In Vitro – In Vivo Extrapolation for High-Throughput Prioritization and Decision-Making

Setting the Stage

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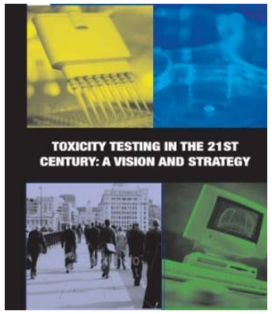
THE HAMNER INSTITUTES FOR HEALTH SCIENCES
WHERE GREAT MINDS & MEDICINE MEET

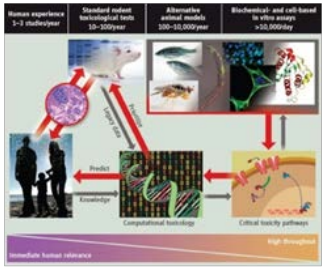
In Vitro-to-*In Vivo* Extrapolation for High-Throughput Prioritization and Decision-Making

- Webinars: First Wednesdays, 11:00AM E.D.T.
  - October 7 – Barbara Wetmore: Setting the Stage
  - November 4 – John Wambaugh: Model Development
  - December 2 – Lisa Sweeney: Model Evaluation
  - January 6, 2016 – TBD: State of the Science

- In-person Meeting: February 17-18, 2016
  - US EPA, Research Triangle Park, NC

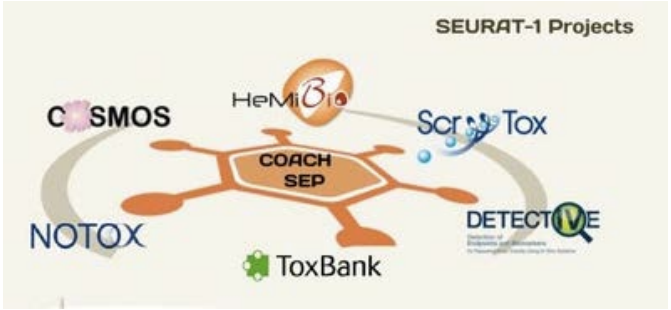
Broad-Based Movement in Toxicology Towards *In Vitro* Testing and Hazard Prediction



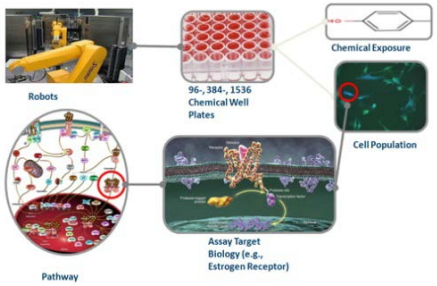


Collins *et al.*, Science 319:906, 2008

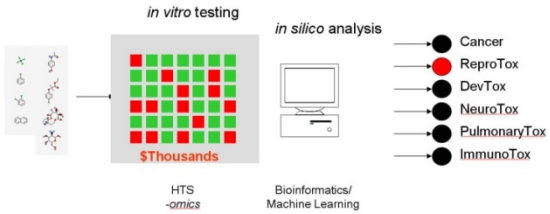
carcinoGENOMICS
a Project of the European Union



ToxCast™ Chemical Screening

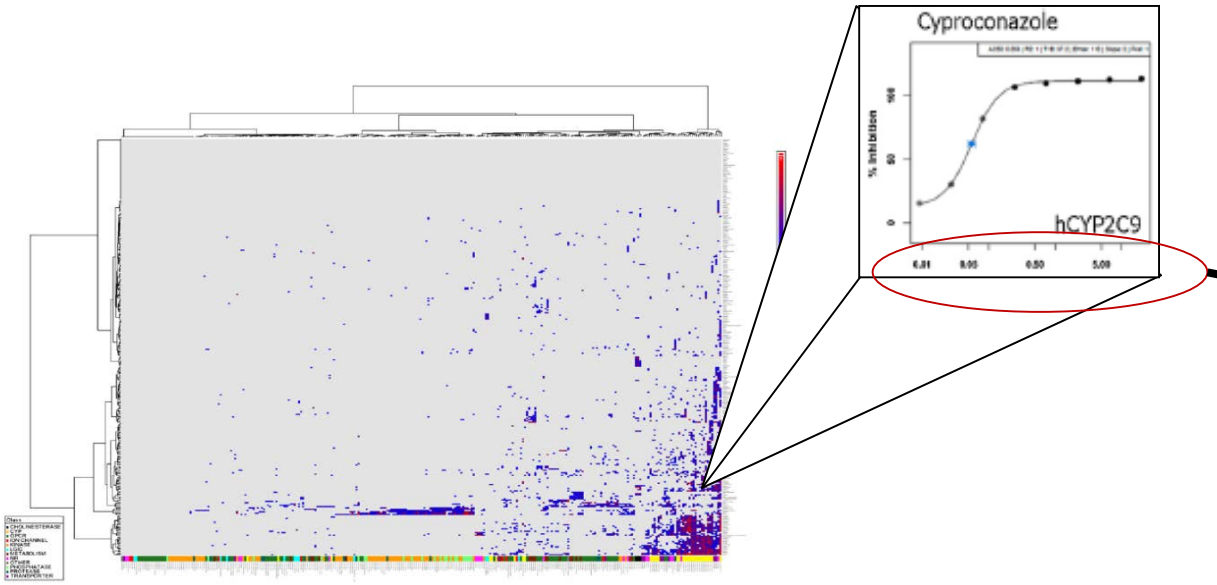


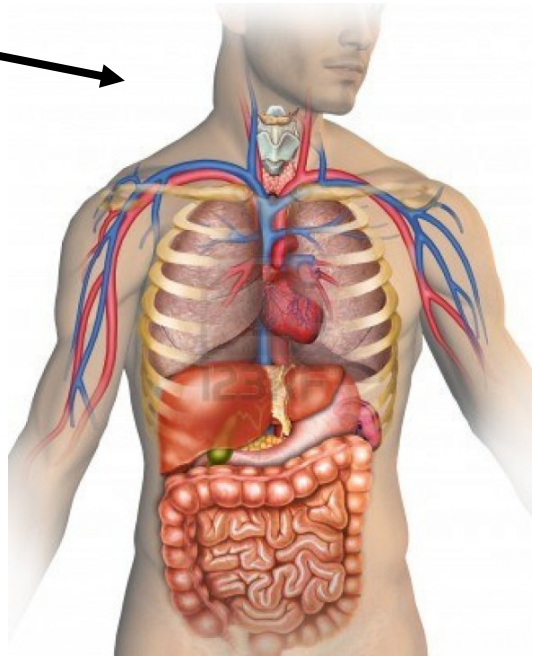
Future of Toxicity Testing



High-Throughput Toxicity Testing Data

Difficulty Translating Nominal Testing Concentrations into *In Vivo* Doses





Knudsen *et al.* Toxicology 282:1-15, 2011

In Vitro - *In Vivo* Extrapolation

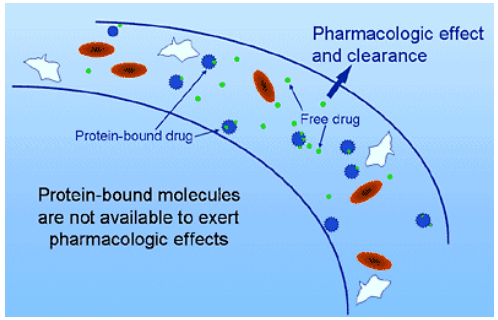
Definition: Utilization of *in vitro* experimental data to predict phenomena *in vivo*

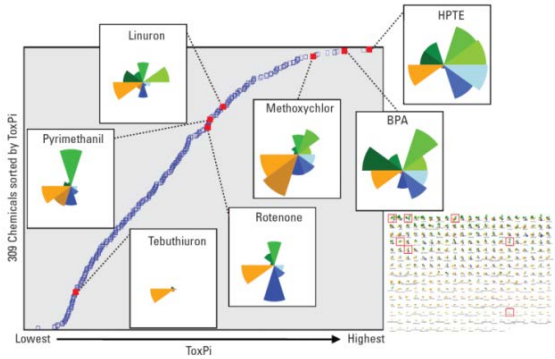
- **IVIVE-PK/TK (Pharmacokinetics/Toxicokinetics):**

  Fate of molecules/chemicals in body
  - Considers ADME; uses PK / PBPK modeling
- **IVIVE-PD/TD (Pharmacodynamics/Toxicodynamics):**
  Effect of molecules/chemicals at biological target *in vivo*
  - Assay design/selection important; perturbation as adverse/therapeutic effect, reversible/ irreversible
- **Both contribute to predict *in vivo* effects**

– IVIVE to Predict Pharmacokinetics – Prioritization and Hazard Prediction Based on Nominal Concentrations Can Misrepresent Potential Health Risks

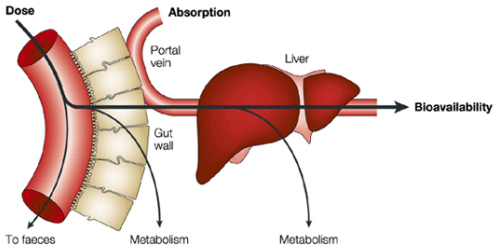
Protein Binding





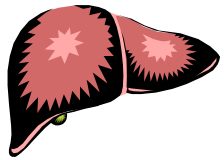
Reif *et al.* Environ Hlth Perspect 118:1714, 2010

Bioavailability

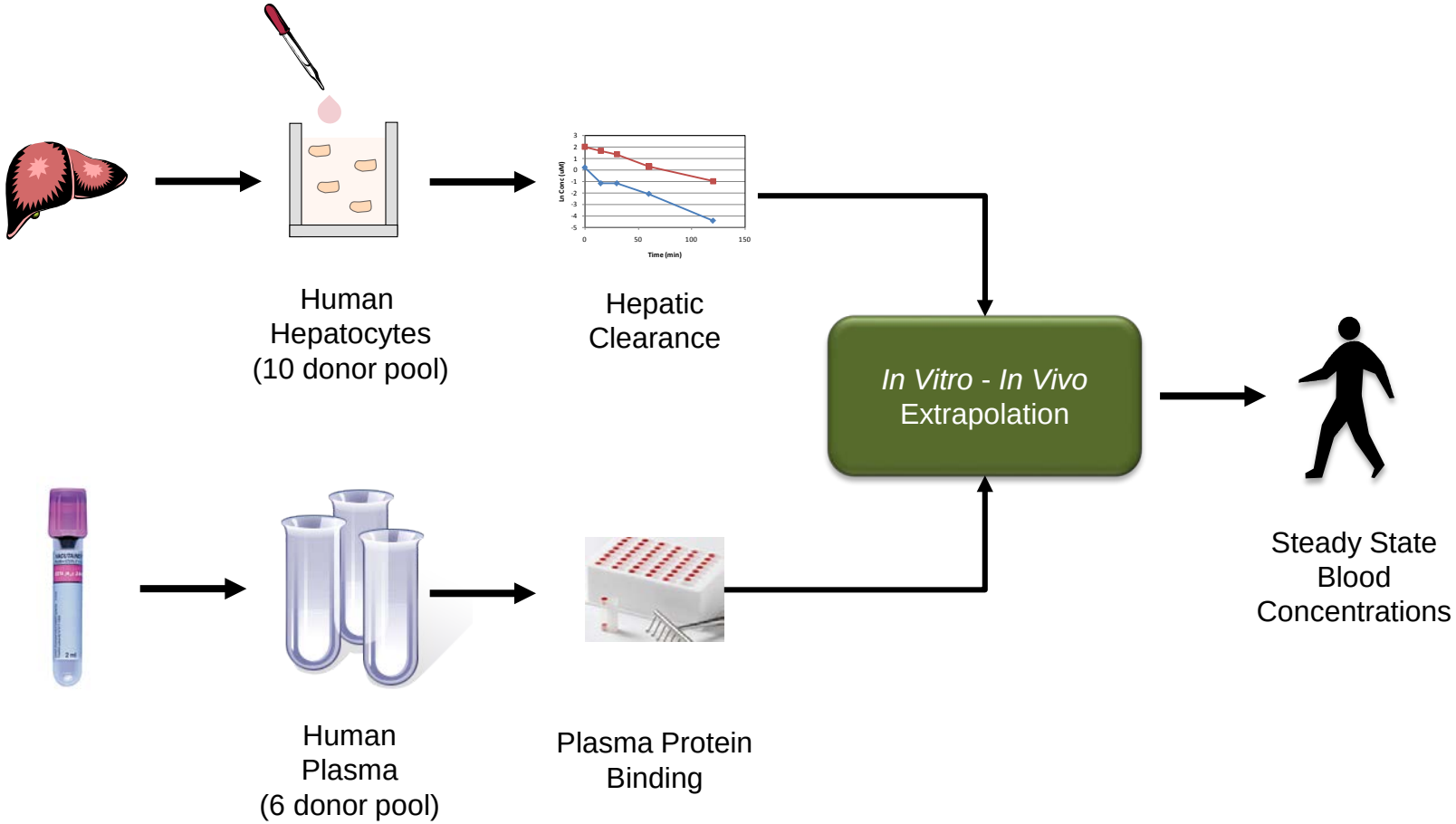


van de Waterbeemd and Gifford, *Nat Rev Drug Disc* 2:192, 2003

Metabolic Clearance



-- IVIVE in a HT Environment --
Modeling *In Vivo* Pharmacokinetics Using *In Vitro* Assays



-- IVIVE in a HT Environment --
Modeling *In Vivo* Pharmacokinetics Using *In Vitro* Assays

In Vitro - In Vivo Extrapolation →

$$[Conc]_{SS} = \frac{\text{Dose Rate} * \text{Body Weight}}{CL_{WholeBody}}$$

$$CL_{WholeBody} = CL_R + CL_H$$

- 100% Oral bioavailability assumed for both CL_R and CL_H
- Kinetics are assumed to be linear

- CL_R: renal clearance (L/hr)
- $CL_{H:}$ hepatic clearance (L/hr)
- $Cl_{int:}$ intrinsic clearance (L/hr)
- GFR: glomerular filtration rate (L/hr)
- F_{uB}: fraction unbound in blood
- Q_L: hepatic blood flow (L/hr)
- HPGL: hepatocytes per gram liver
- $V_{L:}$ volume of liver (g)

$$CL_R = F_{UB} * GFR$$ *where GFR ≈ 6.7 L/hr*

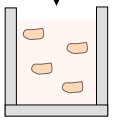
$$CL_H = \frac{F_{UB} * Q_L * CL_{Int}}{Q_L + F_{UB} * CL_{Int}}$$ *where Q_L ≈ 90 L/hr*

$$CL_{Int} = HPGL * V_L * CL_{invitro}$$ *where HPGL ≈ 137 million cells/g*

V_L ≈ 1820 g

Integrating Human Dosimetry and Exposure with the ToxCast *In Vitro* Assays

309 EPA ToxCast Phase I Chemicals



Human Liver Metabolism

Human Plasma Protein Binding

Population-Based IVIVE Model

Upper 95th Percentile Css Among 10,000 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

~600 *In* Vitro ToxCast Assays

ToxCast AC_{50} Value

Plasma Concentration

Oral Exposure

Reverse Dosimetry

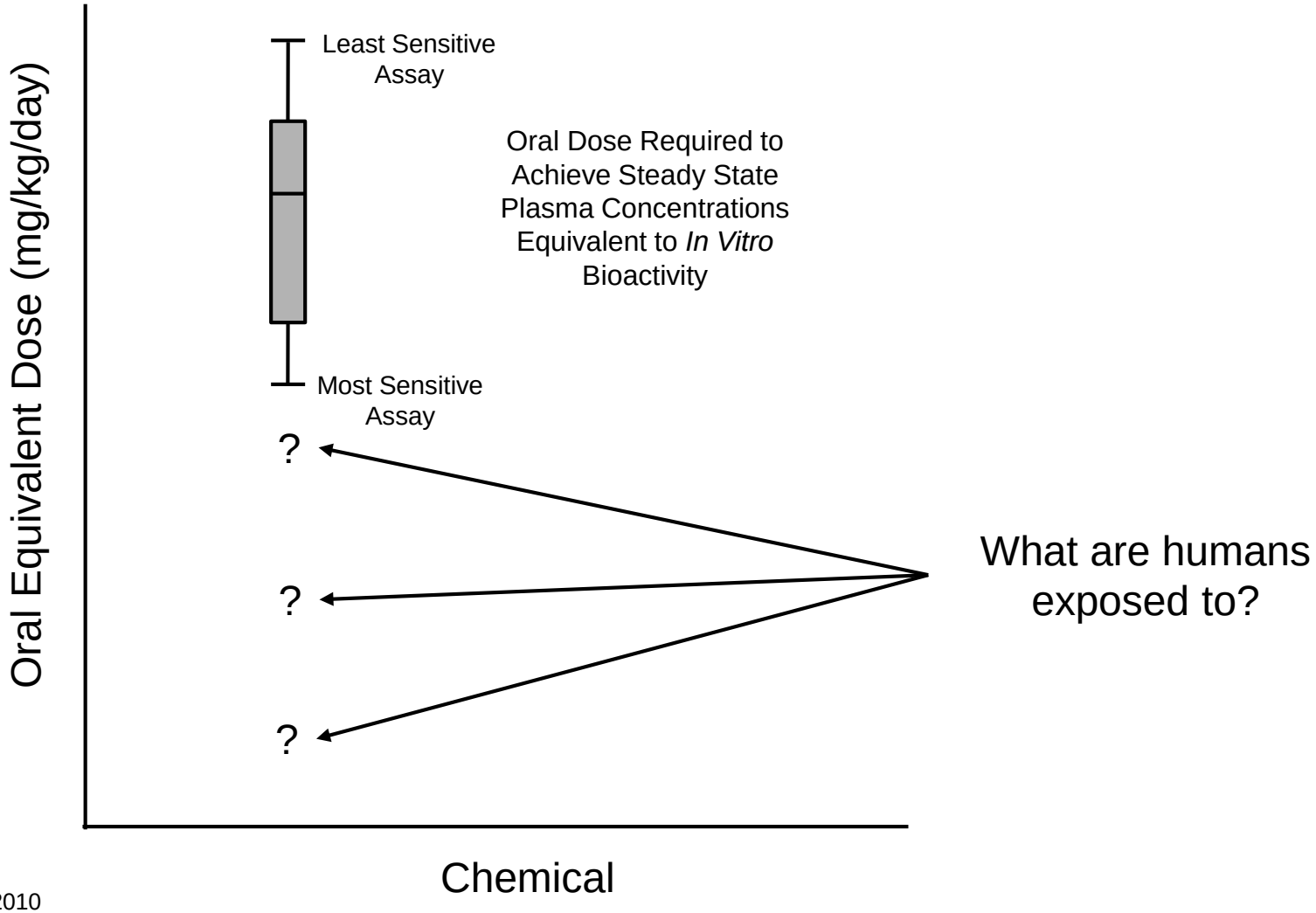
Least Sensitive Assay

Most Sensitive Assay

Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity (mg/kg/day)

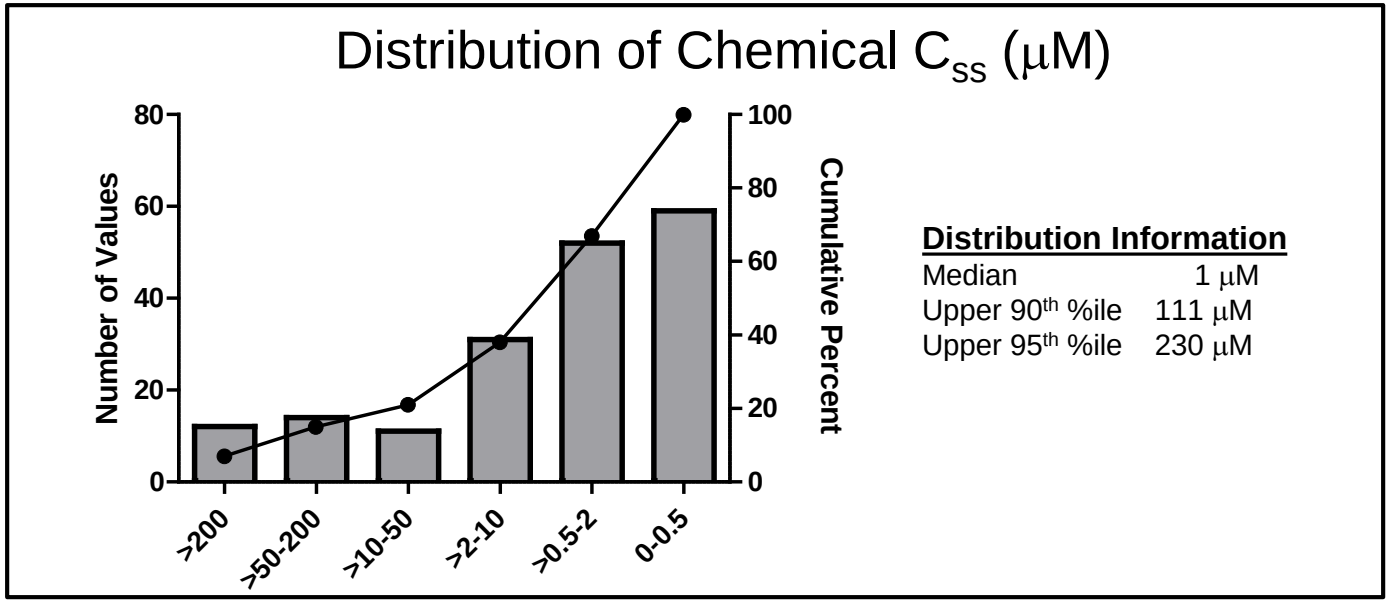
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012

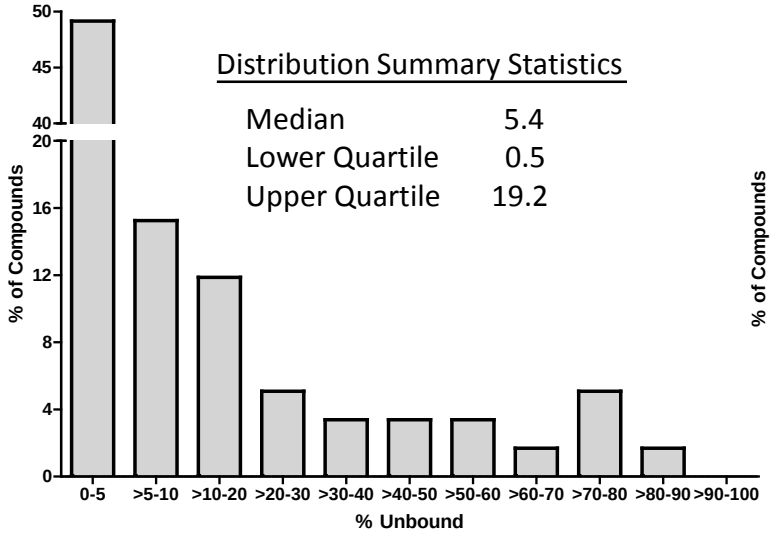
Integrating Human Dosimetry and Exposure with the ToxCast *In Vitro* Assays

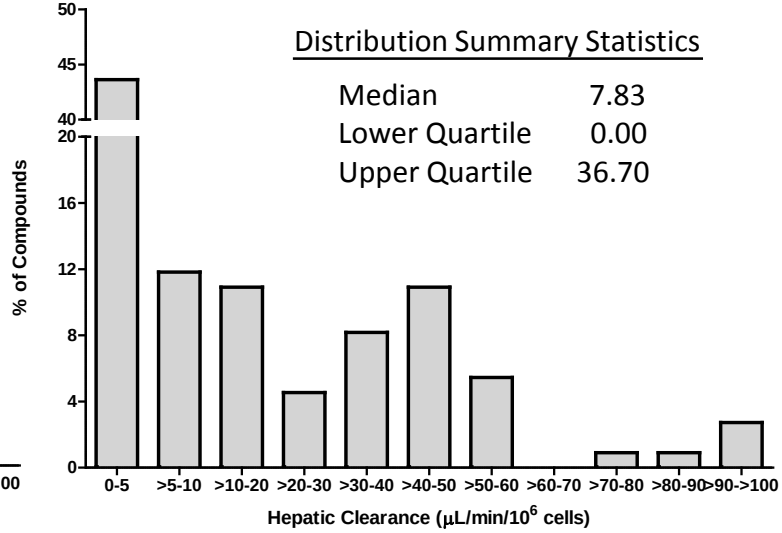


Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012

Pharmacokinetic Data Across 440 Chemicals Provides Insights into Distributions Across Tested Space







How good are we at predicting *in vivo* C_{ss}?

ToxCast Phase I Chemicals

Chemical	*In vivo*-Derived C_{ss} (µM)	*IVIVE* $C_{ss}^{a,b}$ (µM)	*IVIVE* Caco-2[c] $C_{ss}^{a,b}$ µM)
2,4-D	9.05-90.05	39.25	40.43
Bisphenol-A	< 0.13[d]	0.09	0.09
Cacodylic acid	1.80	3.06	--[e]
Carbaryl	0.03	0.01	0.01
Fenitrothion	0.03	2.28	2.28
Lindane	0.46	1.27	1.29
Oxytetracycline dihydrate	0.36	2.00	**0.44**
Parathion	0.17	2.48	2.56
PFOS	19,990[f]	153.23[f]	171.51[f]
PFOA	20,120[f]	13.25[f]	15.92[f]
Picloram	0.27	57.19	**32.01**
Thiabendazole	0.45	13.76	15.20
Triclosan	2-10	0.07	0.07

ToxCast Phase II Chemicals

Chemical	*In vivo*-Derived C_{ss} (µM)	*IVIVE* $C_{ss}^{a,b}$ (µM)	*IVIVE* Caco-2[c] $C_{ss}^{a,b}$ µM)
Acetaminophen	1.1	0.52	0.57
2-Chloro-2'-deoxyadenosine	0.28	1.36	**0.58**
Coumarin	0.01-0.02	13.63	15.40
Diphenhydramine HCl	0.11-0.16	3.18	3.57
6-Propyl-2-thiouracil	1.10	1.58	1.80
Chlorpyrifos	0.022	0.24	0.27
Sulfasalazine	0.2-1.8	11.6	**2.5**
Candoxatril	0.023	0.18	0.14
Flutamide	0.004-0.005	0.57	0.64
PK 11195	0.14	0.58	0.66
5,5'-Diphenylhydrantoin	4.92	1.59	1.59
Triamcinolone	0.05-0.29	0.004	0.002
Volinanserin	0.037	0.03	0.03
Zamifenacin	2.86	0.57	0.64

How good are we at predicting *in vivo* C_{ss}?

ToxCast Pha… Chemicals … ToxCast Phase II Chemicals

27 Chemicals:
~60% are within 10-fold of *in vivo* C_{ss} values
~80% are within 20-fold of *in vivo* C_{ss} values

Chemical	*In* De C_{ss} (µ…	*…ivo-* …ed C_{ss} …M)	*IVIVE* $C_{ss}^{a,b}$ (µM)	*IVIVE* Caco-2[c] $C_{ss}^{a,b}$ µM)
2,4-D	9.05-90	1.1	0.52	0.57
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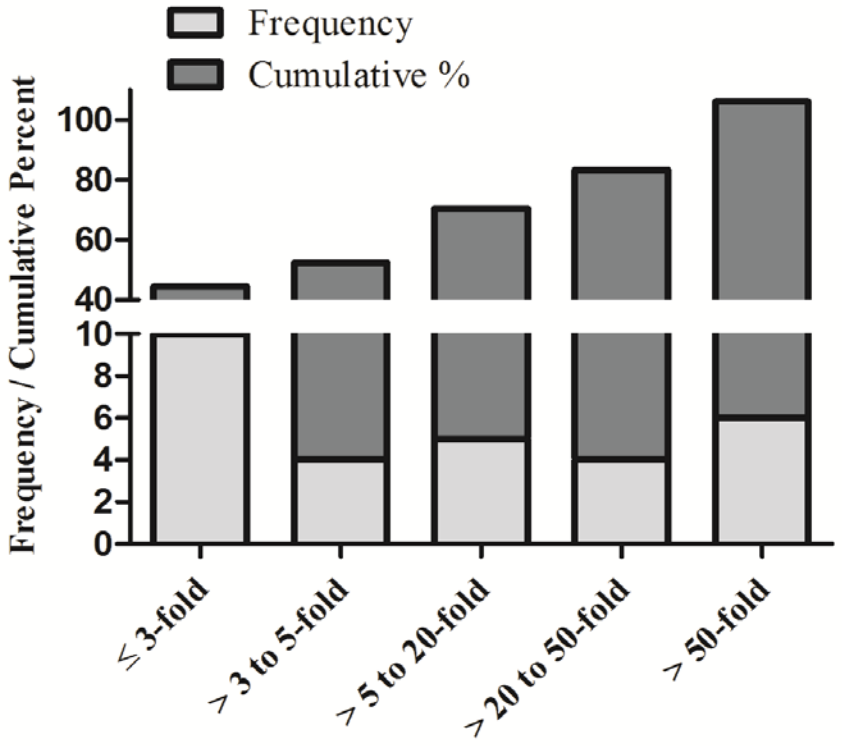
		2.86	0.57	0.64



Reasons for C_{ss} Overprediction - Opportunities for Refinement -

- Not all routes of metabolic clearance are captured
  - Extrahepatic (intestinal, renal, etc.) metabolism
  - Nonhepatocyte-mediated clearance
- Hepatocyte suspensions unable to detect clearance of low turnover compounds
- Absorption / Bioavailability assumed 100%
- Restrictive vs. Nonrestrictive clearance
- Conservative assumptions drive poor predictivity for chemicals known to be rapidly cleared in vivo

Toxicokinetic Triage for Environmental Chemicals

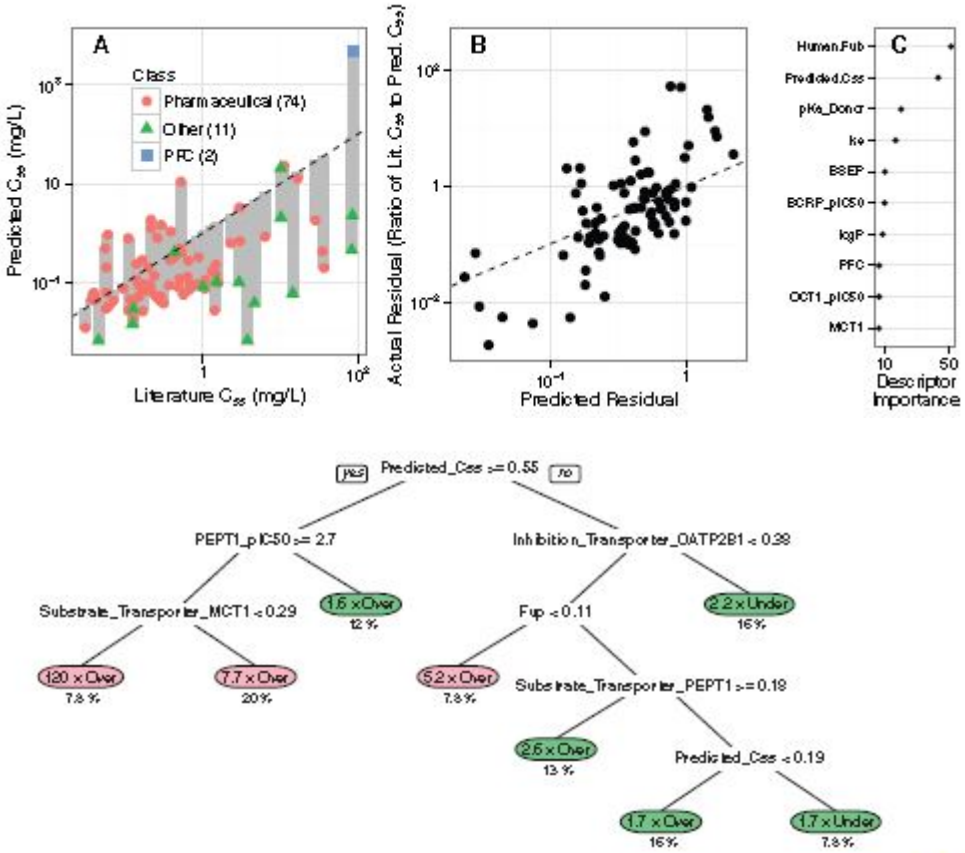


FIG. 5. A recursive partitioning regression tree was used to classify the discrepancy between the C_{ss} predicted from in vitro data and the in vivo C_{ss} (Obach et al., 2008; Wetmore et al., 2012). Each "leaf" of the tree shows a group of chemicals for which HTTK either overestimates C_{ss} (making conservative predictions) or underestimates C_{ss}. For all but 3 groups, the predictions are on the order of the observed C_{ss} (approximately within a factor of 3.2× greater or lesser). For the other 3 groups, the C_{ss} is 5.2×, 7.7×, and 120× overestimated. The dashed line indicates the identity (perfect predictor) line.

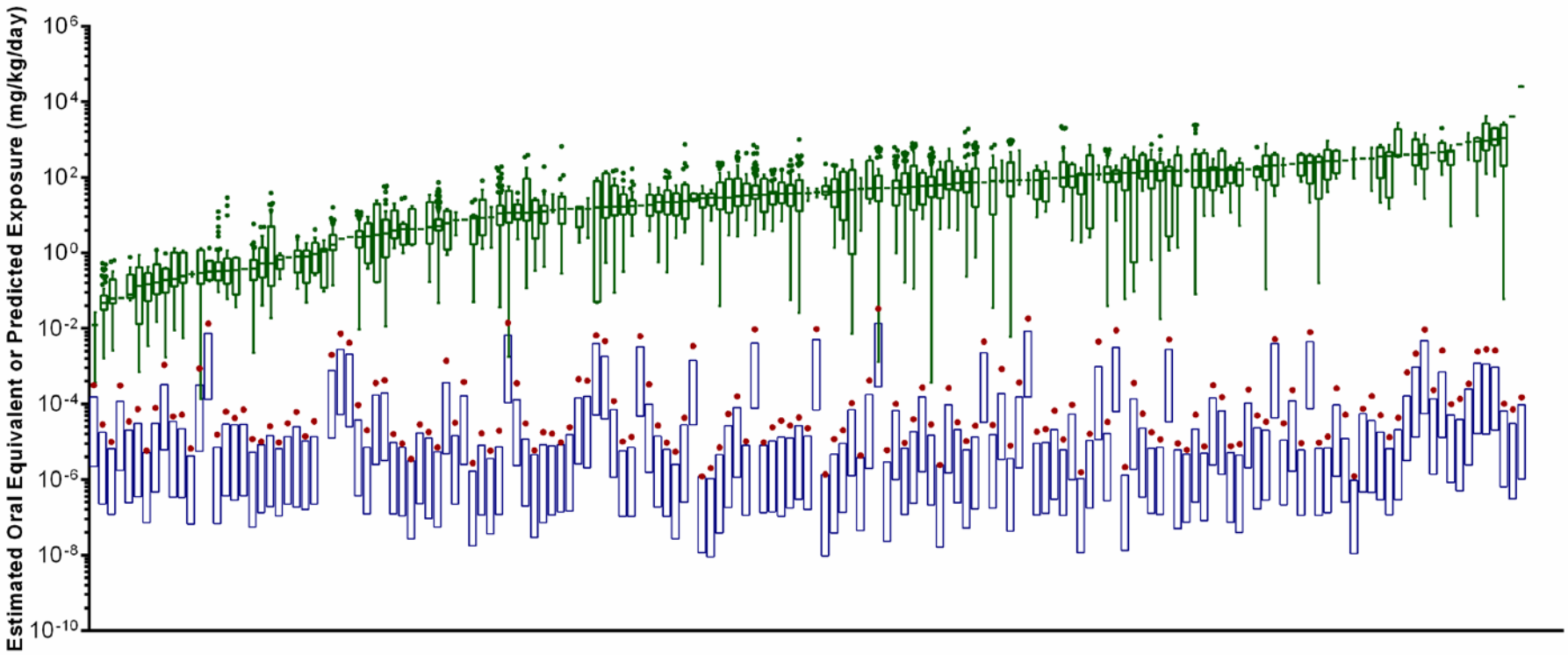
Wambaugh *et al., Tox Sci.,* 2015

Comparing Dosimetry-Adjusted Oral Equivalents against Nominal AC_{50} Concentrations

CAS #	Chemical	Upper 95th %ile Css (μM)	Assay Name (abridged)	AC50 (μM)	Oral Equivalent (mg/kg/day)
4291-63-8	2-Chloro-2'-deoxyadenosine	2.0713	BSK_SAg_PBMCCytotoxicity	1	0.4828
1806-26-4	4-Octylphenol	1.4109	APR_CellCycleArrest	1	0.7088
57-97-6	7,12-Dimethylbenz(a)anthracene	3.9083	APR_CellCycleArrest	1	0.2559
148-24-3	8-Hydroxyquinoline	0.0403	APR_p53Act	1	24.8188
484-17-3	9-Phenanthrol	2.1423	APR_CellLoss	1	0.4668
484-17-3	9-Phenanthrol	2.1423	APR_MitoMass	1	0.4668
484-17-3	9-Phenanthrol	2.1423	APR_MitoticArrest	1	0.4668
120-12-7	Anthracene	0.5800	APR_MitoMembPot	1	1.7241
1912-24-9	Atrazine	0.5998	APR_p53Act	1	1.6672
55285-14-8	Carbosulfan	0.0056	NVS_ENZ_rAChE	1	177.2814
7173-51-5	Didecyl dimethyl ammonium chloride	3.3686	APR_CellLoss	1	0.2969
76-87-9	Fentin hydroxide	318.0339	APR_CellLoss	1	0.0031
99-76-3	Methylparaben	0.1768	APR_CellCycleArrest	1	5.6561
50-65-7	Niclosamide	0.3073	APR_MitoMass	1	3.2544
50-65-7	Niclosamide	0.3073	APR_NuclearSize	1	3.2544
50-65-7	Niclosamide	0.3073	APR_OxidativeStress	1	3.2544
26530-20-1	Octhilinone	0.6864	APR_MitoticArrest	1	1.4569
57-83-0	Progesterone	0.2007	APR_MitoMembPot	1	4.9835
83-79-4	Rotenone	0.3131	APR_MitoticArrest	1	3.1941
79902-63-9	Simvastatin	0.6379	APR_CellCycleArrest	1	1.5677
79902-63-9	Simvastatin	0.6379	APR_MitoMass	1	1.5677
156052-68-5	Zoxamide	168.1532	APR_CellCycleArrest	1	0.0059
156052-68-5	Zoxamide	168.1532	APR_MitoMass	1	0.0059

Same AC_{50}
550-fold lower
Oral Equivalent
after Dosimetry
Adjustment

Incorporating Dosimetry-Adjusted ToxCast Bioactivity Data with HT ExpoCast Predictions



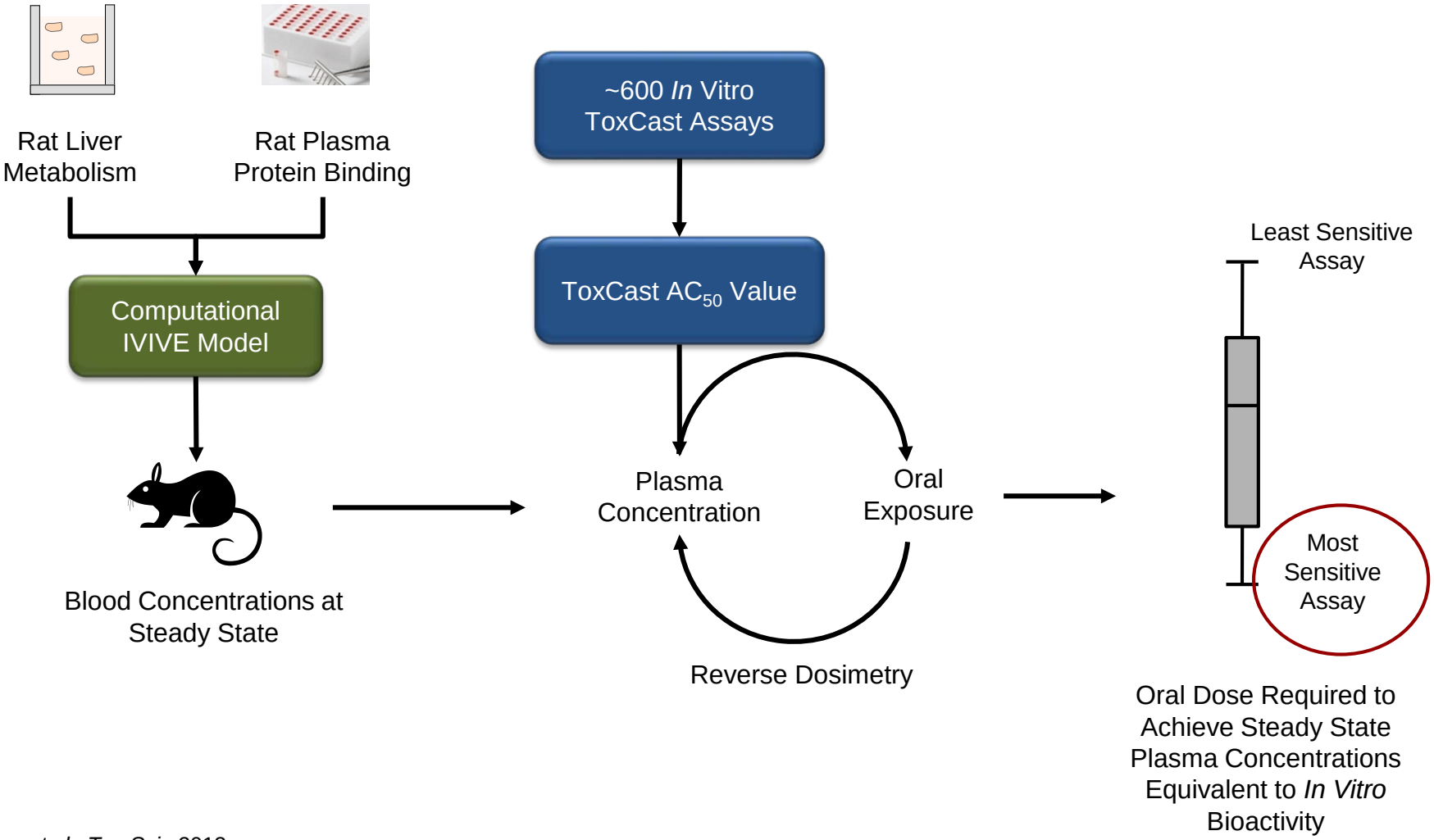
Wetmore *et al.*, Tox. Sci, 2015

Providing an MOE Context to Data

Use and Assay Information for Chemicals with the 20 Lowest Activity:Exposure Ratios

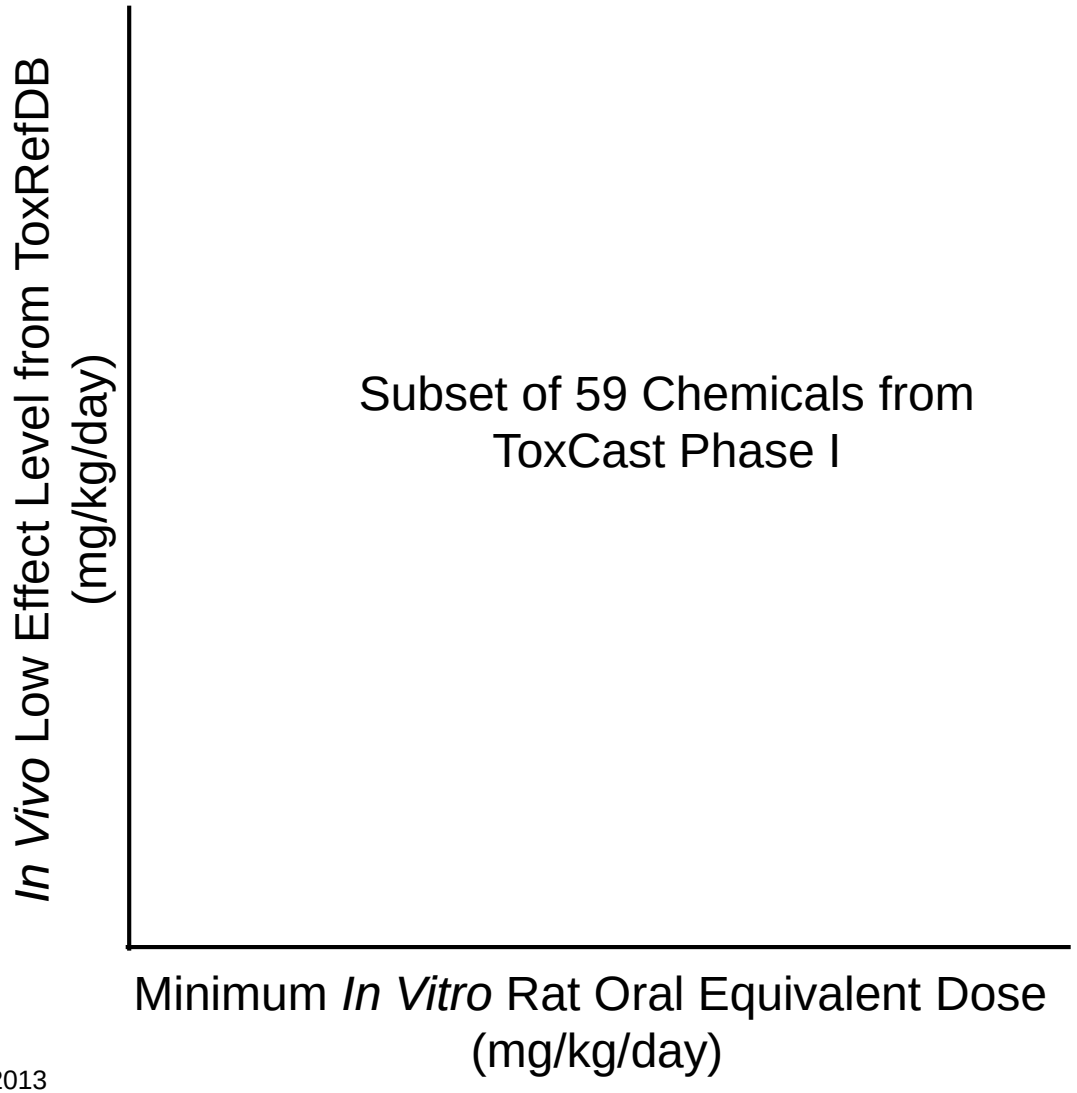
Chemical	Description/Use	No. Assay Hits where MHE[a] AER[b]<100	AC_{50} (μM)[c]	Oral Equivalent[c] (mg/kg/day)	Exposure Total (MHE)	AER (MHE AER)
Tannic acid	Plant polyphenol; food, drug uses; mordant during dyeing process	5	0.0002	5.83E-04	1.35E-02 (3.36E-02)	0.043 (0.02)
Triphenyl phosphate	Plasticizer; fire retardant	3	0.0006	7.66E-04	6.57E-03 (1.41E-02)	0.117 (0.054)
Heptadecafluorooctanesulfonic acid potassium salt	Organofluorine	12	0.013	5.99E-05	3.21E-04 (8.72E-04)	0.187 (0.069)
Mirex	Banned organochlorine insecticide	3	0.01144	1.61E-04	1.55E-04 (3.13E-04)	1.040 (0.516)
Ammonium perfluorooctanoate	Organofluorine	9	0.20182	7.48E-04	3.24E-04 (1.09E-03)	2.310 (0.684)
Tributyl phosphate	Solvent; plasticizer	3	1.28	2.04E-02	4.03E-03 (6.60E-03)	5.05 (3.09)
Potassium perfluorohexanesulfonate	Organofluorine	2	0.0825	3.09E-04	3.09E-05 (7.27E-05)	10.02 (4.26)
Dioctyl phthalate	plasticizer	6	4.88	7.62E-02	7.49E-03 (1.34E-02)	10.18 (5.68)
Diethylstilbesterol	Nonsteroidal estrogen	6	0.000074	1.61E-04	1.49E-05 (2.84E-05)	10.82 (5.68)
Diphenhydramine	Antihistamine drug	2	0.0238	4.91E-03	1.95E-04	25.21

Comparing *In Vitro* ToxCast-derived Points of Departure Against In Vivo Rodent LELs



Wetmore *et al.*, *Tox Sci.*, 2013

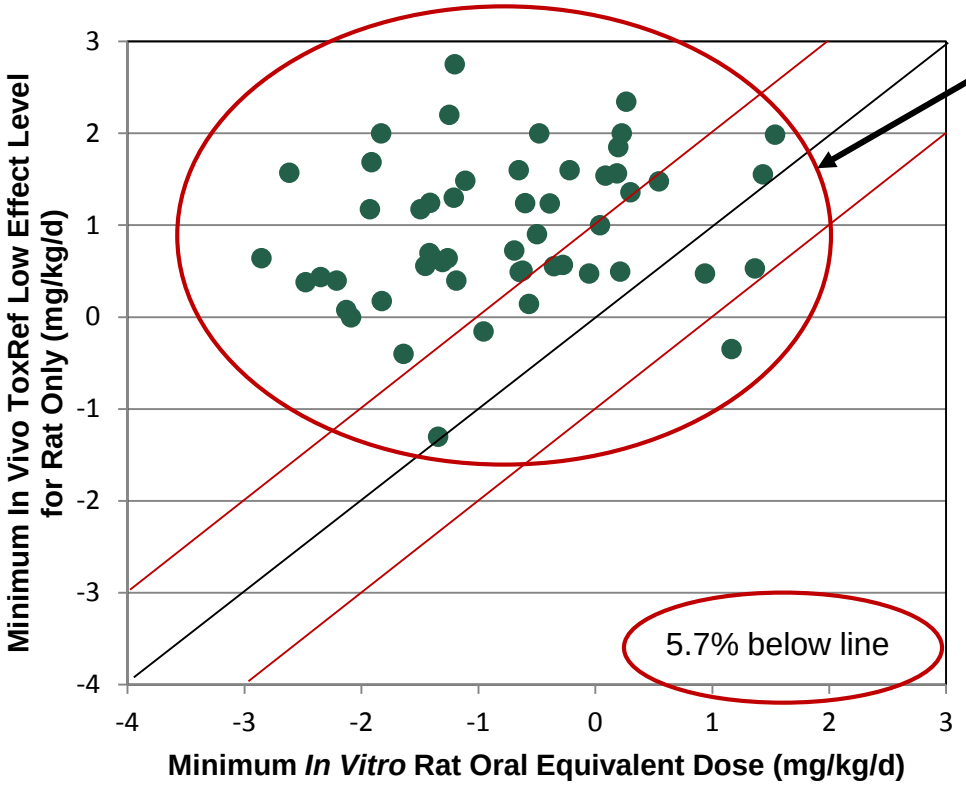
Comparing *In Vitro* ToxCast-derived Points of Departure Against In Vivo Rodent LELs

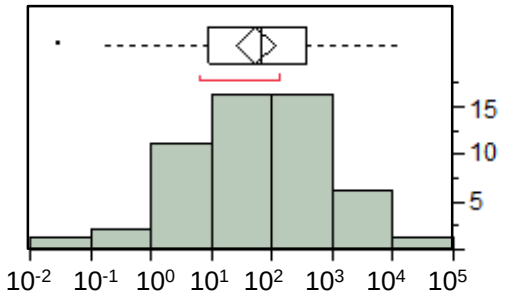


Wetmore *et al., Tox Sci.,* 2013

The Most Sensitive *In Vitro* Assay Provides a Conservative Estimate of the Point-of-Departure

Spanned 38 *In Vivo* Endpoints across Multiple Tissues, Organ Systems, and Study Types (Repro, Chronic, and Dev)





Log Ratio ToxRef Min LEL:ToxCast Min Oral Equivalent Dose

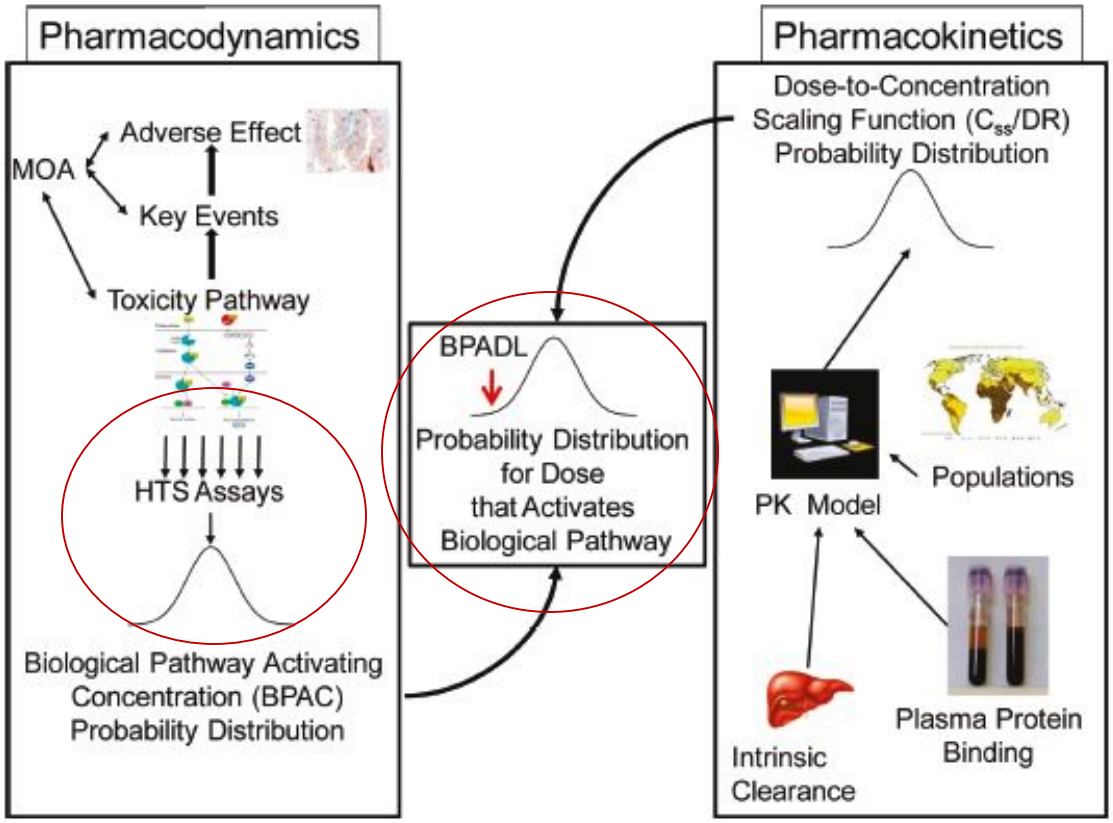
Distribution Summary Statistics

Median	1.82	(66.07)
Upper Quartile	2.55	(354.81)
Lower Quartile	0.95	(8.91)

Wetmore *et al., Tox Sci.,* 2013

High-Throughput Risk Assessment

Transitioning from Potent Hits to Pathway Activating Doses



Judson *et al.*, 2011

Gaps and Limitations of IVIVE Approach to Predict Chemical PK/TK

- Metabolism not considered
  - Transition to metabolically competent systems will require different approach
  - Bioactivating vs. detoxifying metabolism; predictive tools?
- Lack of in vivo PK data to validate IVIVE for environmental chemicals
- Lack of appropriate training sets to validate in silico predictions
  - plasma protein binding, intrinsic clearance, metabolism
- Tissue distribution not considered (blood vs. target tissue)
- C_{max} vs. C_{ss}
- Exposure Routes – dermal, inhalation

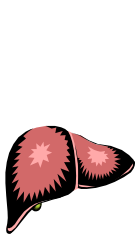
Gaps and Limitations
Relevant for IVIVE to Predict Chemical PK/TK and PD/TD

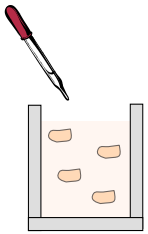
- Mass balance issues
  - Non-specific binding to proteins in incubation
    - PK assays: Cl_{int} underprediction / C_{ss} overprediction
    - PD assays (overestimation of chemical at target site)
  - Non-specific binding to plastics in in vitro system
  - Chemical Volatility, Stability
- Consideration of transporters/uptake
  - Impact on metabolism/absorption (PK/TK)
  - To target site (PD/TD)
- Species differences

In Vitro Assays - Considerations Relevant for IVIVE to Predict Chemical PD/TD

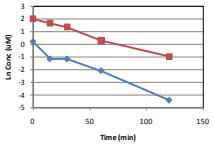
- Span from cell-free to immortalized lines to physiologically relevant systems
- Consideration of relevant mass balance / uptake issues
- Coverage of biological space?
  - Suite of relevant assays
  - Genomics/transcriptomics
  - Sufficient coverage across potential adverse outcomes?
- Ability to discriminate reversible perturbation from irreversible effect, potential adverse outcome
- Temporality – relating in vitro to in vivo

Consideration of Population Variability





Primary
Hepatocytes



Hepatic Clearance
$Cl_{in\ vitro}$

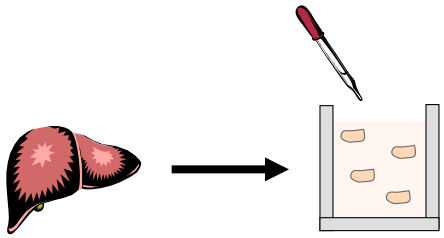
sim CYP
real solutions from virtual populations

Plasma C_{ss}
General Population

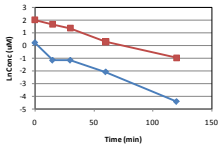
Plasma C_{ss}
General Population

≠

Population-based *In Vitro-In Vivo* Extrapolation



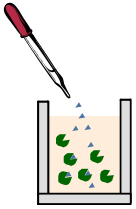
Primary Hepatocytes



Hepatic Clearance
$Cl_{in\ vitro}$

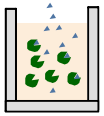
SIMCYP real solutions from virtual populations

Plasma C_{ss}
General Population
Plasma C_{ss} for:



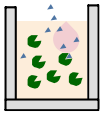
rCYP1A2

$Cl_{rCYP1A2}$



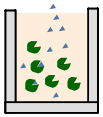
rCYP3A4

$Cl_{rCYP3A4}$



rCYP…

$Cl_{rCYP…}$



rUGT …

$Cl_{rUGT…}$

CYP1A2 CYP2E1 CYP3A4 CYP2C9
CYP… T2B7
CYP2C8 CYP3A5 UGT1A1 CYP2B6

SIMCYP real solutions from virtual populations

Intrinsic Clearance Rates

Neonates

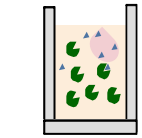
Asians

Northern Europeans

Children

And so on…

Integrating High-Throughput Pharmacokinetics with the ToxCast *In Vitro* Assays



Recombinant Enzyme Metabolism

Human Plasma Protein Binding

Population-Based IVIVE Model

Steady State Plasma Concentrations for Different Subpopulations

~600 *In* Vitro ToxCast Assays

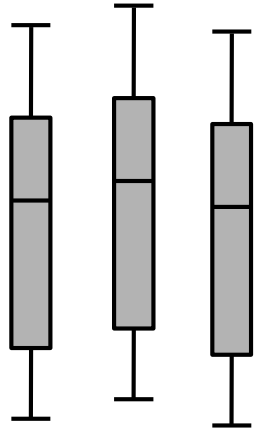
ToxCast AC_{50} Value

Plasma Concentration

Oral Exposure

Reverse Dosimetry

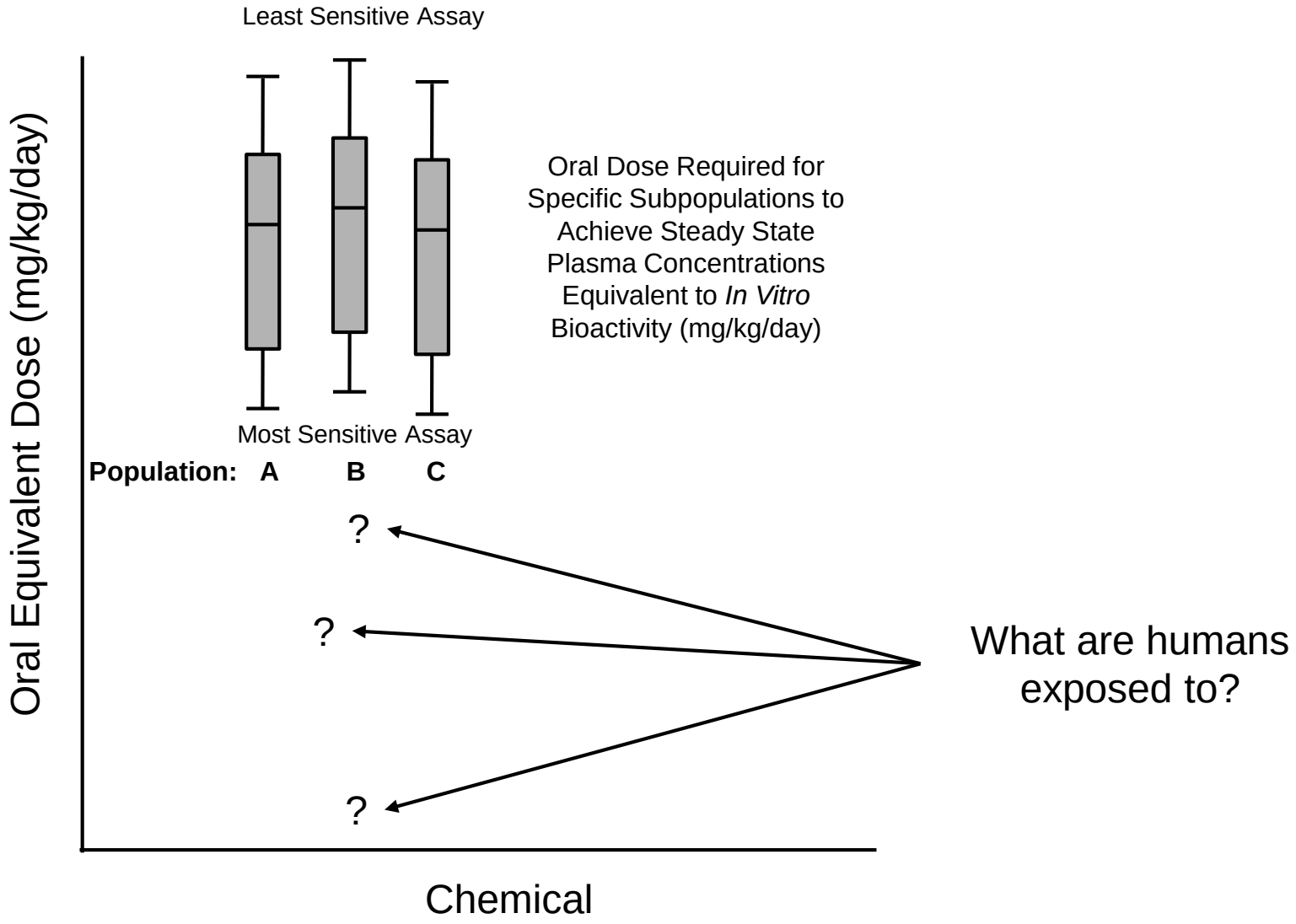
Oral Dose Required for Specific Subpopulations to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity (mg/kg/day)



Population: A B C

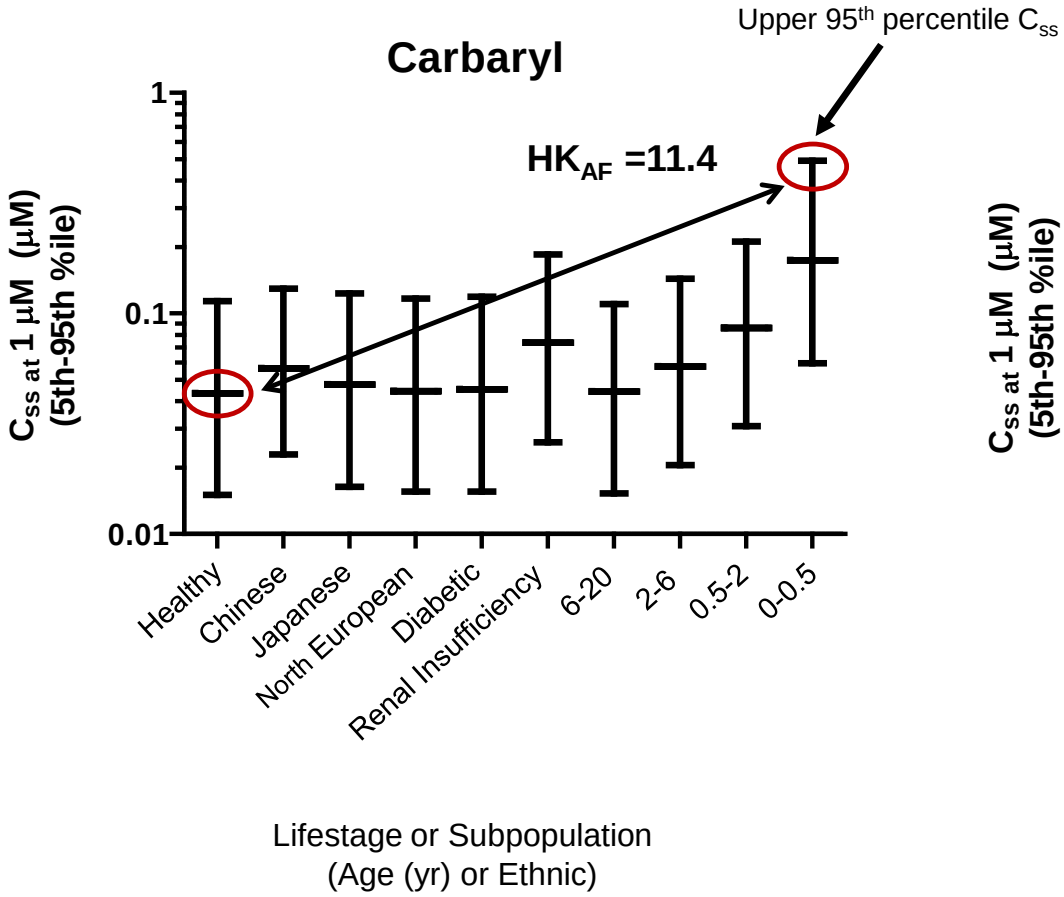
Wetmore *et al.*, 2014, *Toxicol.Sci,* 142(1):210-14

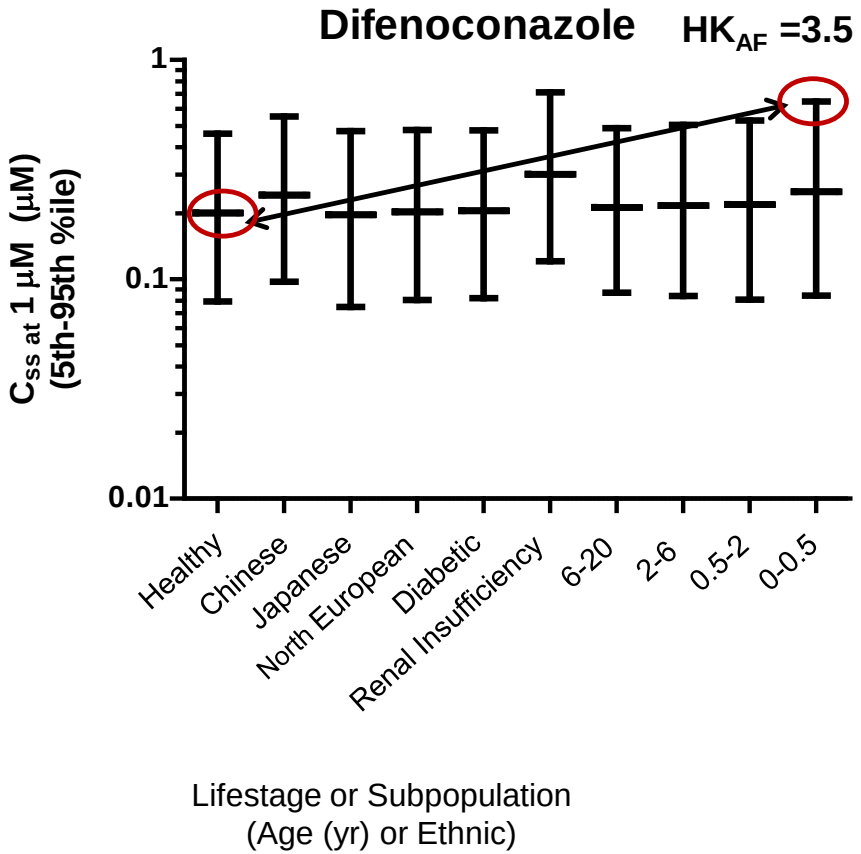
Integrating High-Throughput Pharmacokinetics with the ToxCast *In Vitro* Assays



Wetmore *et al.*, 2014, *Toxicol.Sci,* 142(1):210-14

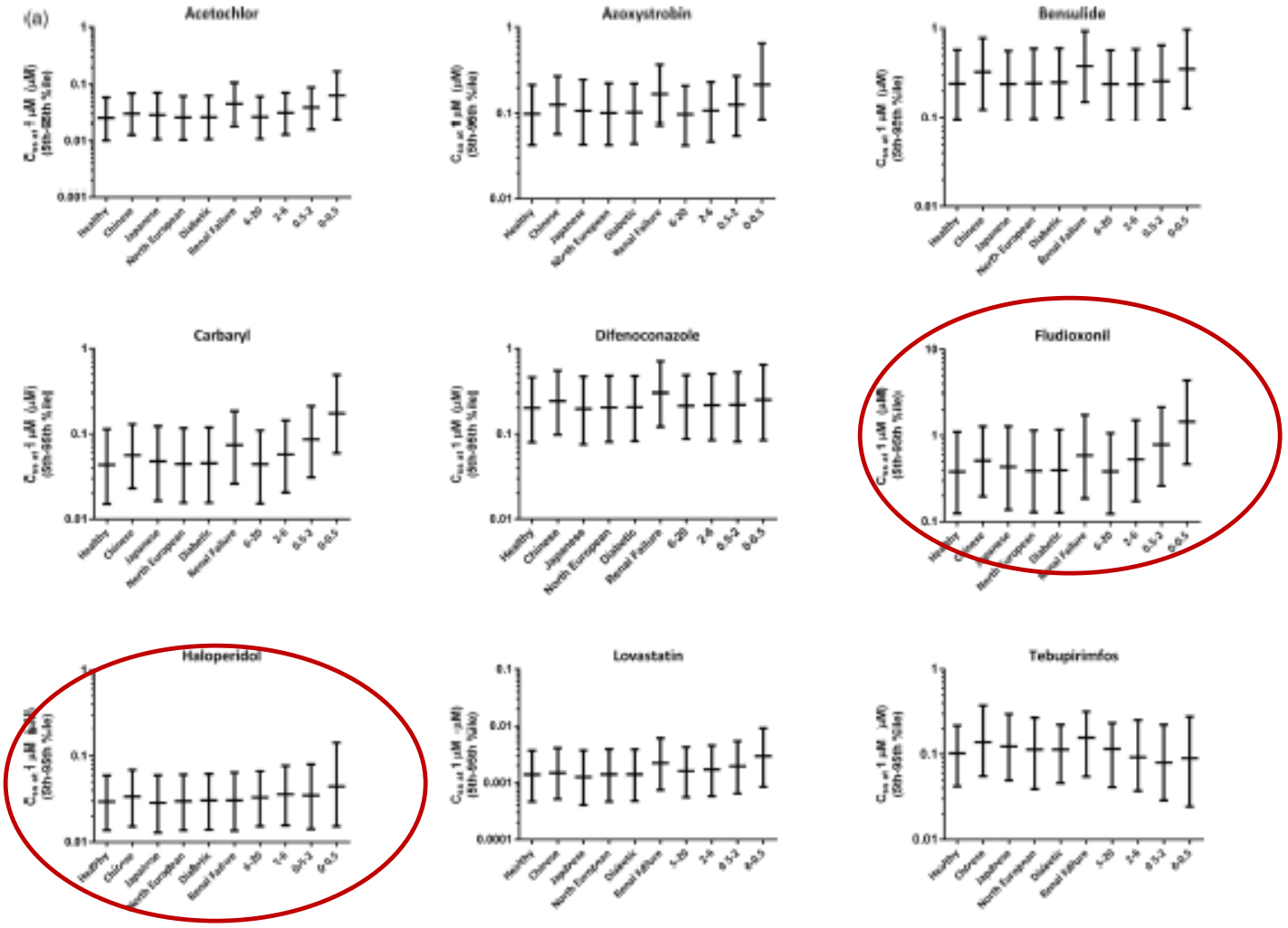
Comparison of C_{ss} Values Derived Across Multiple Lifestages and Subpopulations





HK_{AF}: human toxicokinetic adjustment factor

Comparison of C_{ss} Values Derived Across Multiple Lifestages and Subpopulations

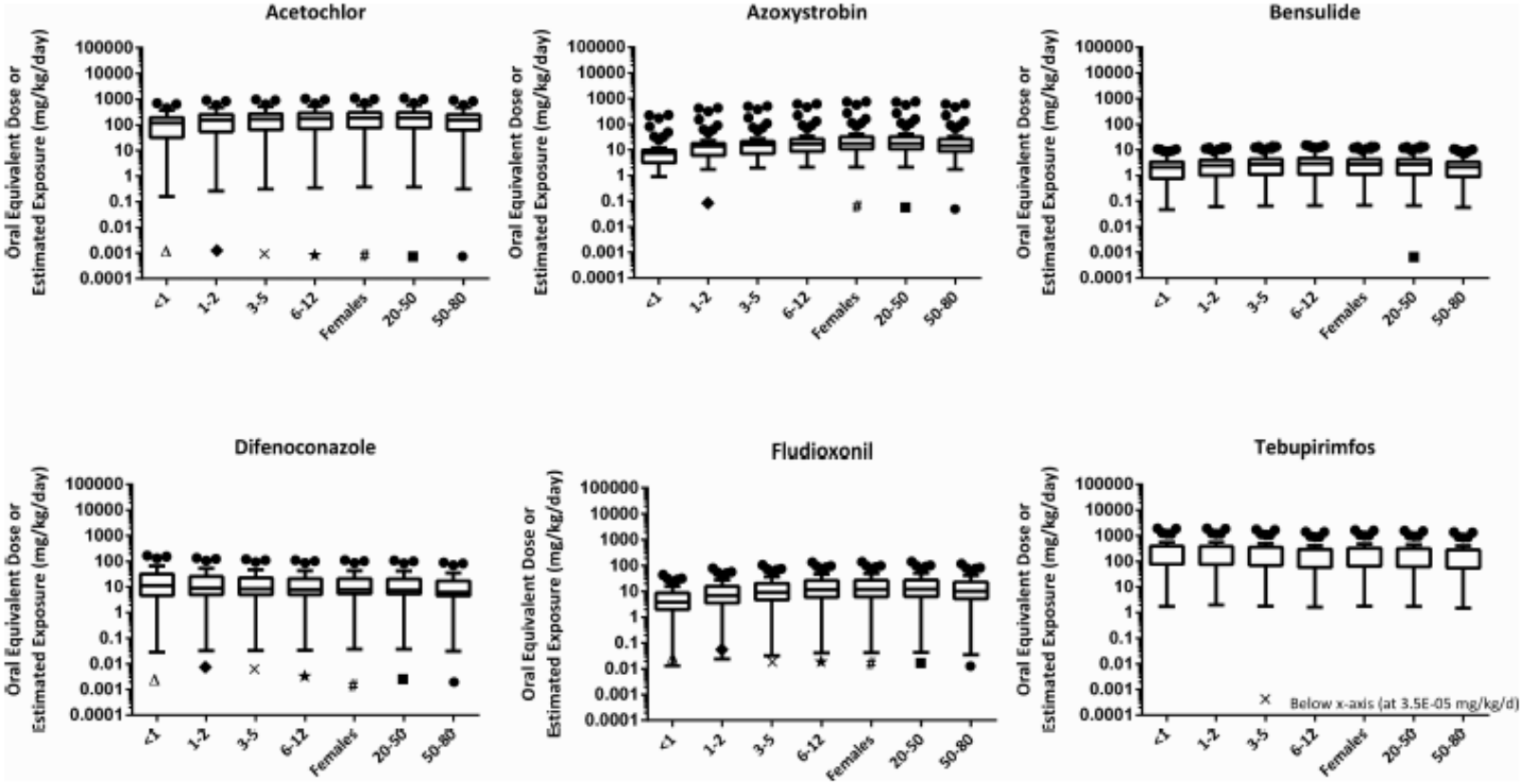


Wetmore *et al*., 2014, *Toxicol Sci.* 142(1):210-214.

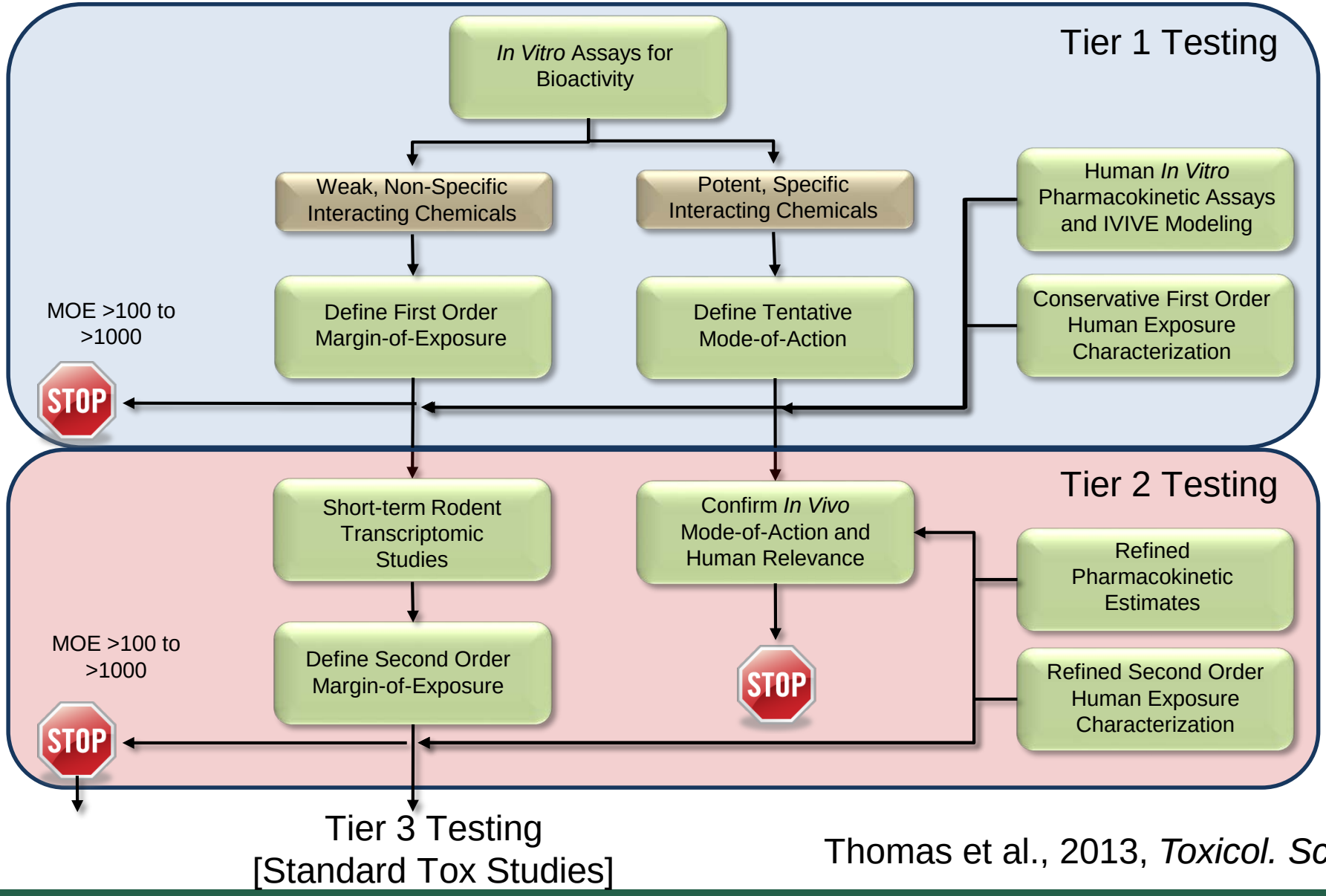
Estimated Chemical-Specific Toxicokinetic Adjustment Factors

Chemical	Median C_{ss} for Healthy Population	95th Percentile C_{ss} for Most Sensitive	Most Sensitive	Estimated HK_{AF}	% Contribution of Isozyme Differences to Average HK_{AF}
Acetochlor	0.026	0.15	Neonatal	6.7	86
Azoxystrobin	0.099	0.66	Neonatal	6.7	86
Bensulide	0.241	0.97	Neonatal	4.0	79
Carbaryl	0.043	0.49	Neonatal	11.4	87
Difenoconazole	0.201	0.49	Renal Insufficiency	3.5	99
Fludioxonil	0.38	4.37	Neonatal	11.5	87
Haloperidol	0.029	0.14	Neonatal	4.9	83
Lovastatin	0.001	0.009	Neonatal	6.5	90
Tebupirimfos	0.107	0.38	Renal Insufficiency	3.5	15

Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations



Utility in a Tiered Testing Approach



Thomas et al., 2013, *Toxicol. Sci.*

Key Points

- Use of IVIVE tools to incorporate dosimetry has enabled a shift from a hazard-based to a risk-based interpretation of HTS data.
- Current in vitro – in vivo assessments for environmental chemicals point to need for tools trained against relevant space for prediction refinement.
- IVIVE effort to evaluate PK variability in a manner that could 1) identify sensitive populations and 2) replace use of default safety factors in risk assessment.
- Using IVIVE in PD/TD will require additional considerations to understand chemical concentration at target.

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In Vitro-to-*In Vivo* Extrapolation for High-Throughput Prioritization and Decision-Making

- Webinars: First Wednesdays, 11:00AM E.D.T.
  - October 7 – Barbara Wetmore: Setting the Stage
  - November 4 – John Wambaugh: Model Development
  - December 2 – Lisa Sweeney: Model Evaluation
  - January 6, 2016 – TBD: State of the Science

- In-person Meeting: February 17-18, 2016
  - US EPA, Research Triangle Park, NC