Rapid Exposure and Dosimetry Research and the ExpoCast Project

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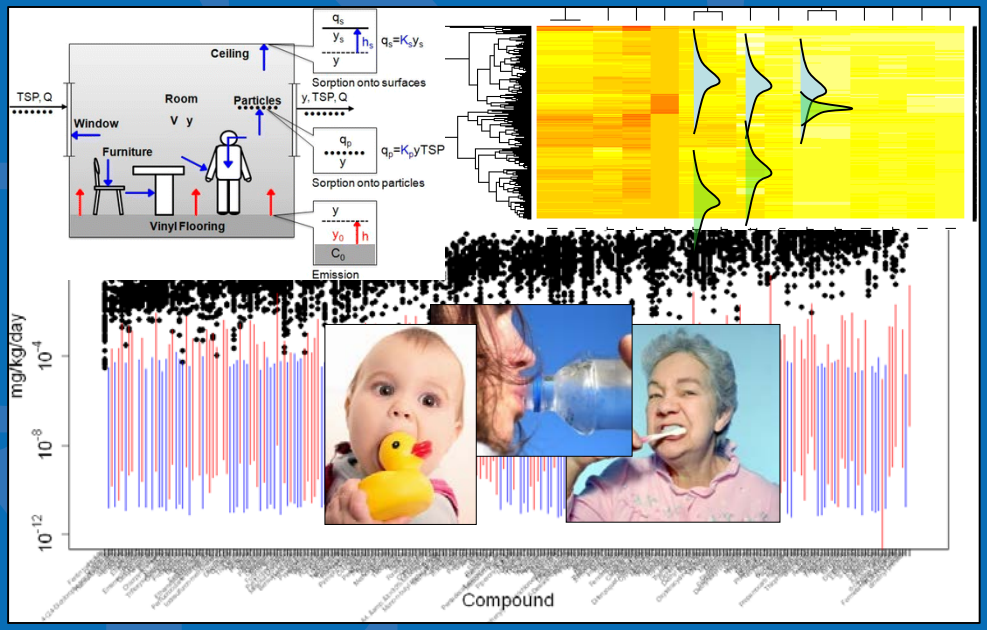
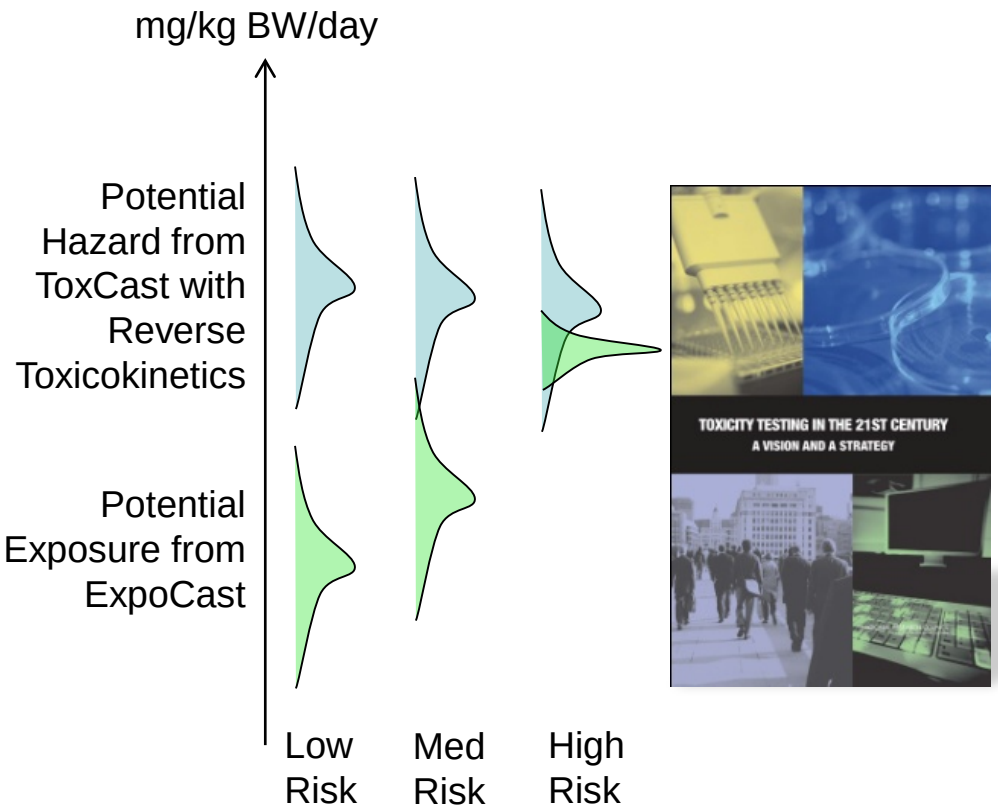


Webinar Presentation to L'Oreal
October 22, 2014

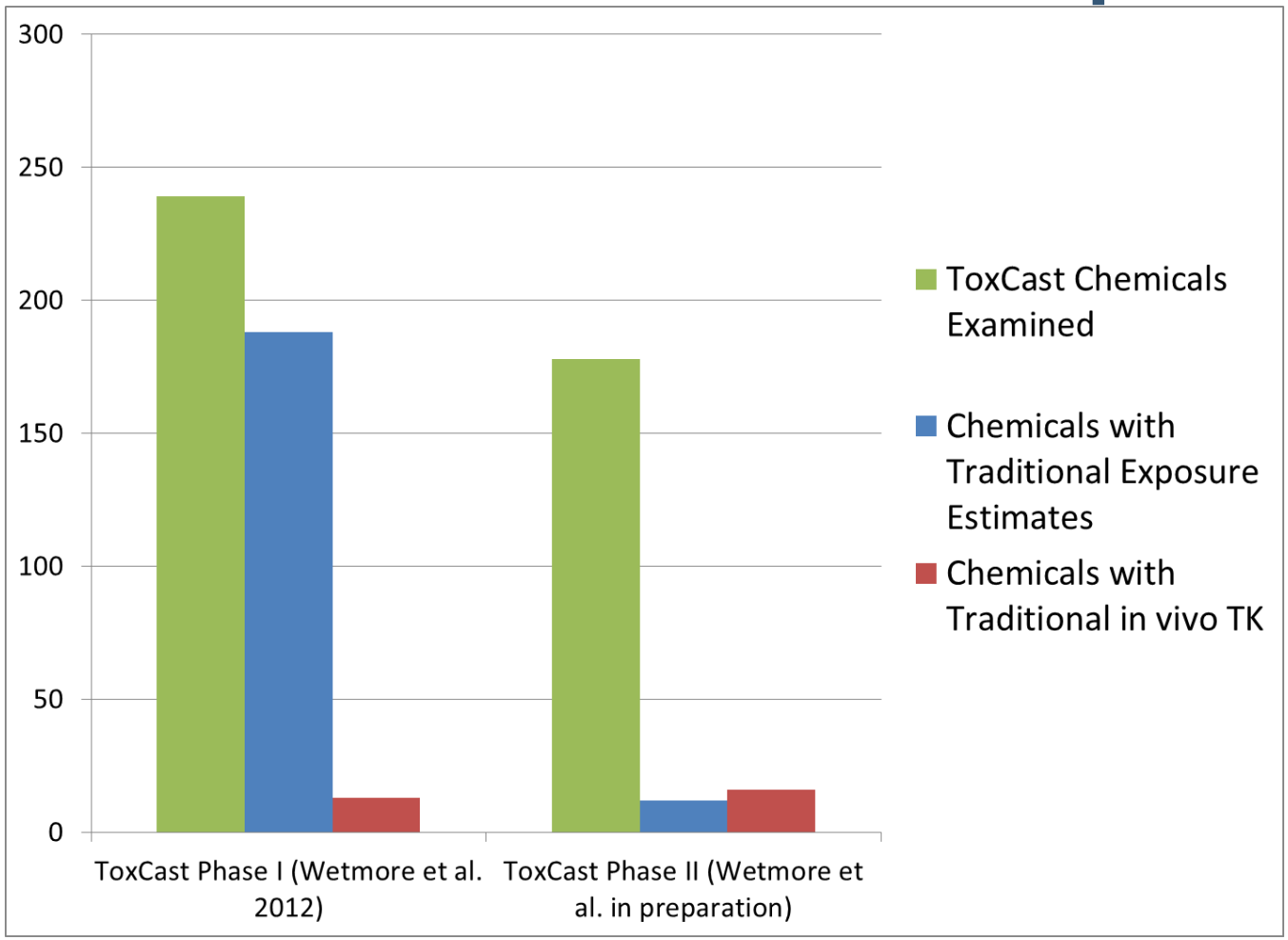
Risk Prioritization Requires Exposure

- **Tox21/ToxCast**: Examining thousands of chemicals using high throughput screening assays to identify *in vitro* concentrations that perturb biological pathways (Schmidt, 2009)
- In Wetmore *et al.* (2012), High throughput toxicokinetic *in vitro* methods are used to approximately convert *in vitro* bioactive concentrations (µM) into daily doses needed to produce similar levels in a human (mg/kg BW/day)
- These doses can then be directly compared with exposure rates, ***where available***



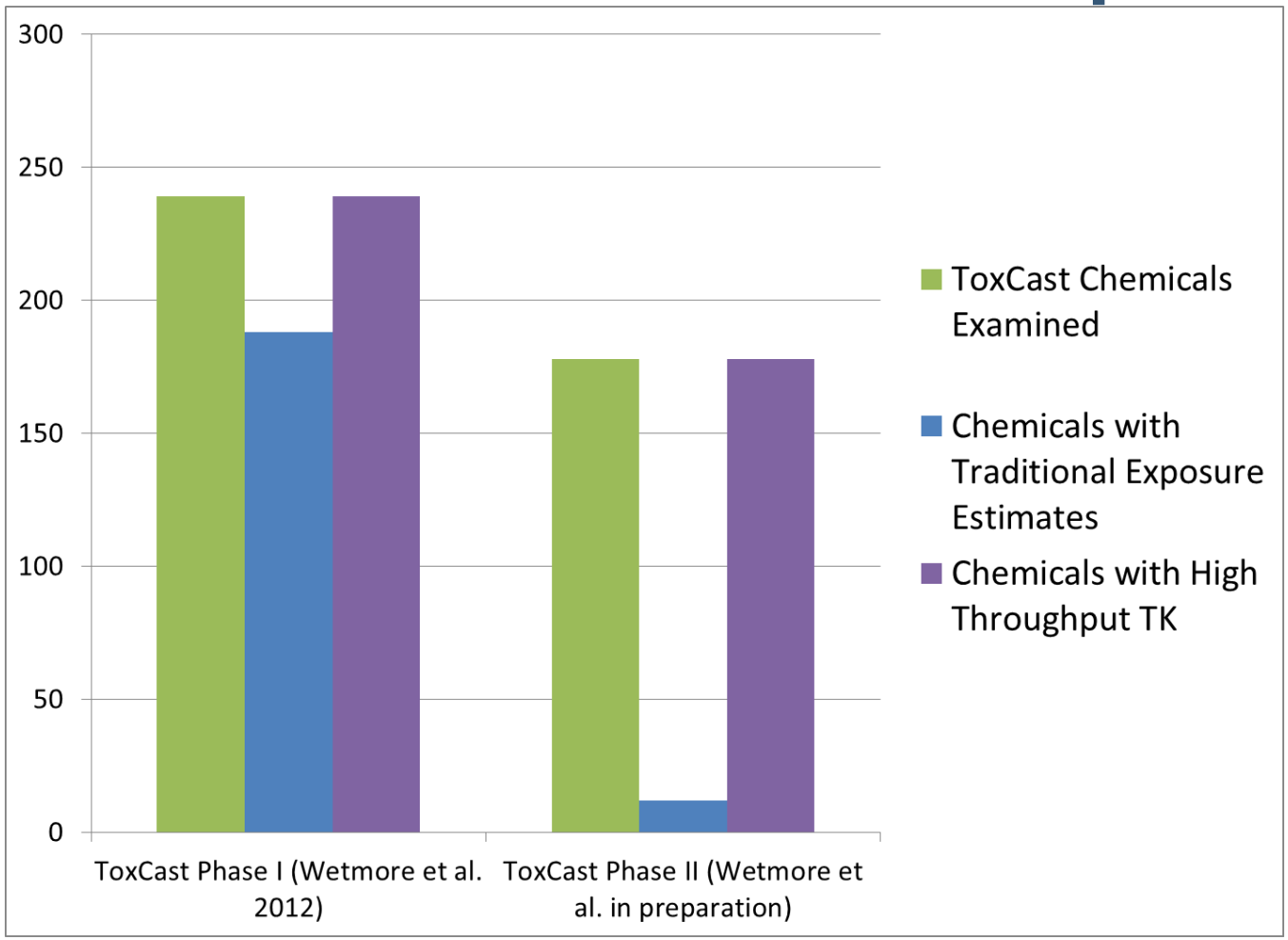
e.g. Judson *et al.*, (2011)

In Vitro Bioactivity, *In Vivo* Toxicokinetics, and Exposure



- Studies like Wetmore *et al.* (2012), addressed the need for toxicokinetic data

In Vitro Bioactivity, *In Vitro* Toxicokinetics, and Exposure



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

Goals for High Throughput Exposure

- Incorporate multiple models into consensus predictions for 1000s of chemicals
- Evaluate/calibrate predictions with available measurement data across many chemical classes
- Empirically estimate uncertainty in predictions

Exposure Space

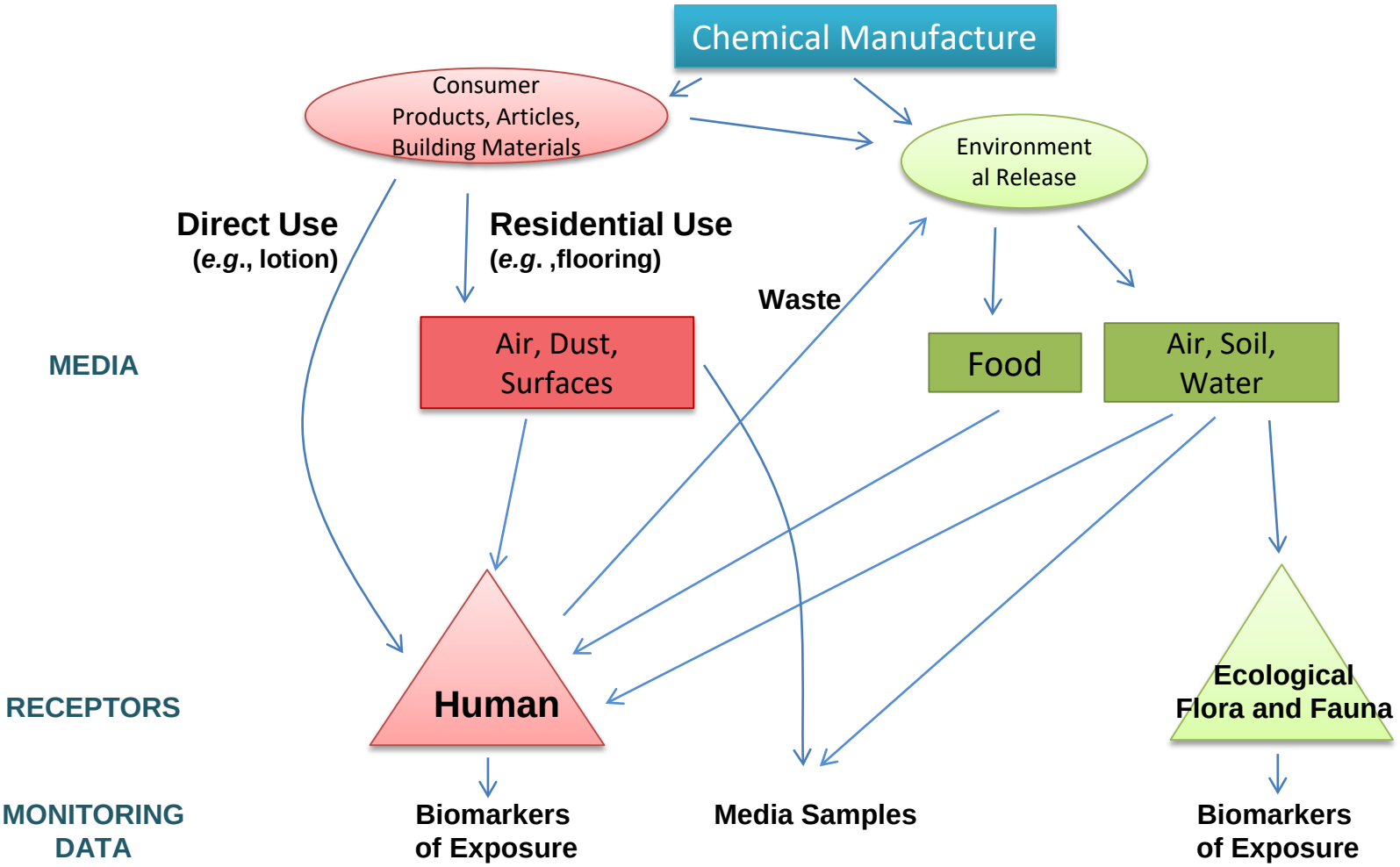


Figure from Kristin Isaacs

Exposure Pathways

United States Environmental Protection Agency

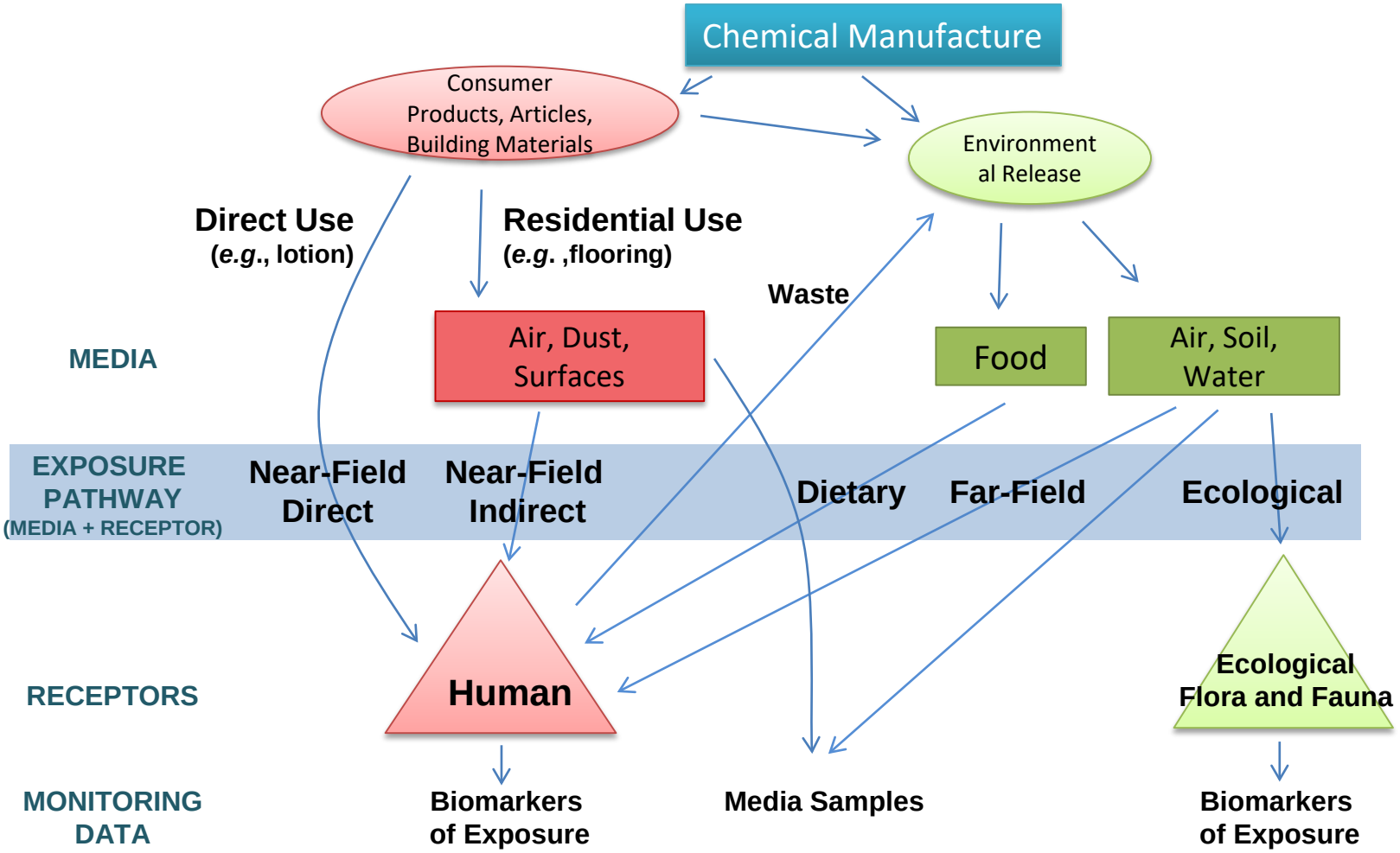
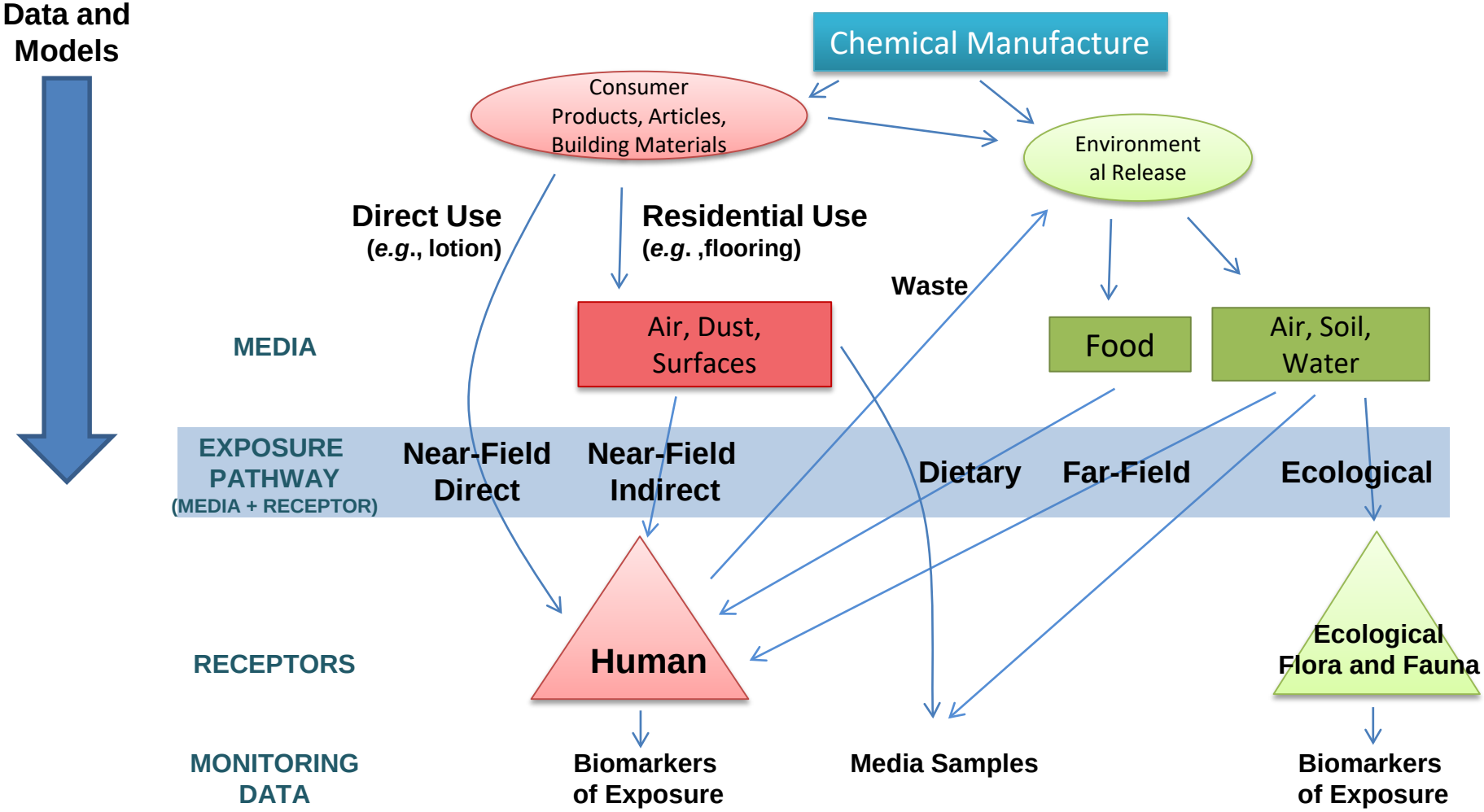


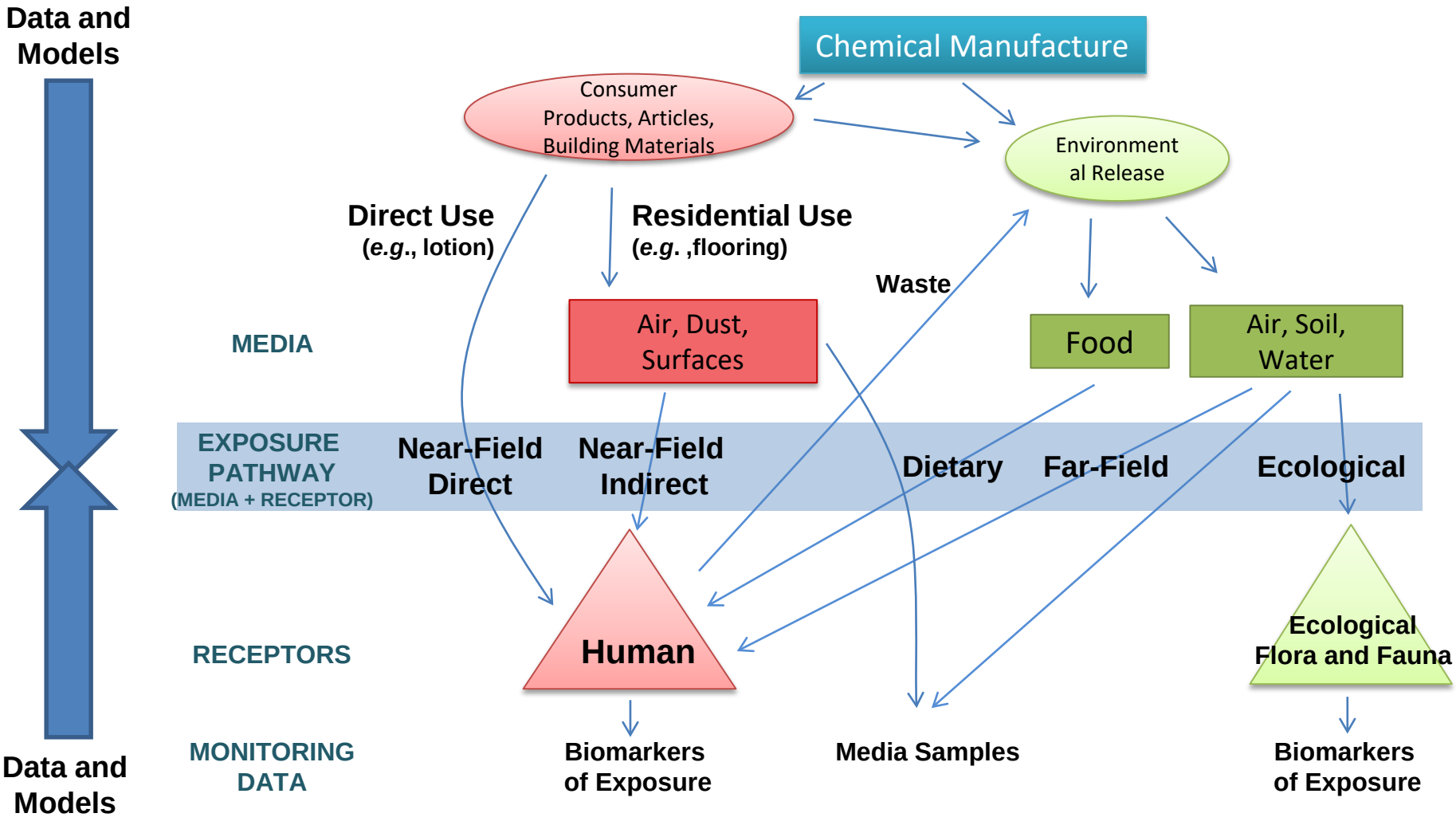
Figure from Kristin Isaacs

Forward Modeling of Exposure Pathways

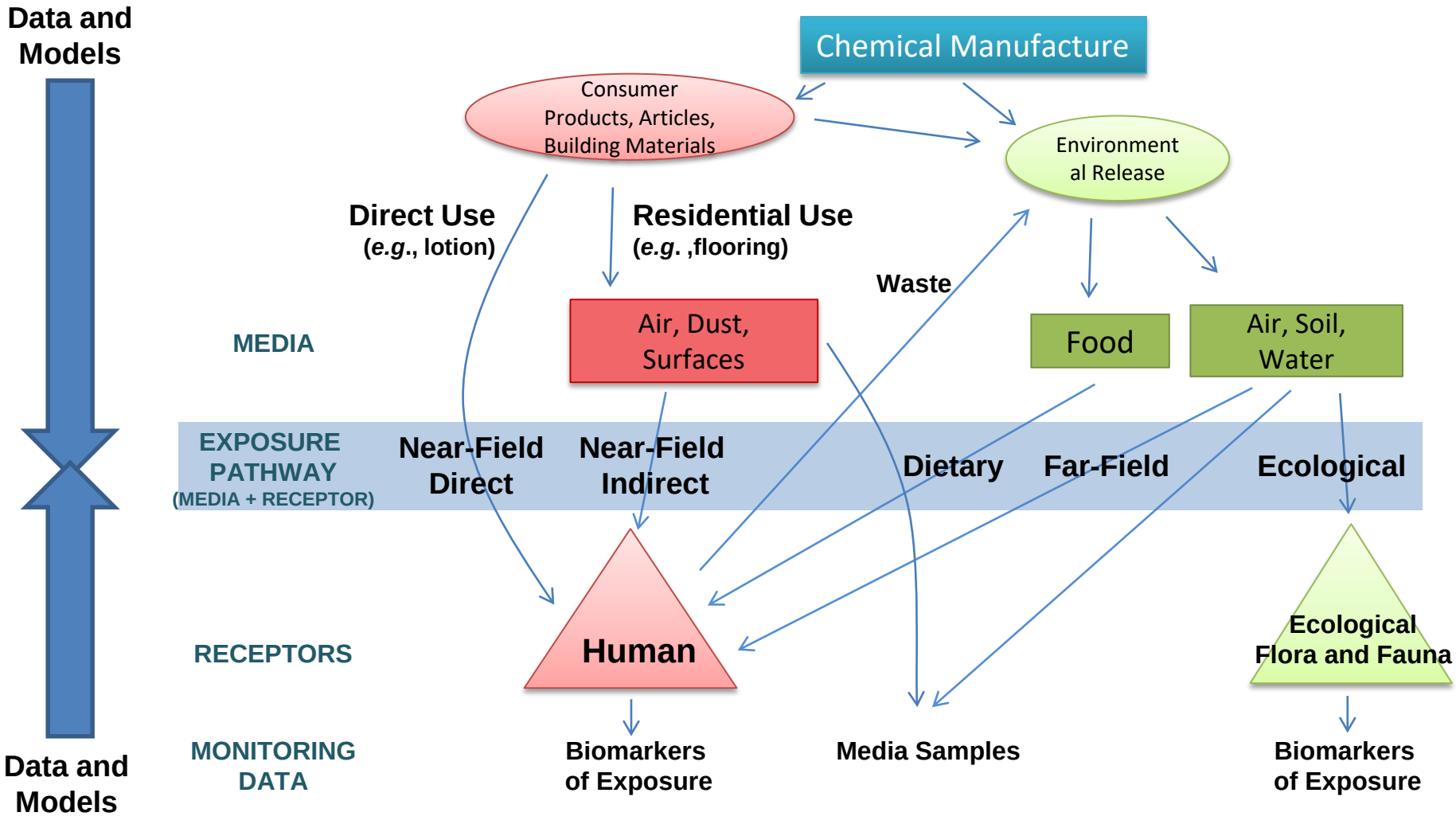
United States Environmental Protection Agency



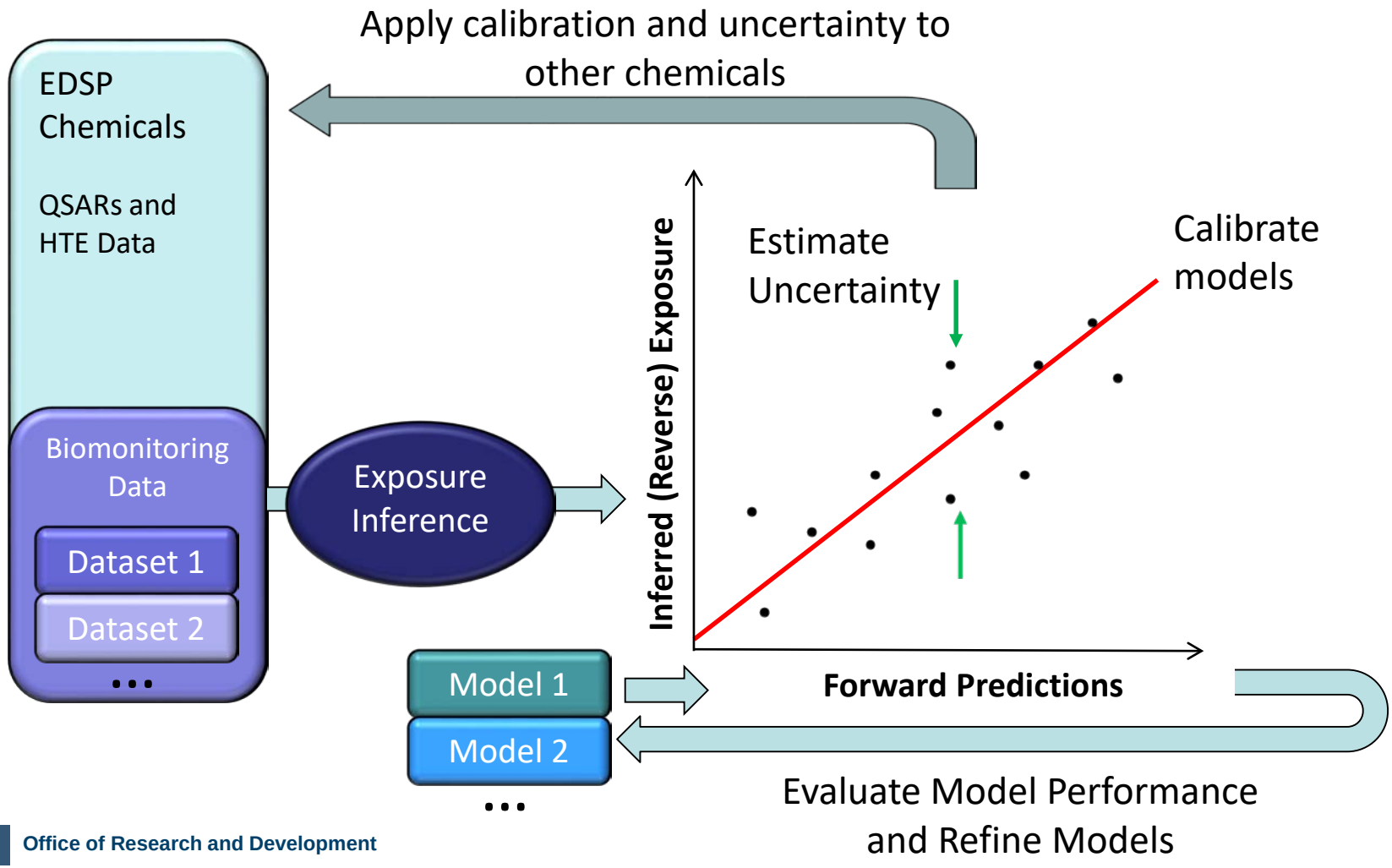
Inference of Exposure Pathways



Evaluation of Forward Predictions with Inferred Exposure

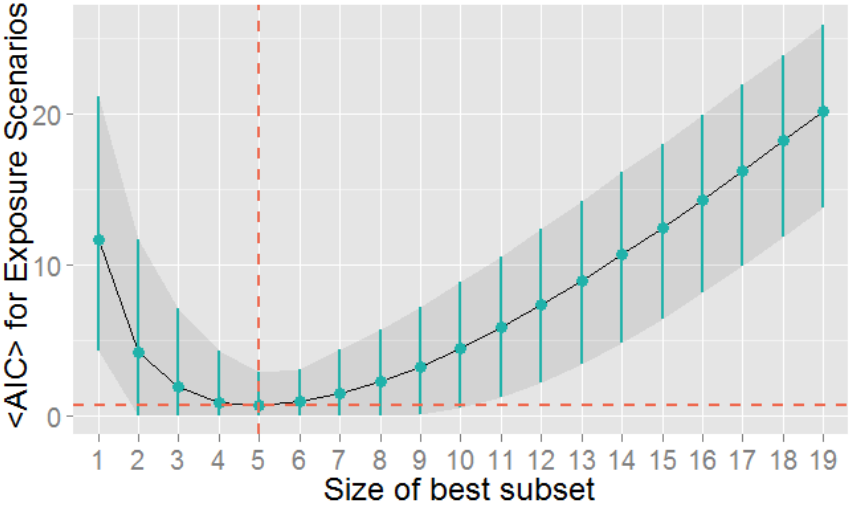


Systematic Empirical Evaluation of Models



High Throughput Descriptors for Exposure

United States Environmental Protection Agency



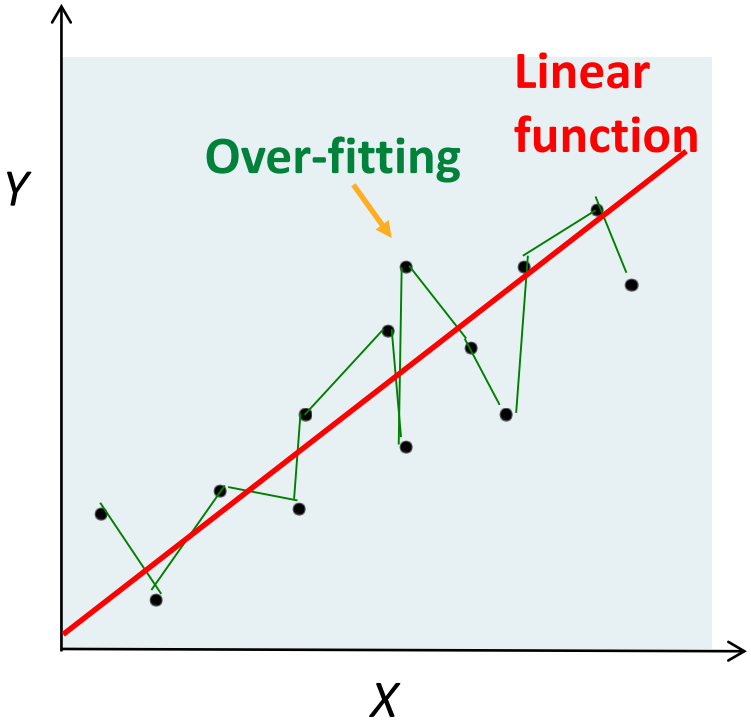
- Antimicrobial
- Colorant
- Food Additive
- Fragrance
- Herbicide
- Personal Care
- Pesticide Active
- Pesticide Inert
- Flame Retardant
- Other
- Industrial no Consumer
- Consumer no Industrial
- Consumer & Industrial
- log(Vapor Pressure)
- log(Hydrophobicity)
- Molecular Weight
- log(Production Volume)
- Random 50%
- Random 10%

Yes / No Use Descriptors

Physico-chemical Properties (EPI Suite)

- The average relative AIC (smaller is better) for models made with different numbers of parameters for explaining 1500 different combinations of chemical exposures

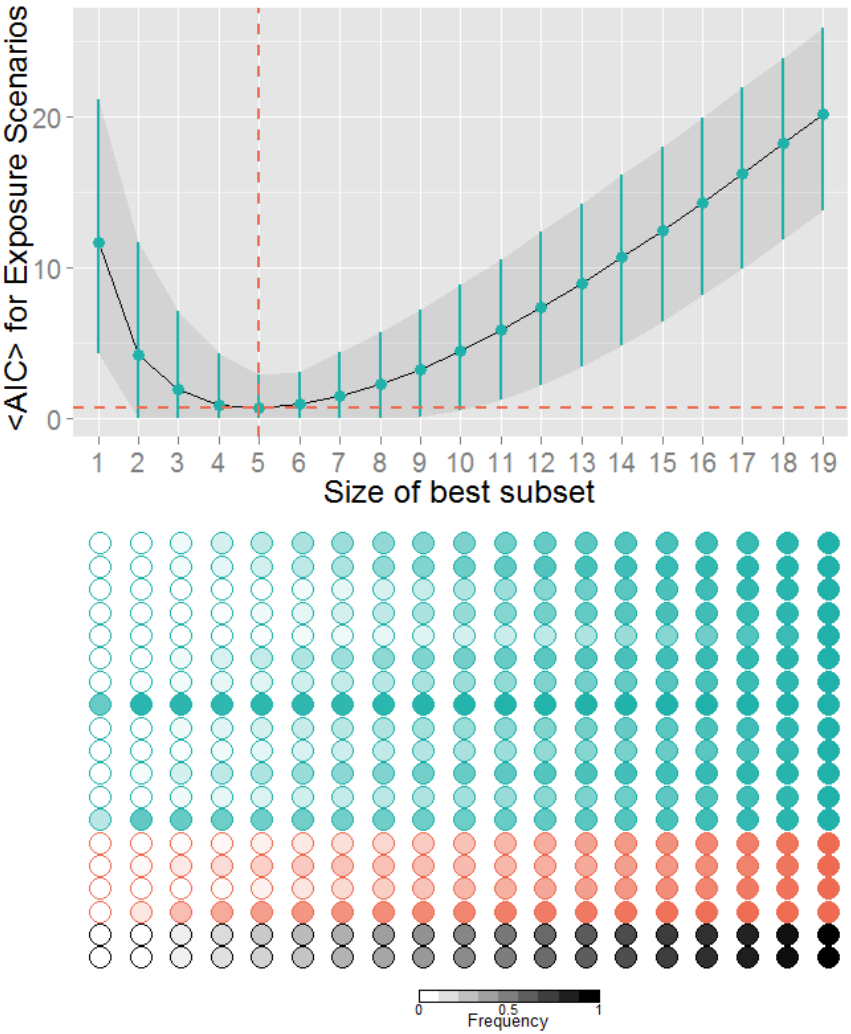
Noisy data and the danger of over-fitting



Environmental Science & Technology, *in press*

Not All Descriptors Are Useful

United States Environmental Protection Agency

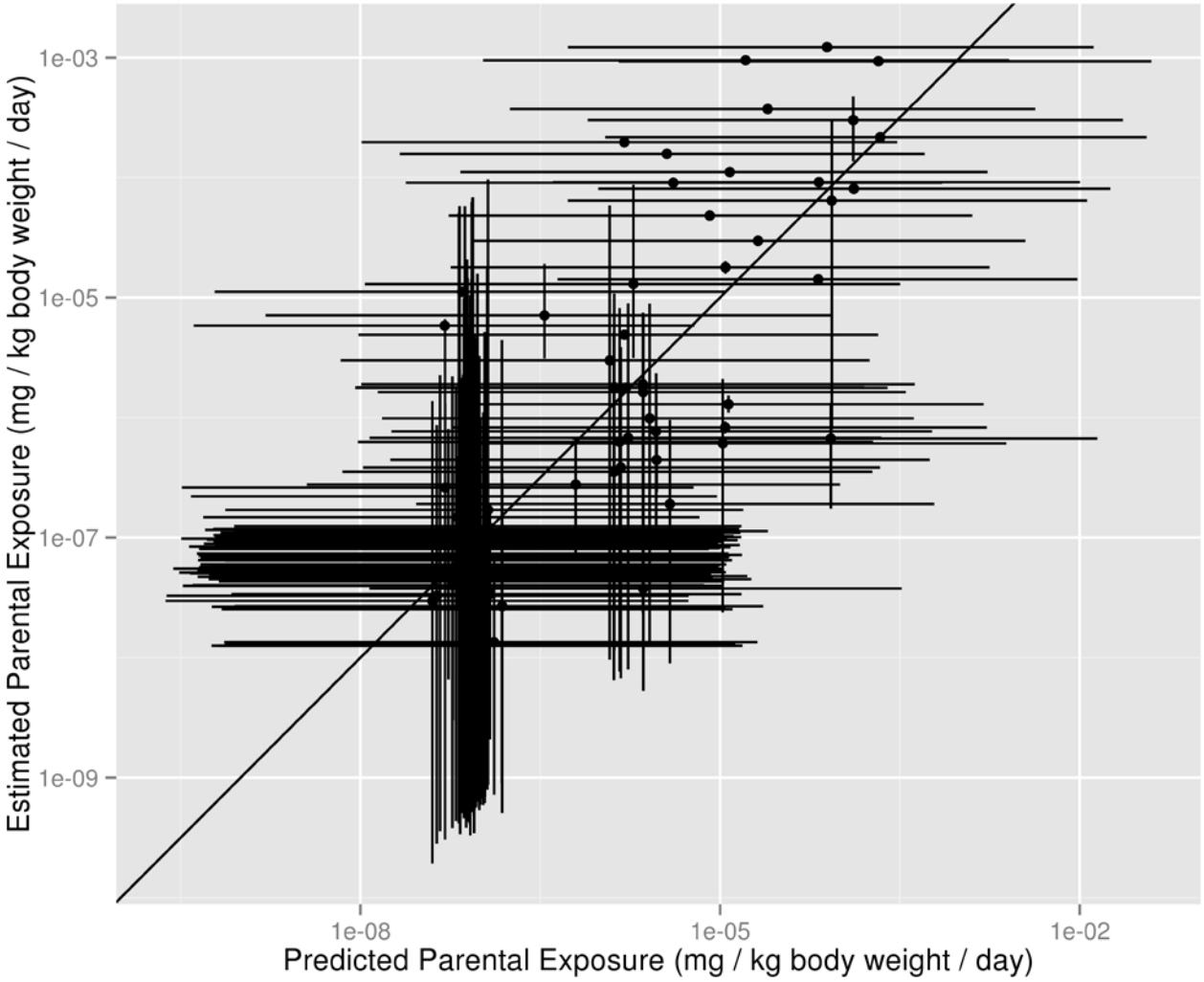


- The average relative AIC (smaller is better) for models made with different numbers of parameters for explaining 1500 different combinations of chemical exposures
- The predictors involved in the optimal model with higher frequencies are represented by darker circles, and those with lower frequencies by lighter circles
- As a sanity check, two random variables generated from binomial distribution with probability 50% and 10% of obtaining 1, are not selected as optimal descriptors in the five factor model

Environmental Science & Technology, *in press*

Predicting NHANES exposure rates

United States Environmental Protection Agency



$R^2 \approx 0.5$ indicates that we can predict 50% of the chemical to chemical variability in mean NHANES exposure rates

Same five predictors work for all NHANES demographic groups analyzed – stratified by age, sex, and body-mass index

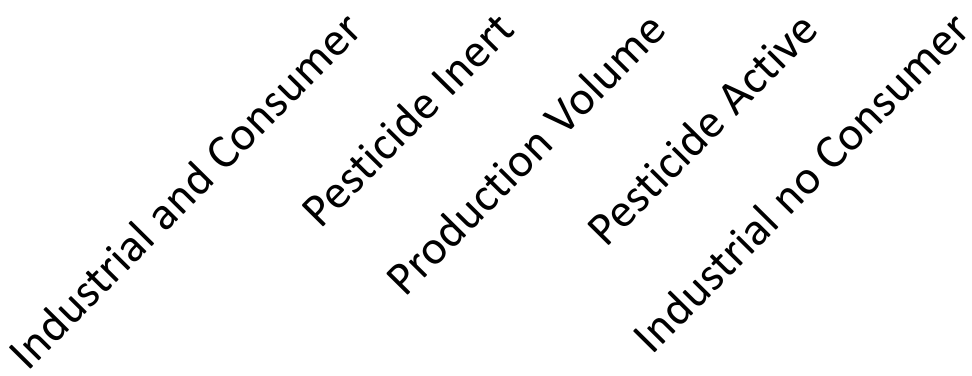
Environmental Science & Technology, *in press*

High-throughput exposure heuristics

Heuristic	Description	Number of Chemicals	
		Inferred NHANES Chemical Exposures (106)	Full Chemical Library (7784)
ACToR "Consumer use & Chemical/Industrial Process use"	Chemical substances in consumer products (*e.g.*, toys, personal care products, clothes, furniture, and home-care products) that are also used in industrial manufacturing processes. Does not include food or pharmaceuticals.	**37**	683
ACToR "Chemical/Industrial Process use with no Consumer use"	Chemical substances and products in industrial manufacturing processes that are not used in consumer products. Does not include food or pharmaceuticals	**14**	282
ACToR UseDB "Pesticide Inert use"	Secondary (*i.e.*, non-active) ingredients in a pesticide which serve a purpose other than repelling pests. Pesticide use of these ingredients is known due to more stringent reporting standards for pesticide ingredients, but many of these chemicals appear to be also used in consumer products	**16**	816
ACToR "Pesticide Active use"	Active ingredients in products designed to prevent, destroy, repel, or reduce pests (*e.g.*, insect repellants, weed killers, and disinfectants).	**76**	877
TSCA IUR 2006 Total Production Volume	Sum total (kg/year) of production of the chemical from all sites that produced the chemical in quantities of 25,000 pounds or more per year. If information for a chemical is not available, it is assumed to be produced at <25,000 pounds per year.	**106**	7784

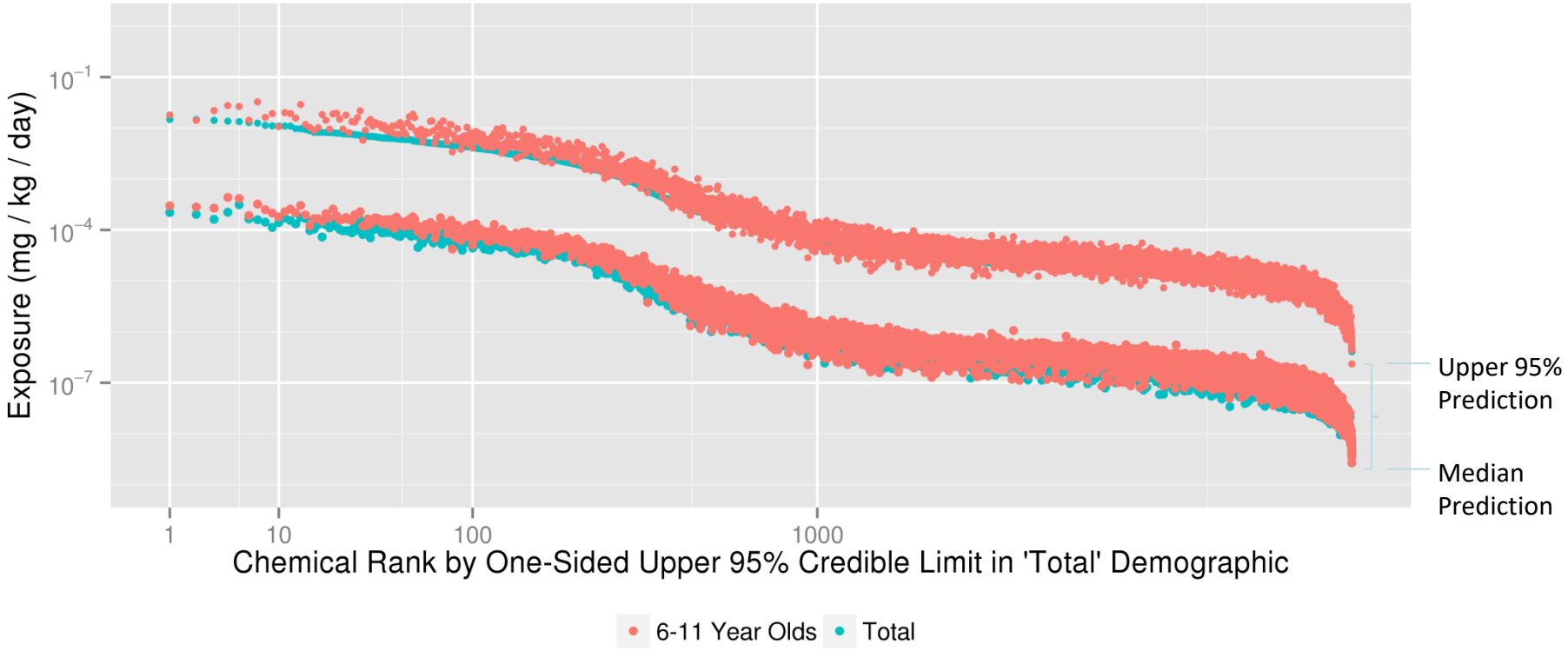
Environmental Science & Technology, *in press*

Predictors Do Not Vary Between Groups



- The vertical lines indicate the 95% credible interval across the 1500 different exposure scenarios inferred from the NHANES urine data
- SHEDS-HT (Isaacs et al., 2014) should help explain some remaining NHANES variability

Calibrated Exposure Predictions for 7968 Chemicals



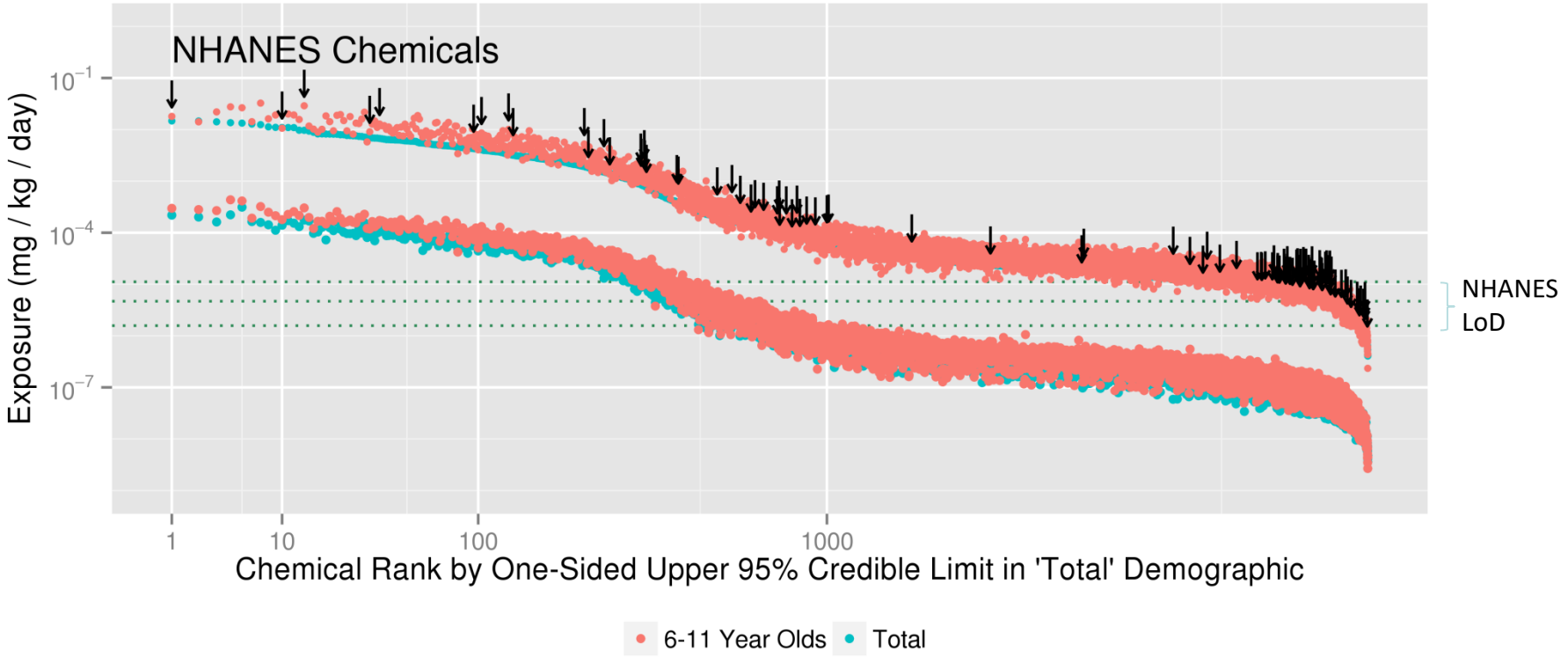
Environmental Science & Technology, *in press*

Calibrated Exposure Predictions for 7968 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

Environmental Science & Technology, *in press*

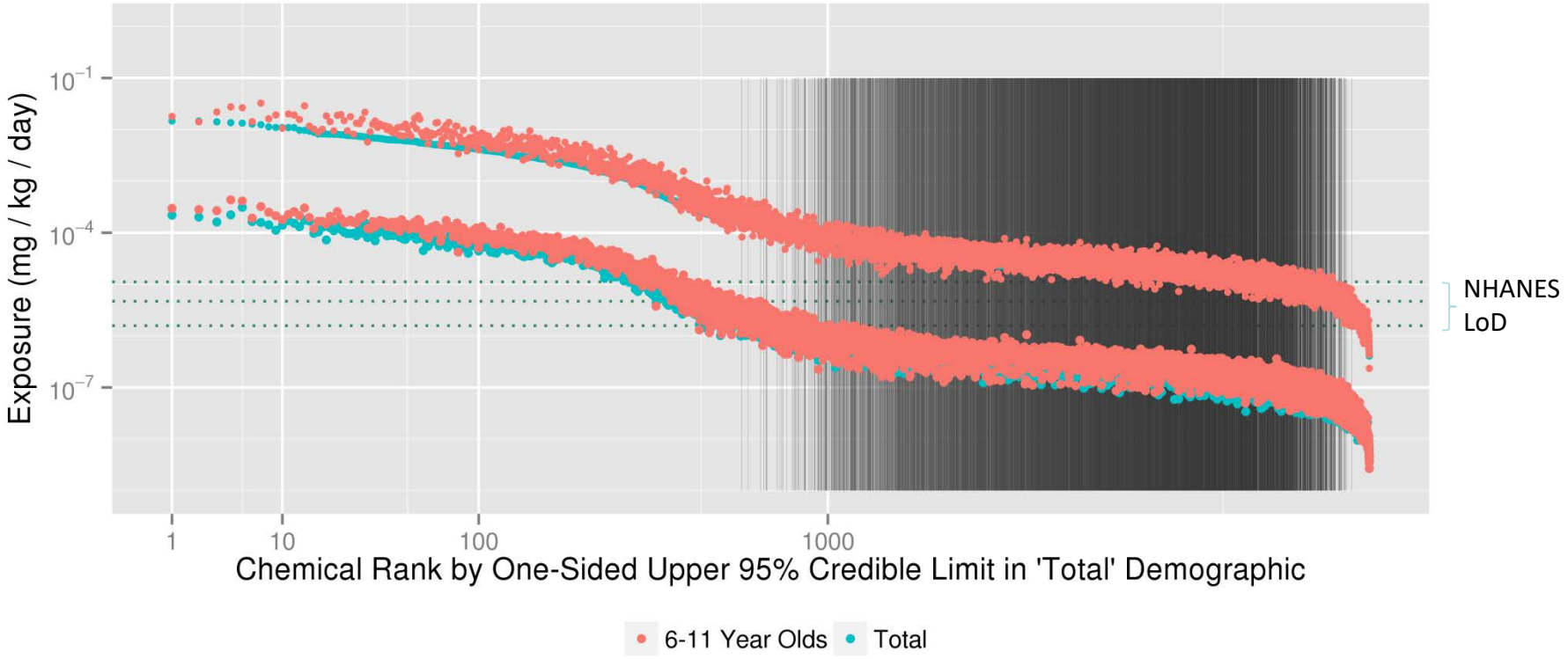
Calibrated Exposure Predictions for 7968 Chemicals



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

Environmental Science & Technology, *in press*

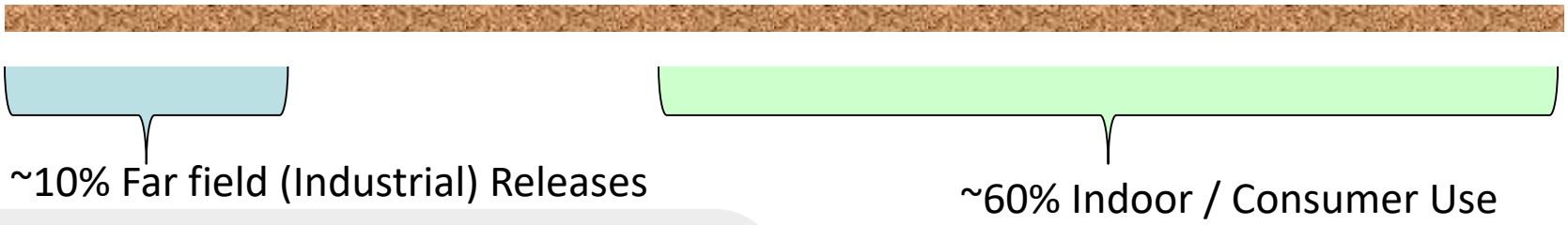
Calibrated Exposure Predictions for 7968 Chemicals



- The grey stripes indicate the 4182 chemicals with no use indicated by ACToR UseDB for any of the four use category heuristics

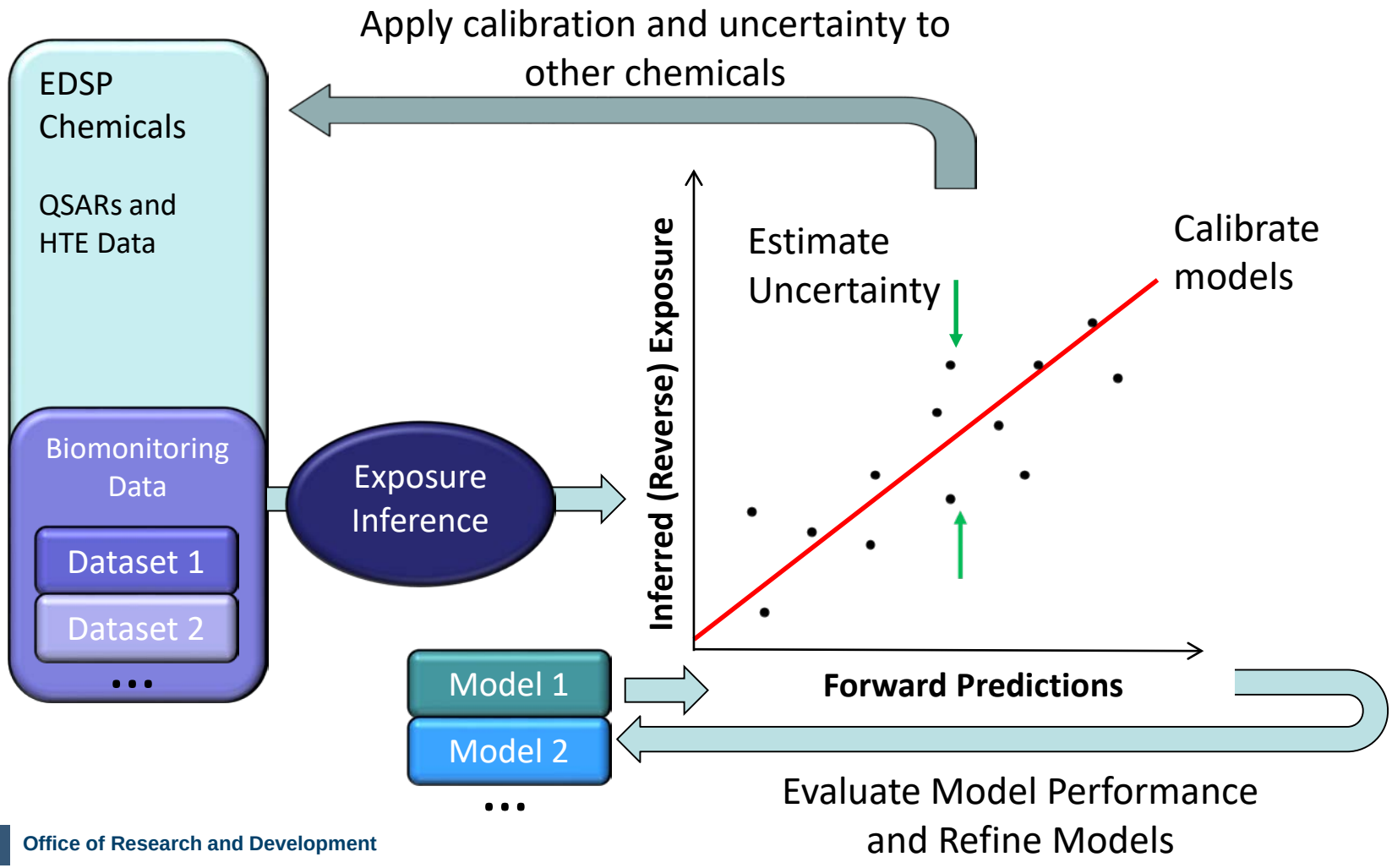
Environmental Science & Technology, *in press*

Better Models and Data Should Reduce Uncertainty



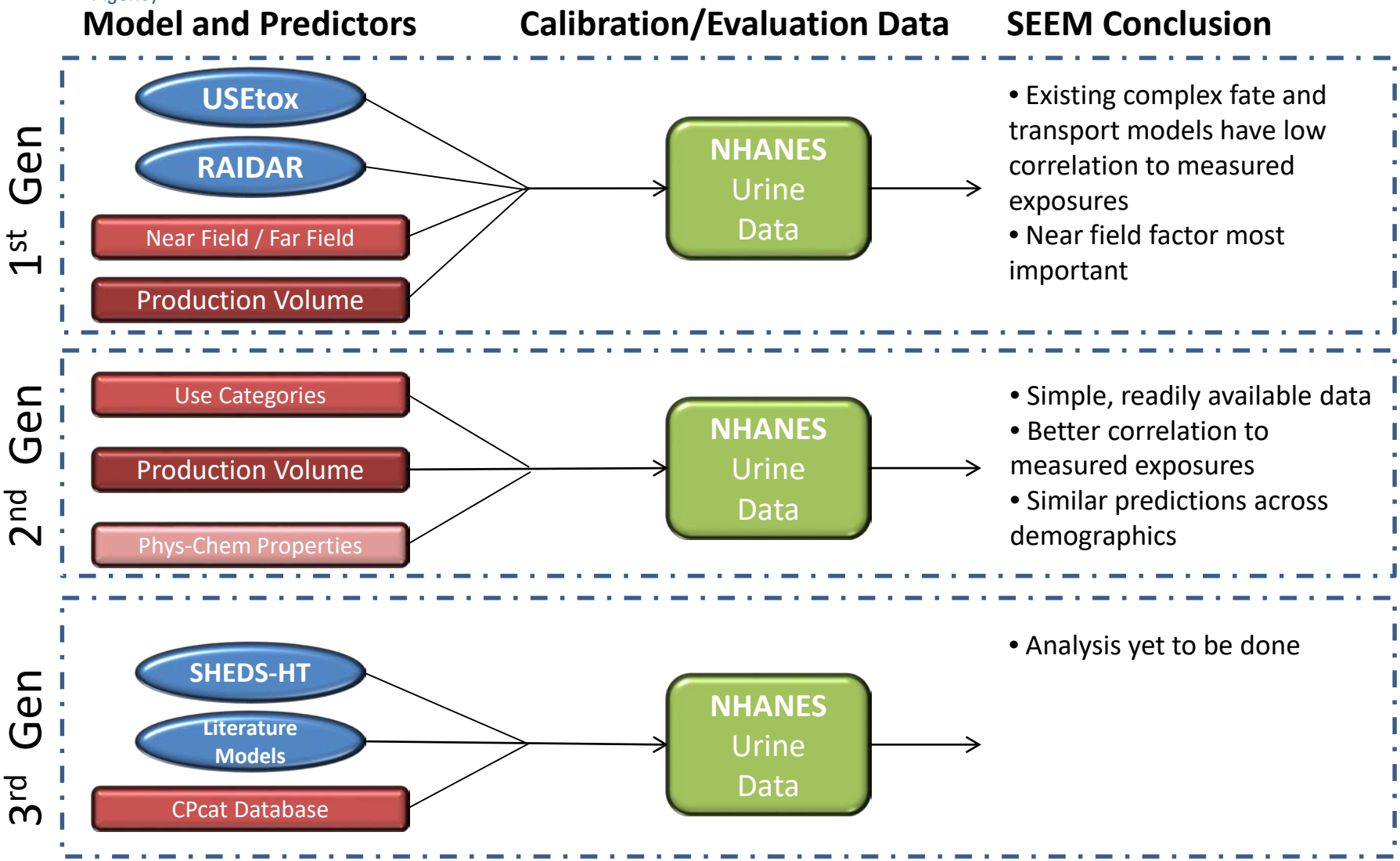
Consumer product database and two new near field models in 2014

Systematic Empirical Evaluation of Models



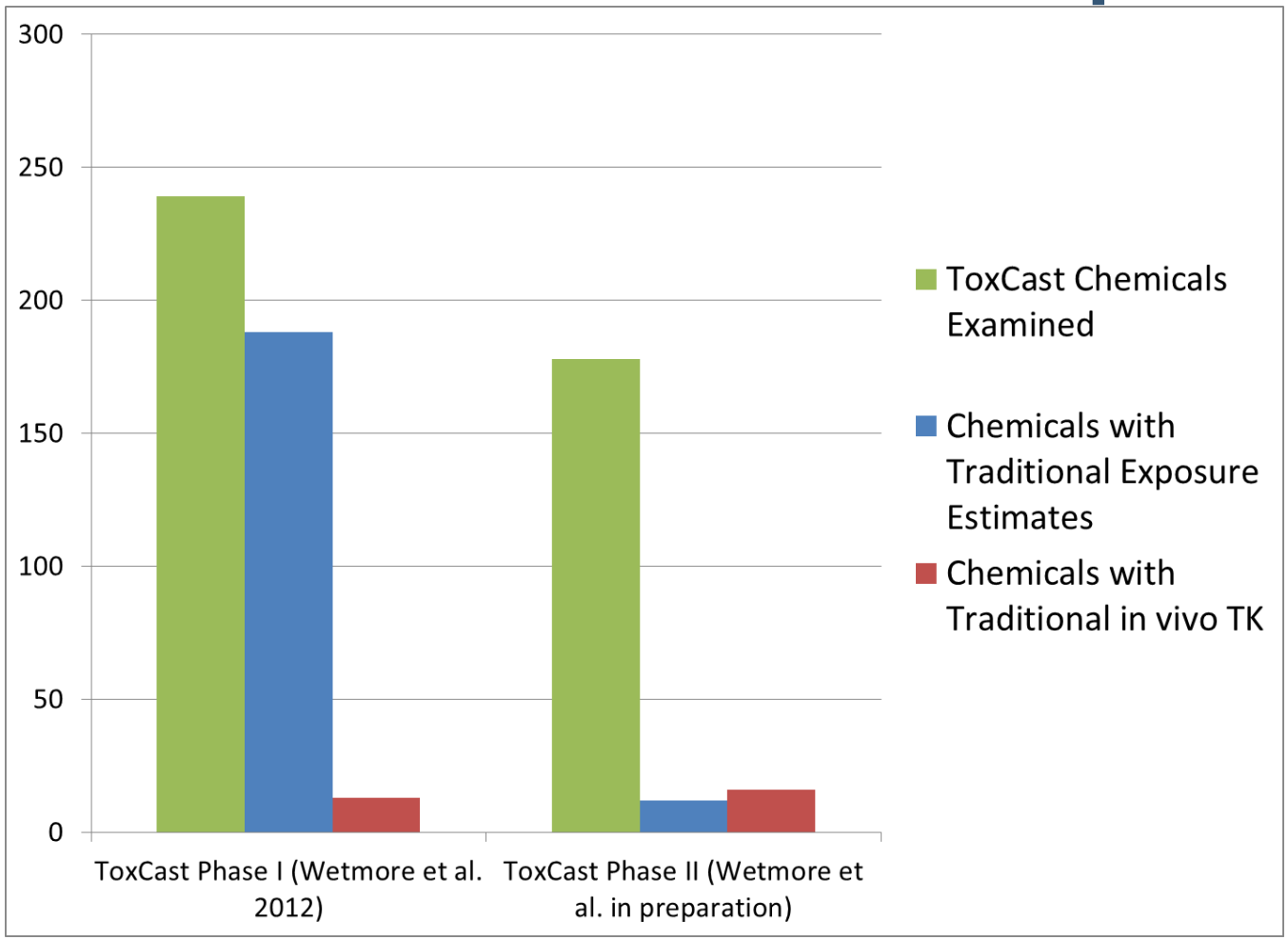
SEEM Evolution – Human Exposure

United States Environmental Protection Agency



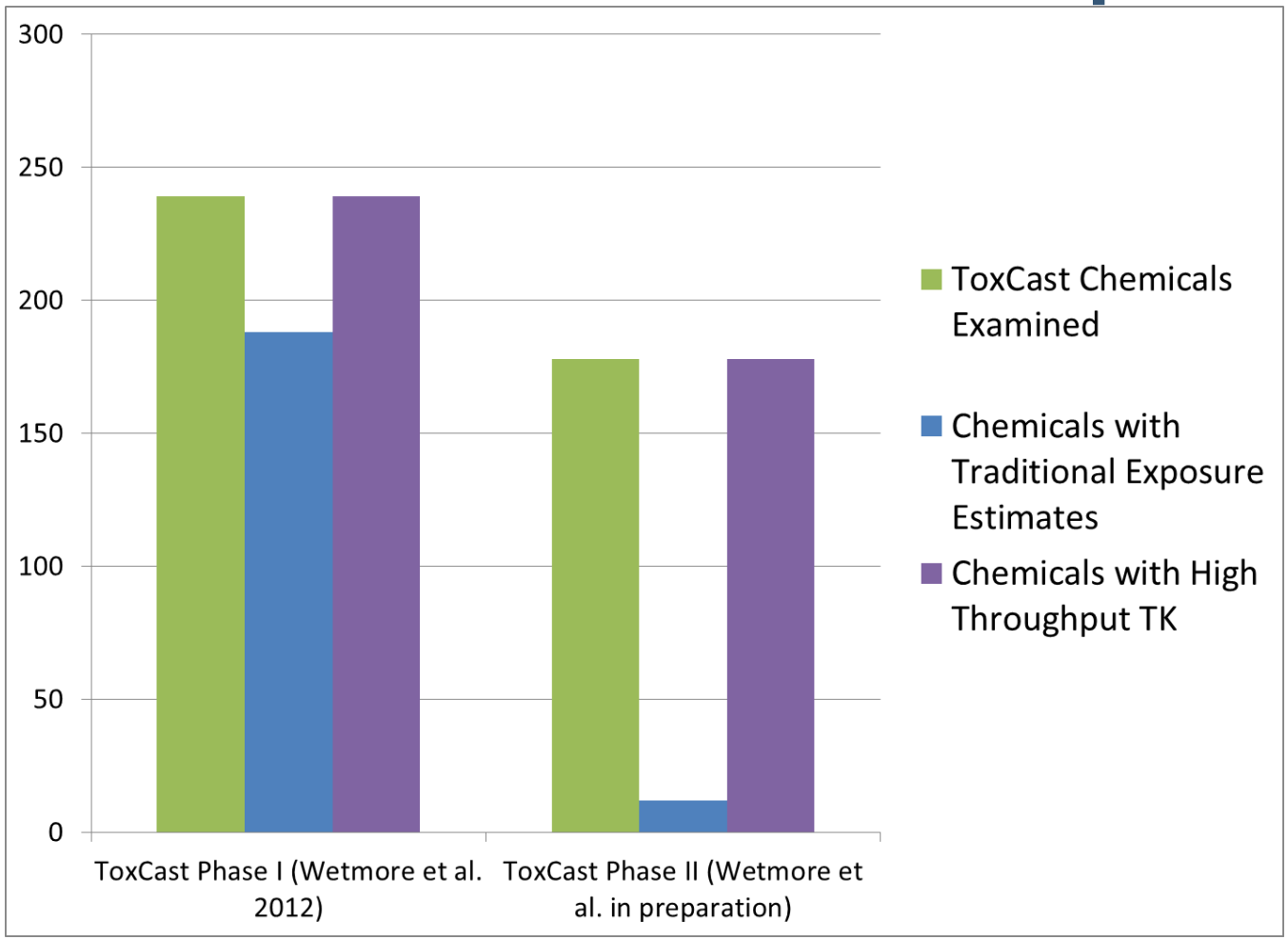
Approach described in Wambaugh *et al.* (2013) ExpoCast Framework Paper

In Vitro Bioactivity, *In Vivo* Toxicokinetics, and Exposure



- Studies like Wetmore et al. (2012),addressed the need for toxicokinetic data

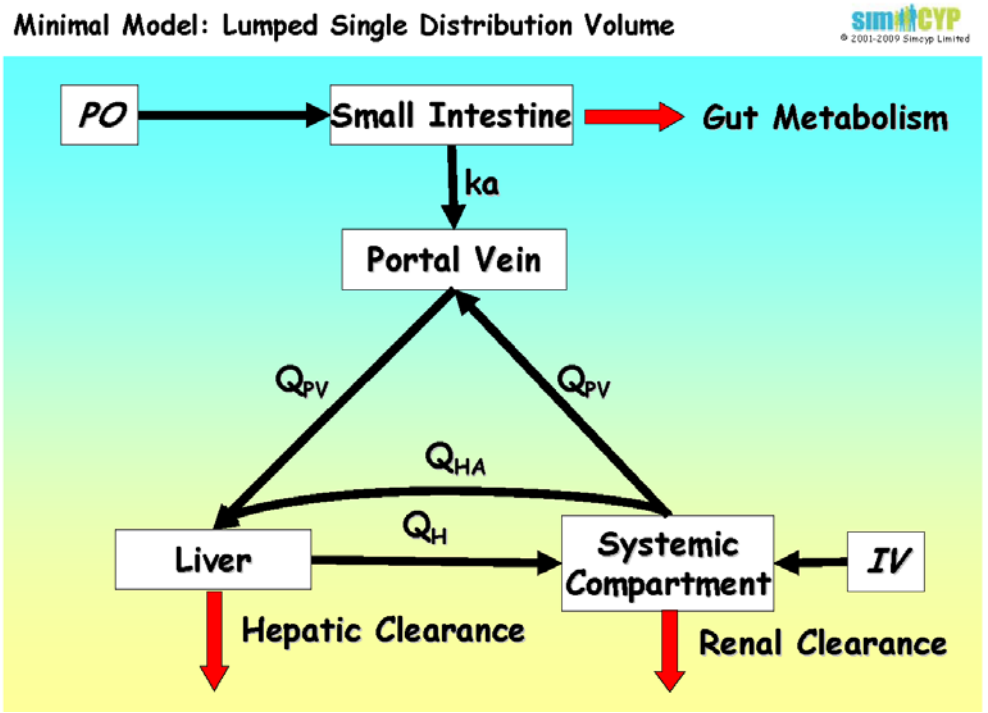
In Vitro Bioactivity, *In Vitro* Toxicokinetics, and Exposure



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

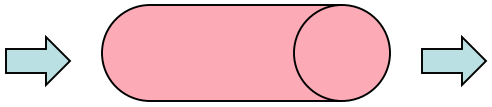
Steady-State Plasma Concentration

- *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



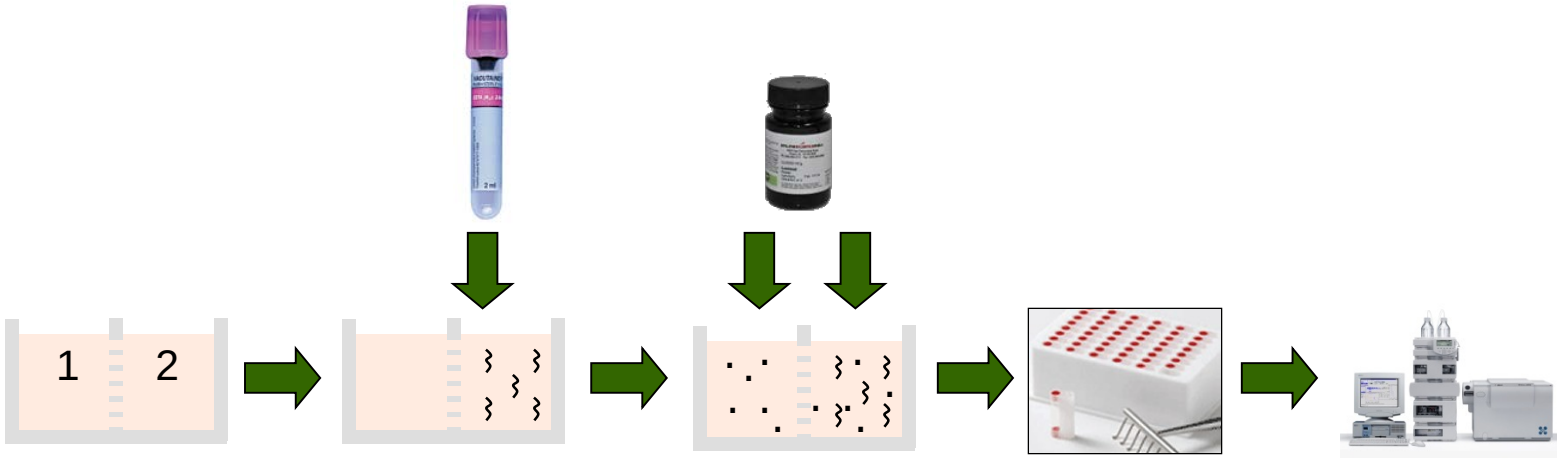
$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day)



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

Plasma Protein Binding (Fraction Unbound in Plasma)



Double-wells connected by semi-permeable membrane on a Rapid Equilibrium Dialysis (RED) Plate

Add plasma (6 donor pool for human) to one well

Add chemical

Incubate plates to allow wells with and without protein to come to equilibrium

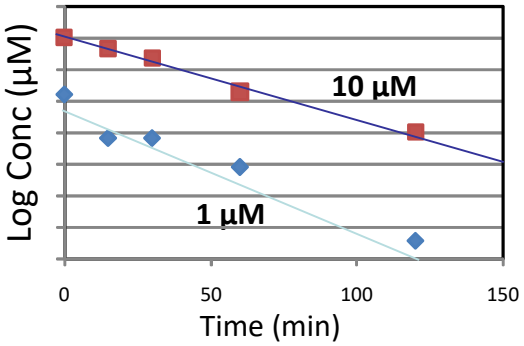
Determine concentration in both wells (analytical chemistry)

$$F_{ub,p} = \frac{C_{well1}}{C_{well2}}$$

- Data on ToxCast chemicals initially collected at Hamner Institutes
- Published:
  - Rotroff et al. (2010) - Pilot study using 38 Phase I ToxCast Chemicals
  - Wetmore et al. (2012) - Remainder of easily analyzed Phase I chemicals
  - Wetmore et al. (2013) Rat PK for 50 ToxCast/ToxRefDB compounds

RED Method: Waters *et al.* (2008)

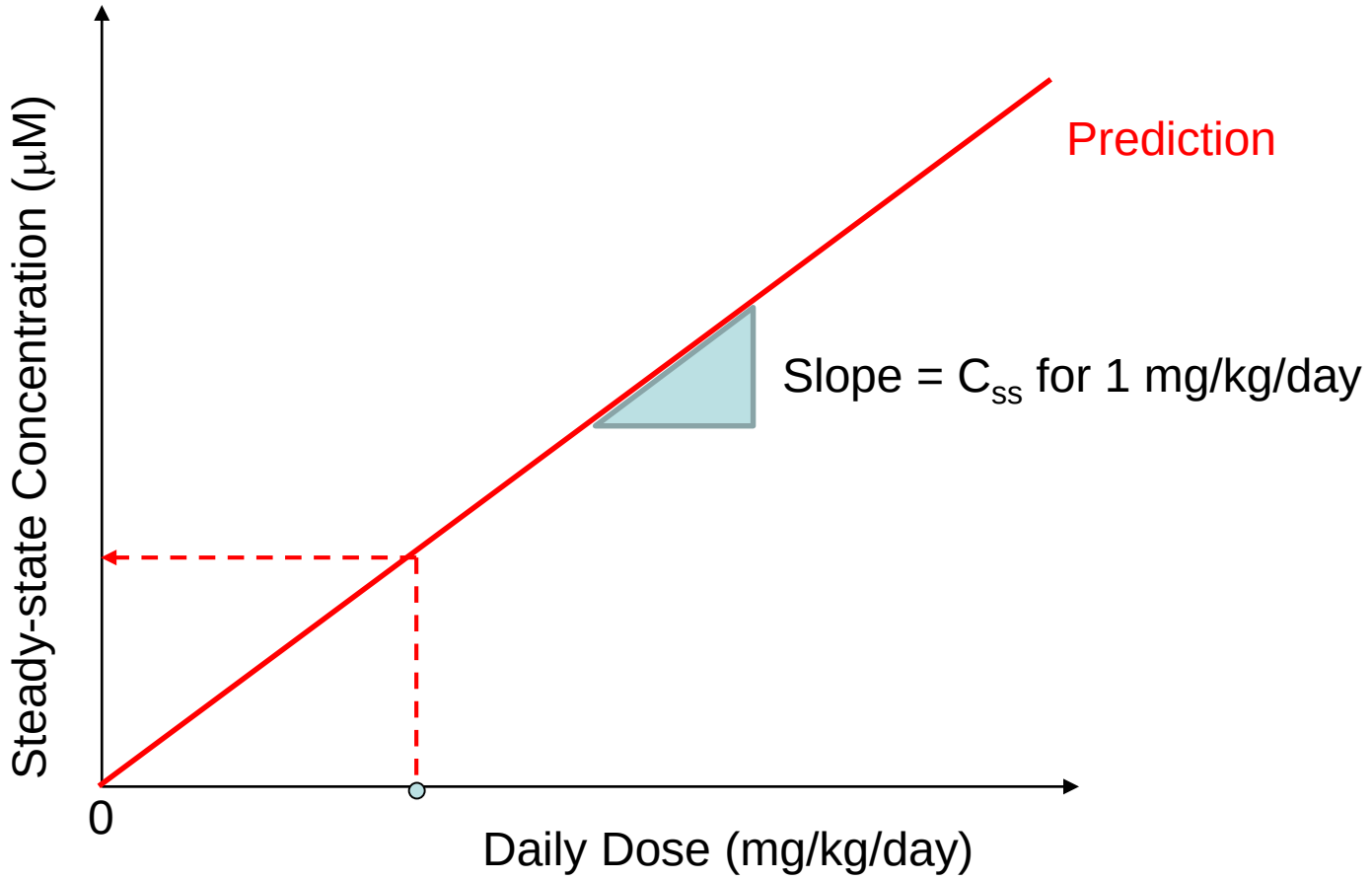
The rate of disappearance of parent compound (slope of line) is the **hepatic clearance** (µL/min/10^6 hepatocytes)



We perform the assay at 1 and 10 µM to check for saturation of metabolizing enzymes.

Cryopreserved hepatocyte Method: Shibata *et al.* (2002)

Steady-State Model is Linear

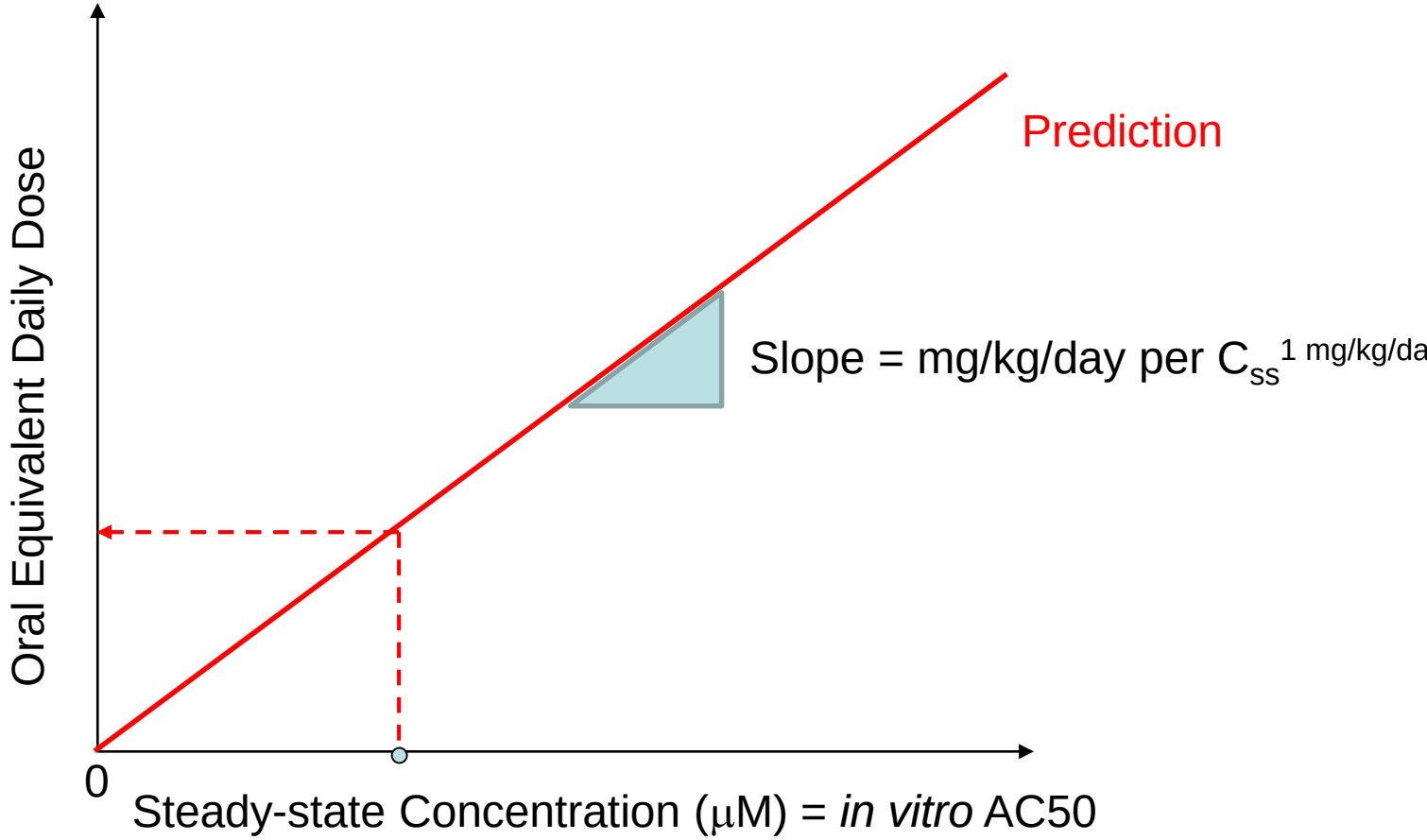


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

Wetmore *et al*. (2012)

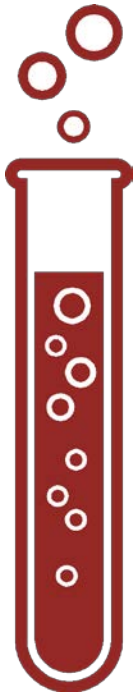
- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

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
  Wetmore *et al*. (2012)

High Throughput Toxicokinetics (HTTK)



High Throughput *In Vitro* Bioactive Concentration

HTTK *in vitro* data

Simulated Human *In Vivo* Doses

Monte Carlo Simulation of Biological Variability

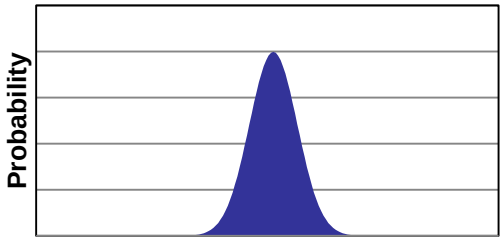
Combination of higher exposure and sensitivities

Populations that are More Sensitive

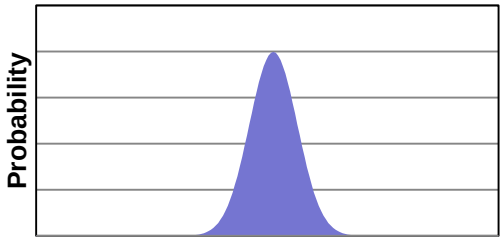
Approach described in Wetmore *et al*. (2012) Bioactivity, Dosimetry, and Exposure Paper

Images from Thinkstock

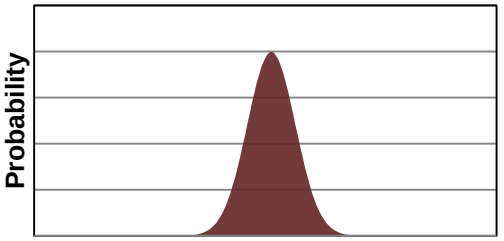
Monte Carlo (MC) Approach to Variability



log Liver Flow (Q_l)

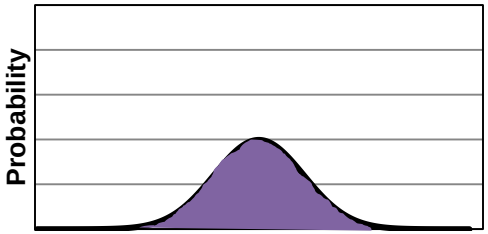


log Glomerular Filtration Rate…

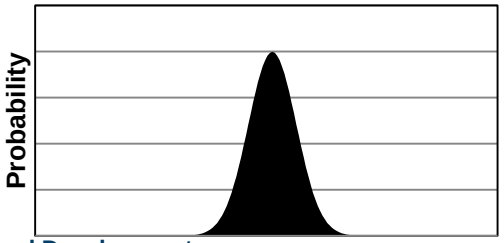


log Liver Volume

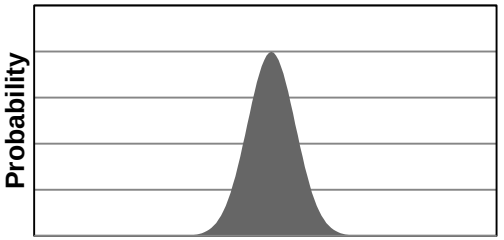
$$C_{ss} = \frac{\text{oral dose rate}}{\left(\text{GFR} * \text{F}_{ub}\right) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$



C_{ss}



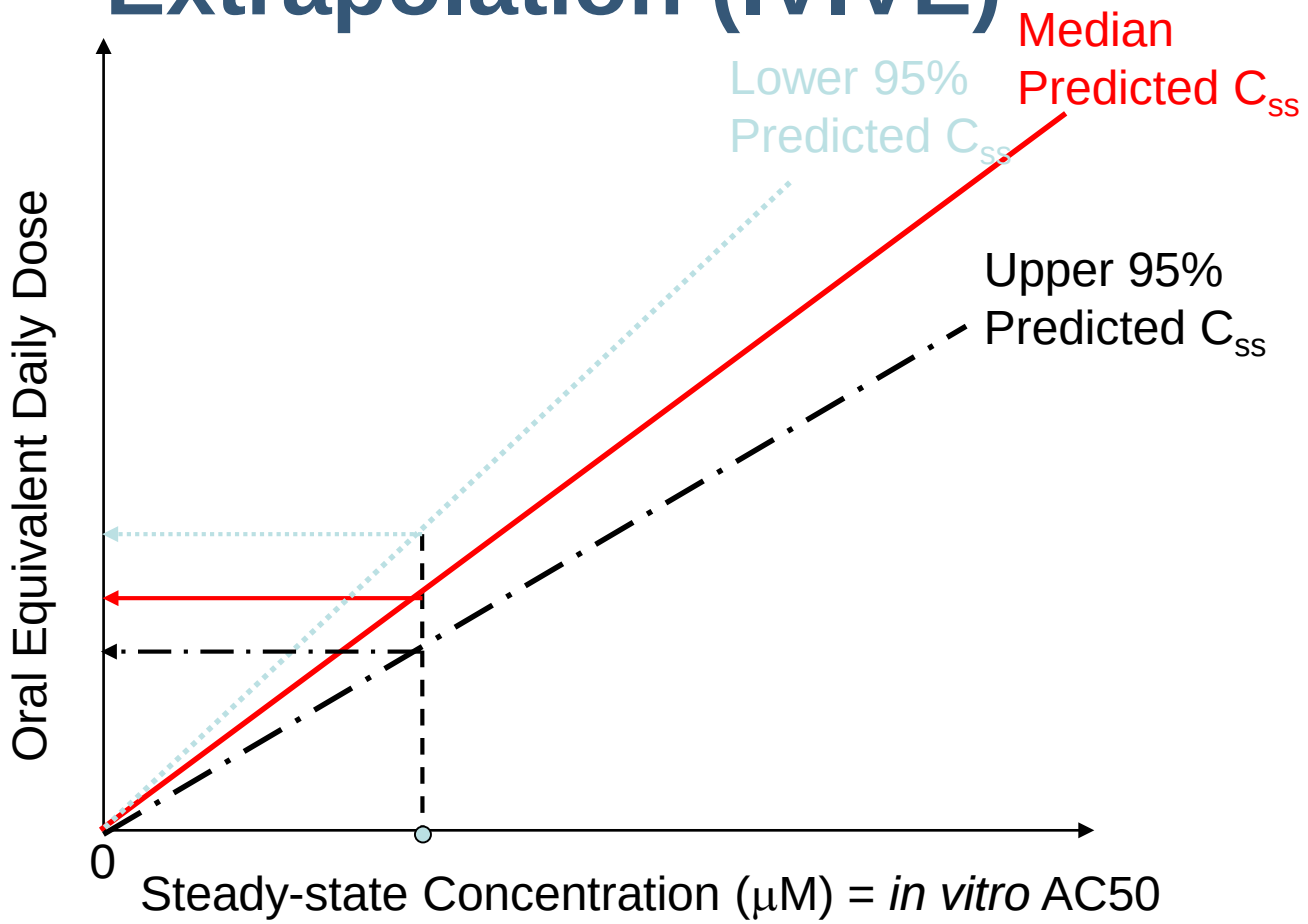
log Cl_{int} in vitro



log f_{ub}

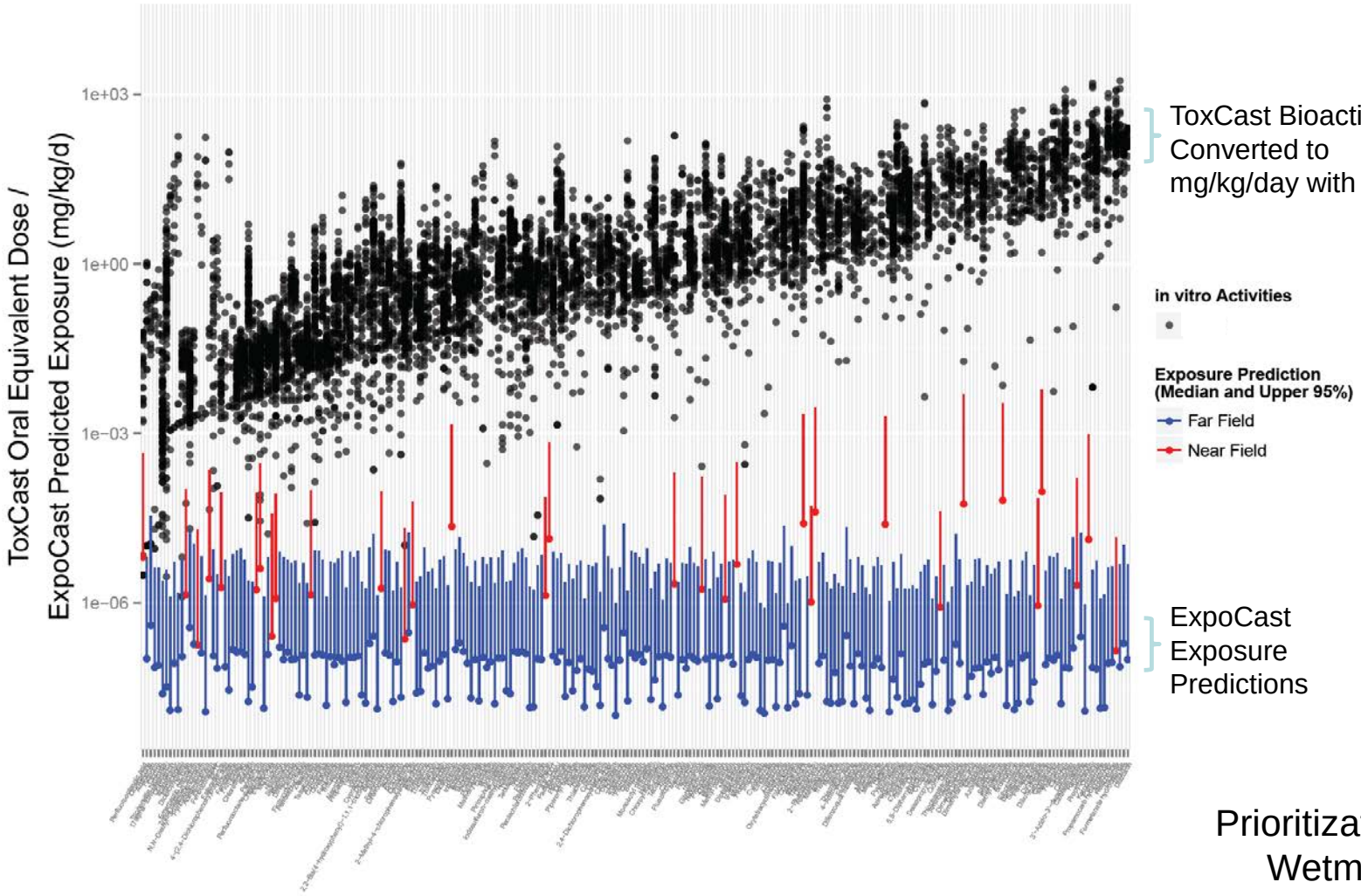
Wetmore *et al*. (2012)

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



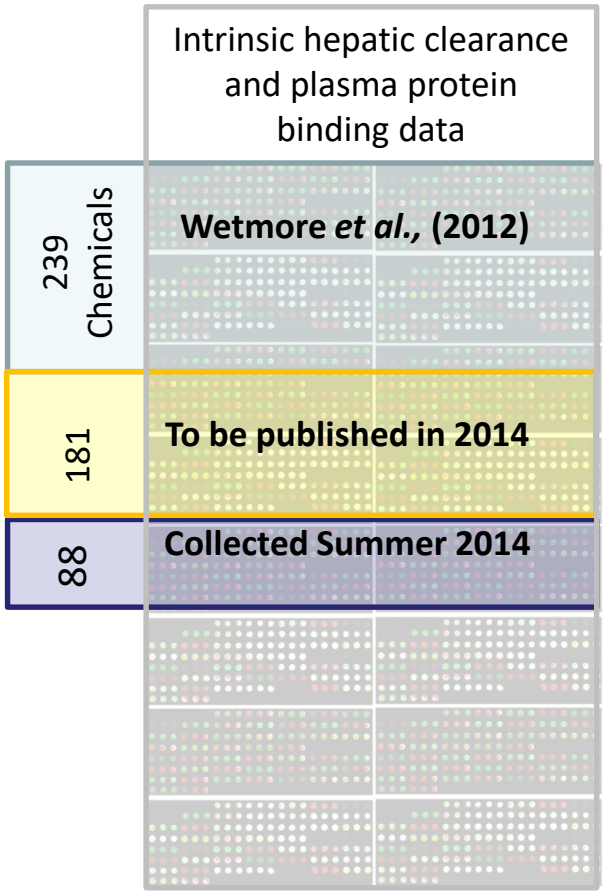
- The higher the predicted C_{ss}, the lower the oral equivalent dose, so the upper 95% predicted C_{ss} from the MC has a lower oral equivalent dose

High Throughput Risk Prioritization



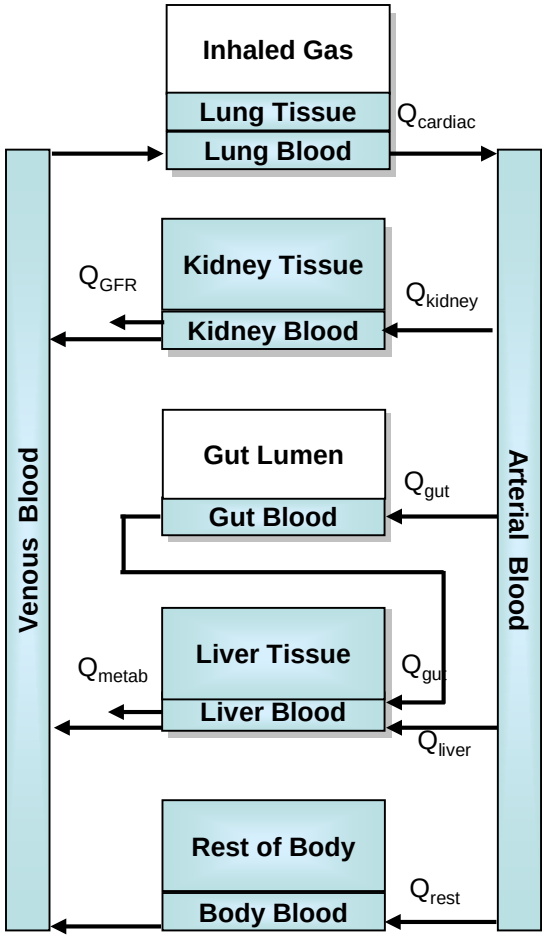
Prioritization as in Wetmore *et al*. (2012) Bioactivity, Dosimetry, and Exposure Paper

New HTTK Data



- ToxCast HTTK testing:
  - Measuring metabolism by human hepatocytes
  - Improved assays for measuring binding of chemicals to human plasma protein
  - Obtain data on ToxCast chemicals not investigated by the Hamner Institute studies
  - Reinvestigate chemicals that proved difficult in previous efforts
- This data will eventually allow determination of human oral equivalent doses (mg/kg BW/day) for most ToxCast chemicals.

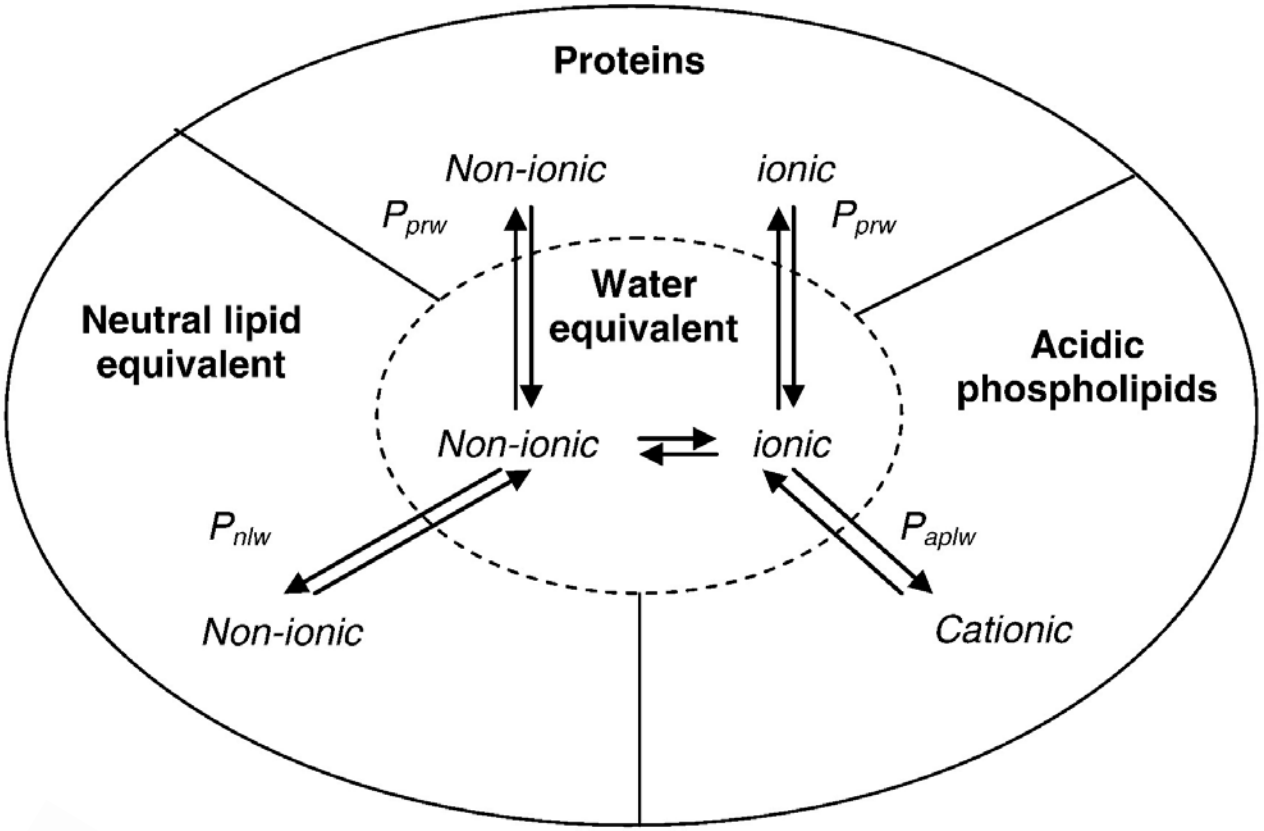
High Throughput Physiologically-based Toxicokinetics (HTPBTK)

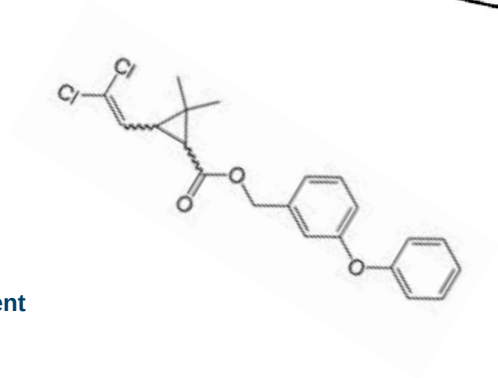


- Some tissues (*e.g.*, arterial blood) are simple compartments, while others (*e.g.*, kidney) are compound compartments consisting of separate blood and tissue sections.
- Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (*e.g.*, fat, brain, bones) are lumped into the "Rest of Body" compartment.
- Chemical enters the body primarily through oral absorption, but we don't know absorption rate and bioavailability (assume "fast", *i.e.* 1/h and 100%)
- The only ways chemicals "leaves" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

Predicted Partition Coefficients

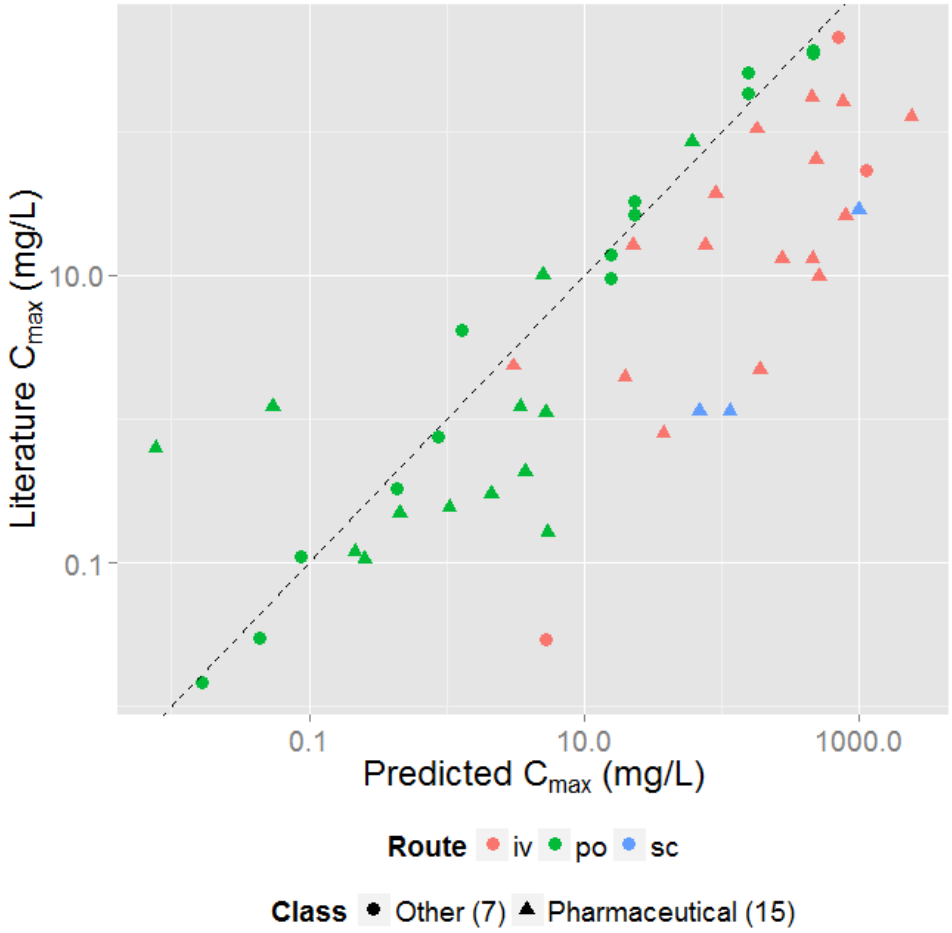
- Tissue-specific partitioning estimated (Schmitt, 2008) using:
  - Physicochemical properties (logP, pKa) predicted from structure (EPI Suite)
  - Measured fraction unbound in plasma (f_{ub})





Partitioning figure is from Peyret (2010),

Evaluating HTPBTK Predictions from *In Vitro* Data



- HTPBTK predictions for the peak plasma concentration (C_{max})
- *in vivo* measurements from the literature for various treatments (dose and route) of rat
- C_{max} predictions and *in vivo* data are correlated (R^2 ~ 0.65)

Conclusions

- By evaluating performance of high throughput exposure models against monitoring data we develop a calibration and estimate of uncertainty that we can apply to thousands of chemicals (ExpoCast)
- Currently analyzing the output of the first generation (2014) of mechanistic high throughput near field (*e.g.*, consumer use) models parameterized from minimal chemical-specific information
- Already know that this information alone can explain roughly half of the chemical-to-chemical variance in exposure inferred from biomonitoring data
- Also need HTTK data to convert *in vitro* bioactivity (*e.g.*, ToxCast) to exposures for comparison with ExpoCast
- Can use this data to build HTPBTK models, and need to develop high throughput dermal exposure approach

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