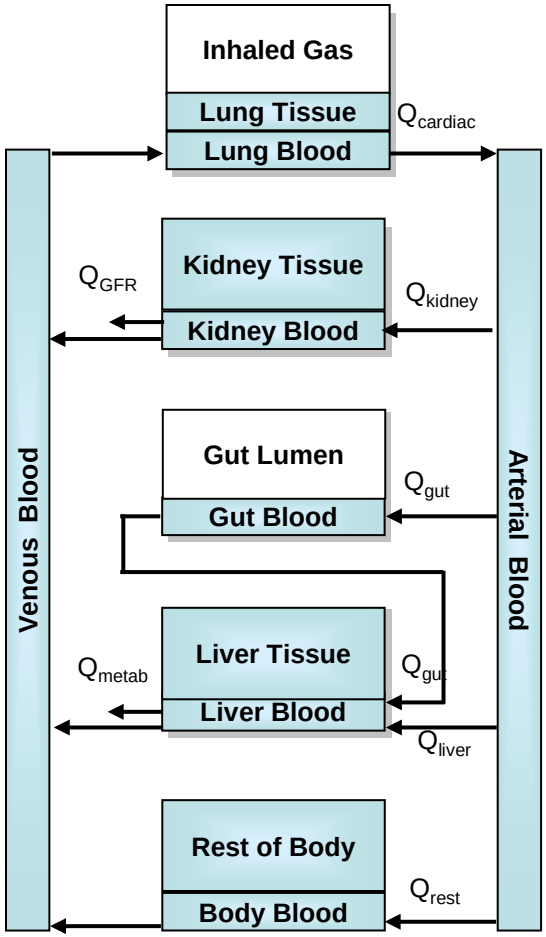
RTK Oral Equivalents



- Translation from *in vitro* to steady-state oral equivalent doses allow greater discrimination between effective chemical potencies

Results from Wetmore et al. *(2012)*

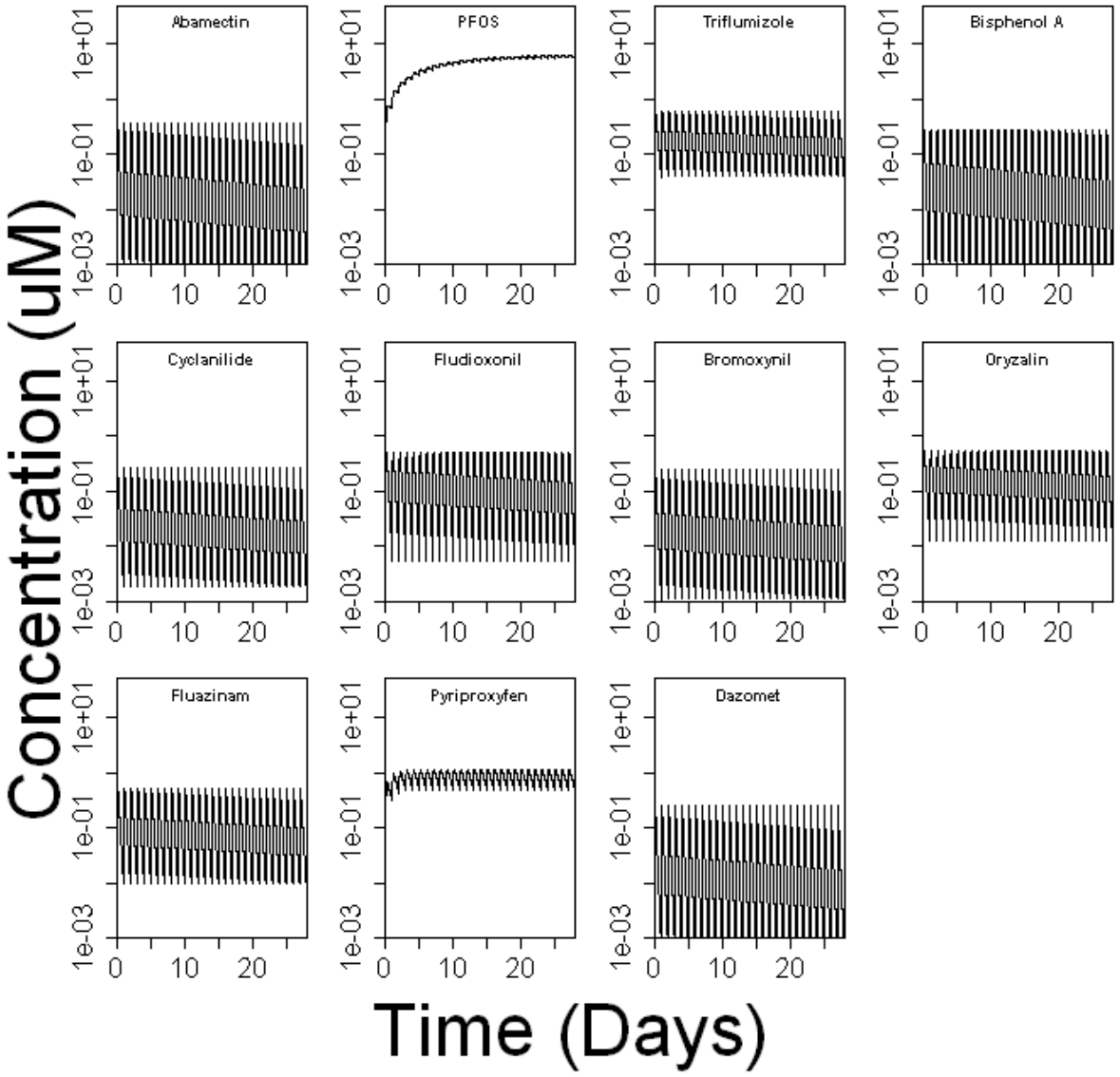
Physiologically-based Toxicokinetic (PBPK) Model



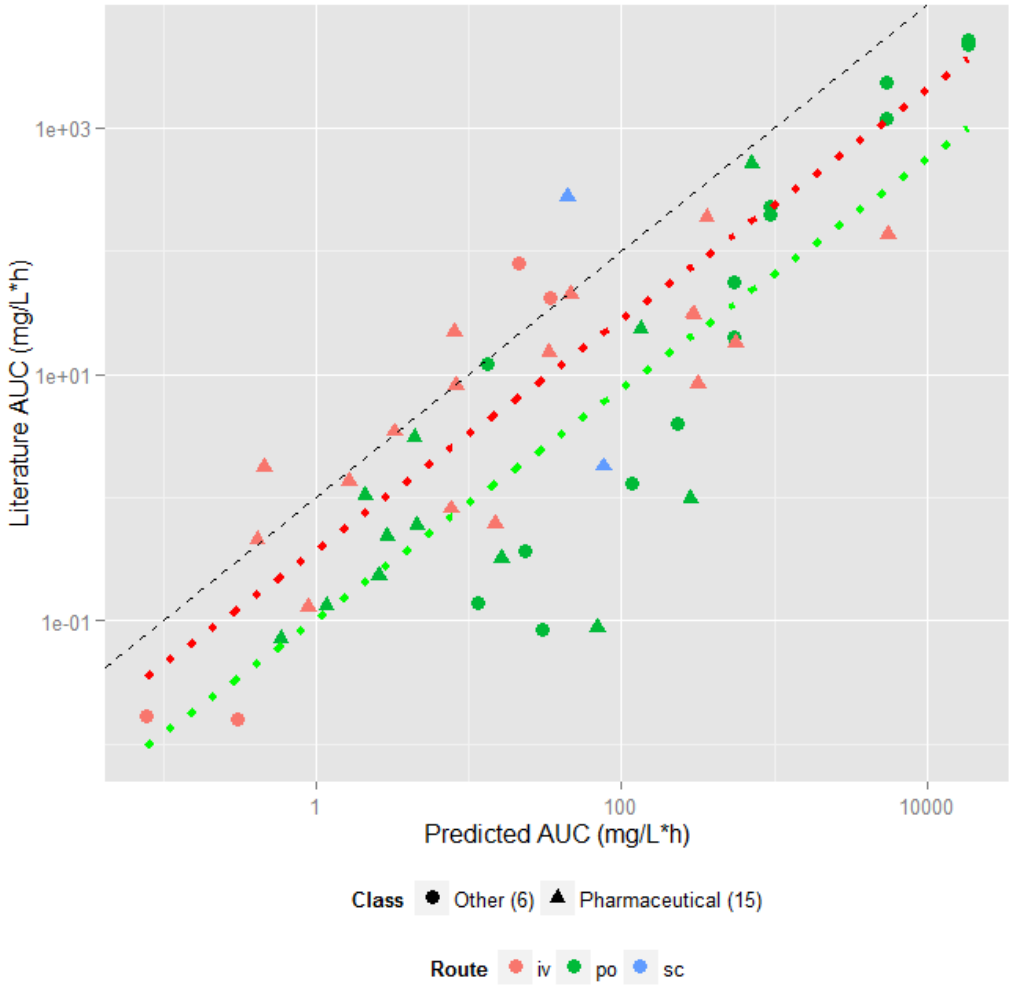
- Out of 239 ToxCast chemicals examined by Wetmore et al. (2012), only 11 had some sort of human-relevant TK data or model
- HTTK predictions of steady-state behaviors were generated in Wetmore et al. (2012) using *in vitro* TK methods
- Can build generic, high throughput PBPK (HTPBPK) models parameterized with
  - the same *in vitro* HTTK data used for steady-state work, **plus**
  - QSARs for tissue-specific properties
  - Assumptions about unknown dynamic processes, such as absorption
- These HTPBPK models can provide a simulated in vivo context for tissue simulations

Predicted PK Metrics

- Human hepatic concentration of various chemicals as a function of 28 daily doses (10 mg/kg/day)
- Can predict mean and peak concentration and time integrated area under the curve (AUC) for various tissues

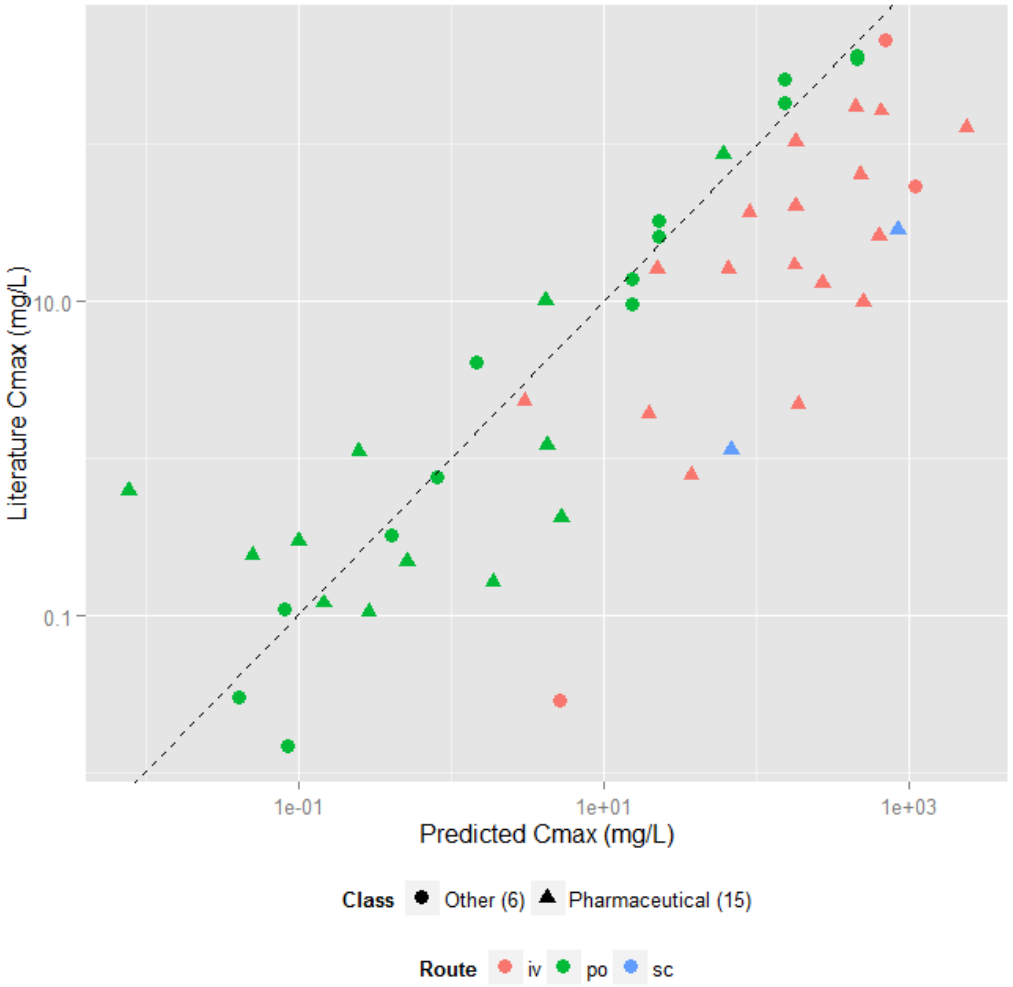


Evaluating HTPBPK Predictions from *In Vitro* Data



- HTPBPK predictions for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat.
- Predictions are generally conservative – *i.e.*, predicted AUC higher than measured
- Oral dose AUC ~3.6x higher than intravenous dose AUC (p-Value 0.021)

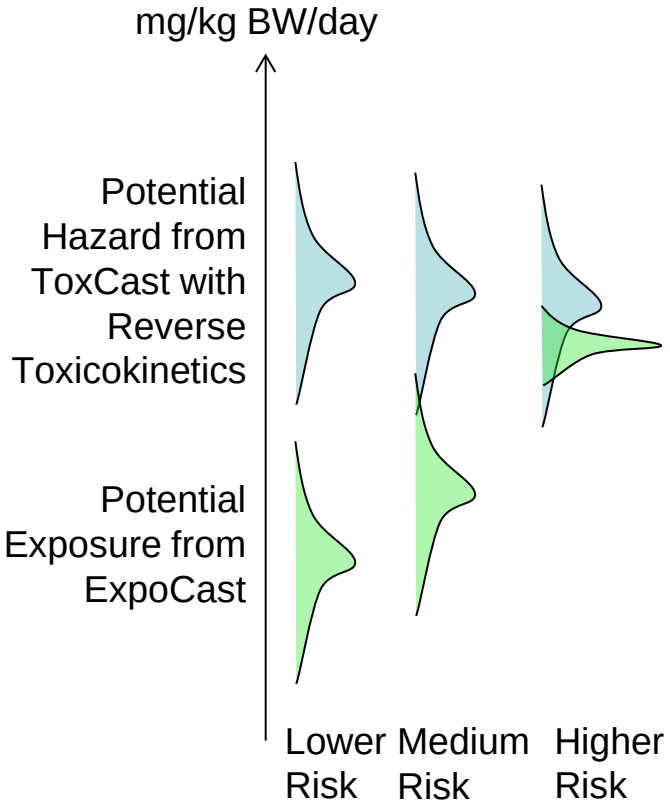
Evaluating HTPBPK Predictions from *In Vitro* Data



- C_{max} predictions relatively decent (R^2 ~ 0.69)

The Exposure Component of Risk

- Ultimately hope to do a rapid risk prioritization of chemicals with minimal information
- Identify chemicals most in need of additional resources and traditional methodologies
- Risk is the product of hazard and exposure
- High throughput exposure forecasting (ExpoCast) can bound mean human exposures for key populations



e.g. Judson *et al.*, (2011)

Systematic Empirical Evaluation of Models (SEEM)

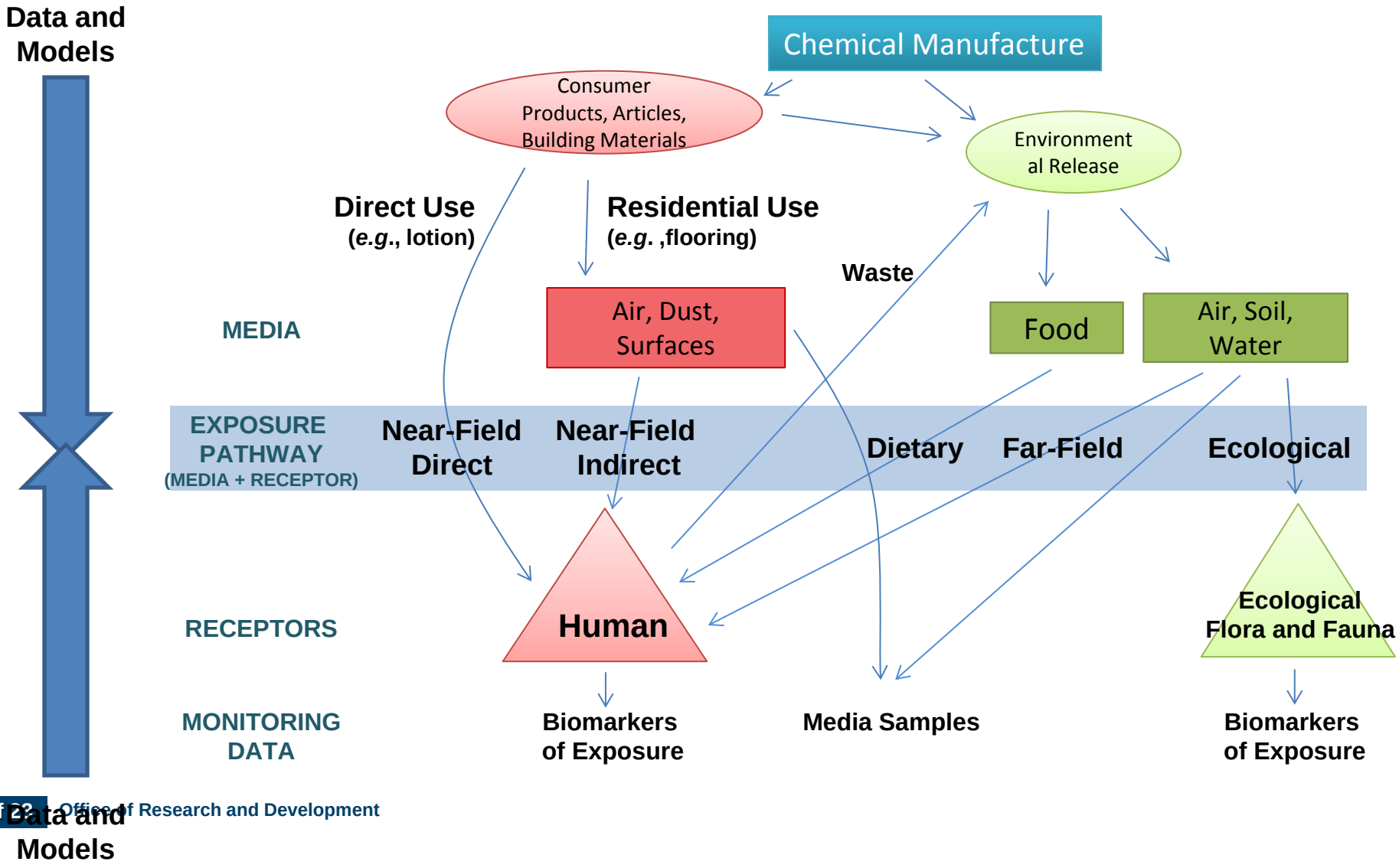
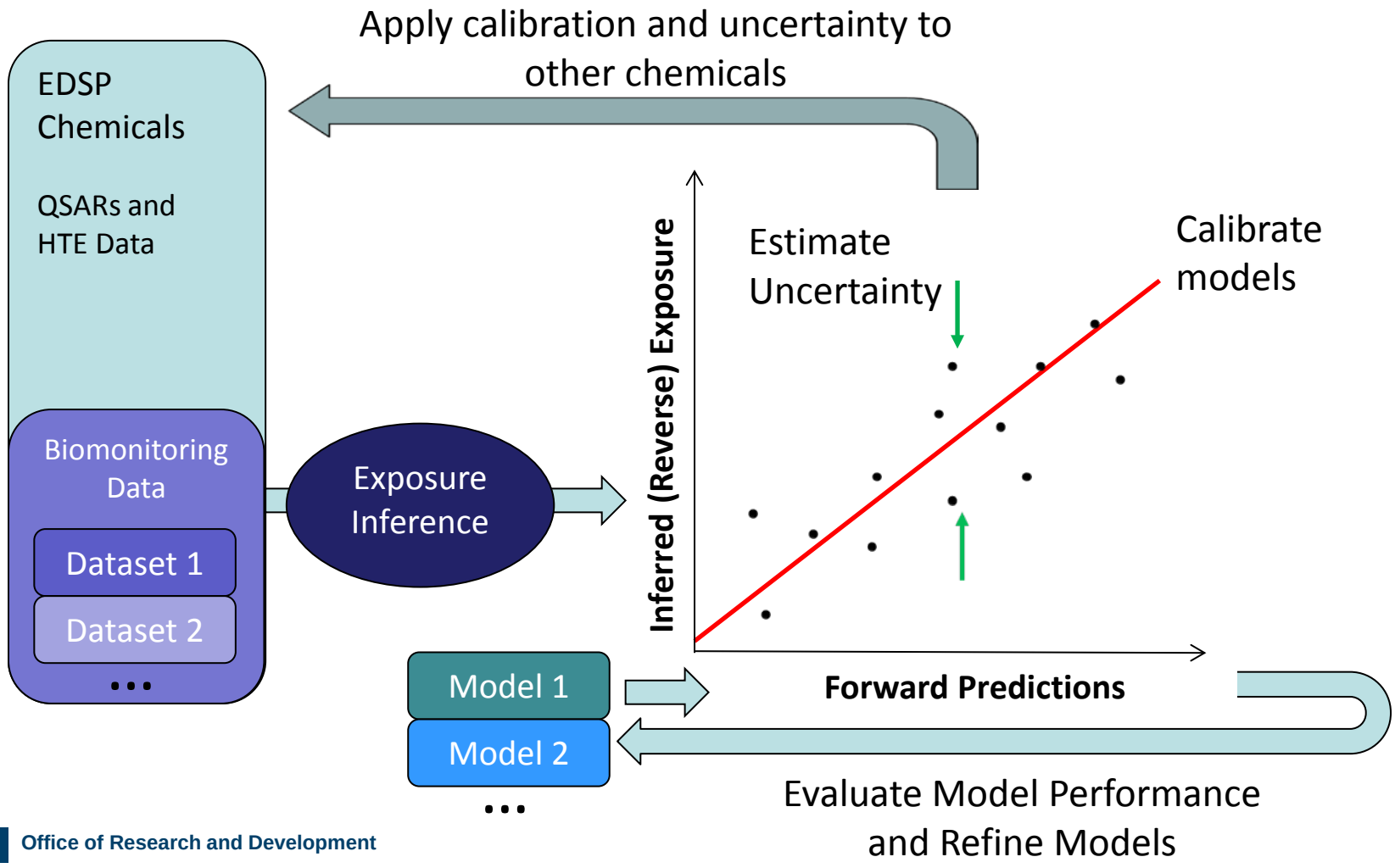
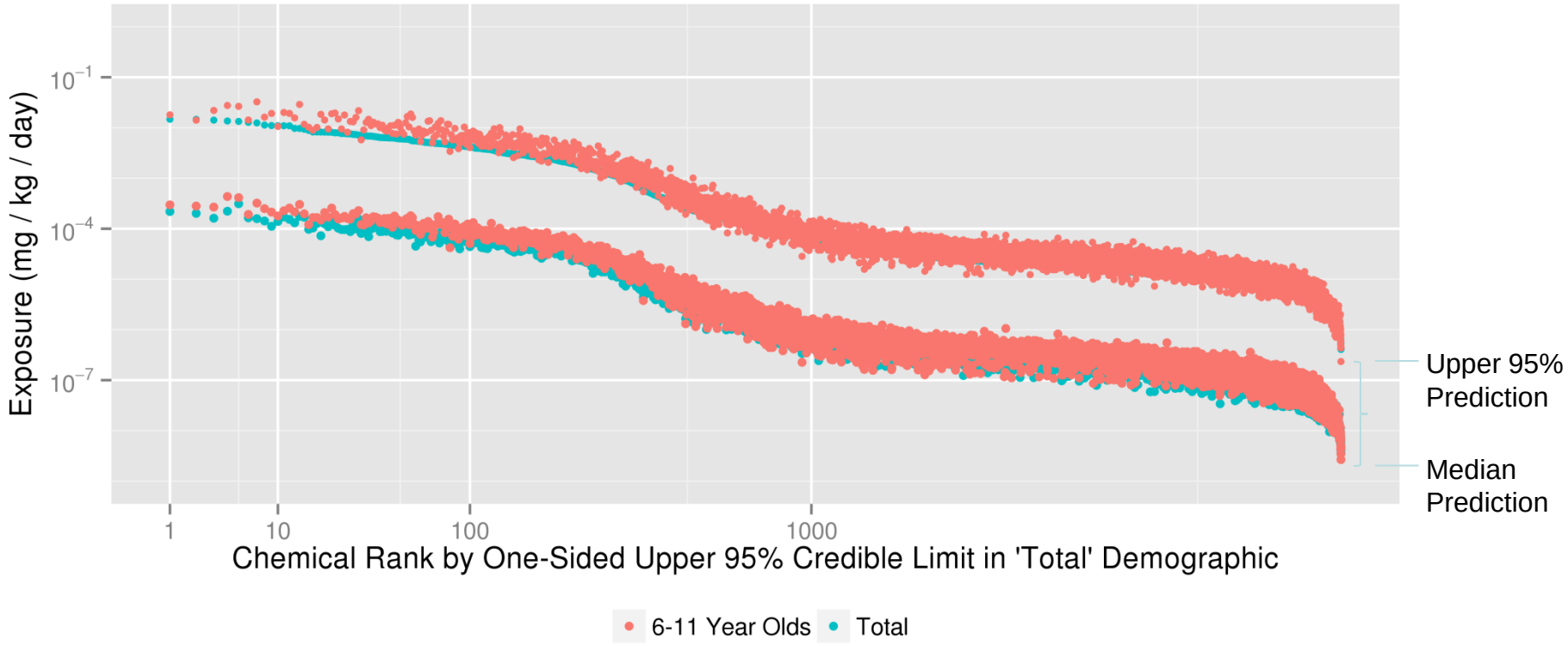


Illustration of the SEEM Framework



Exposure Predictions for 7968 Tox21 Chemicals

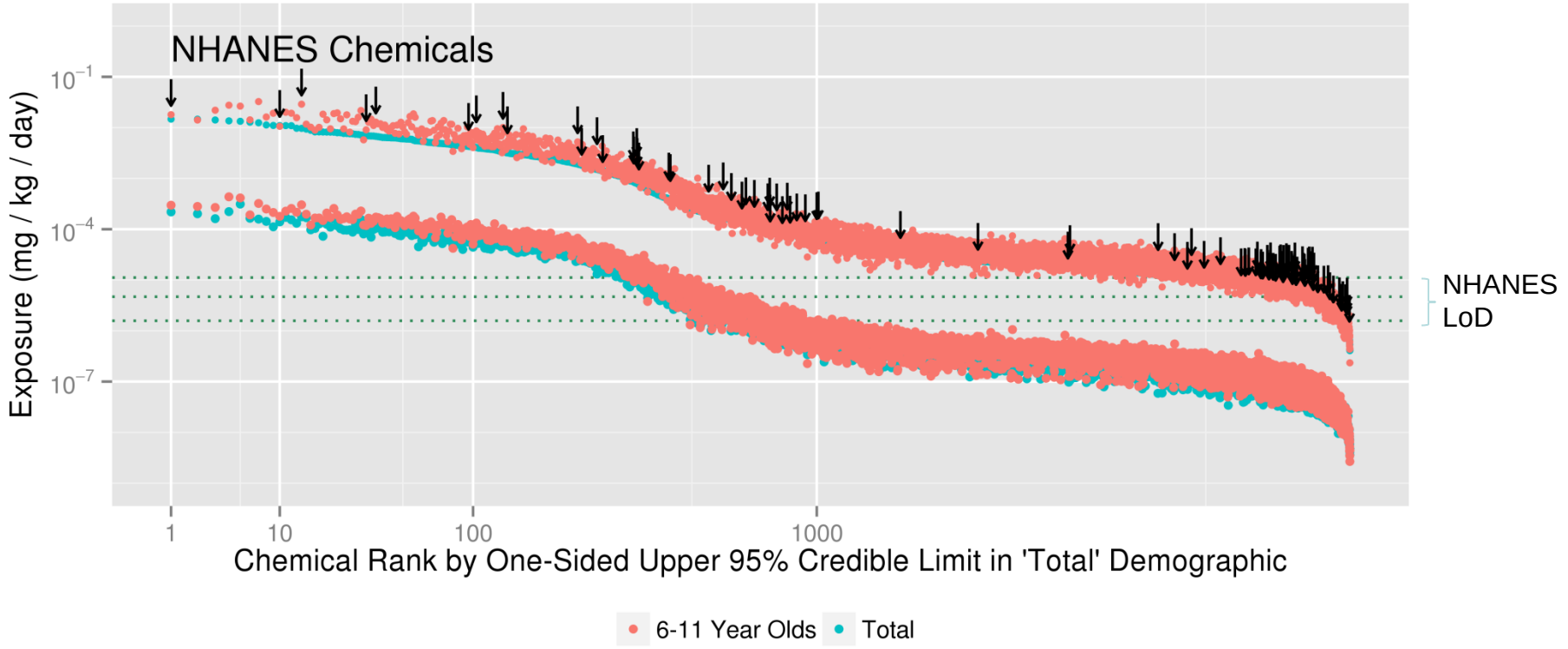


- Five factors can explain roughly 50% of the chemical-to-chemical variance in NHANES chemical exposures across demographics, including women of child-bearing age and children aged 6-11

Exposure Predictions for Tox21 Chemicals

- We focus on the median and upper 95% predictions because the lower 95% is below the NHANES limits of detection (LoD)
- Dotted lines indicate 25%, median, and 75% of the LoD distribution

Exposure Predictions for 7968 ToxCast Chemicals



- Chemicals currently monitored by NHANES are distributed throughput the predictions
- Chemicals with the first and ninth highest 95% limit are monitored by NHANES

Conclusion

- Using in vitro TK methods developed for pharmaceuticals, we can parameterize HTPBPK models
- We can model the difference between *in vivo* measurements and HTTK predictions (*i.e.*, the residuals or errors)
- We can connect HTPBPK models to tissue simulations to provide simulated in vivo context for assessing the impact of chemical perturbations identified by high throughput screening assays

EPA Office of Research and Development
Chemical Safety for Sustainability Research Plan

United States Environmental Protection Agency

Rapid Exposure and Dosimetry

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ToxCast HepaRG Assay

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