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

Setting the Stage

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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
Broad-Based Movement in Toxicology Towards *In Vitro* Testing and Hazard Prediction
High-Throughput Toxicity Testing Data
Difficulty Translating Nominal Testing Concentrations into In Vivo Doses

**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

Bioavailability

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

-- IVIVE in a HT Environment --

Modeling *In Vivo* Pharmacokinetics Using *In Vitro* Assays

Human Hepatocytes (10 donor pool) → Hepatic Clearance → *In Vitro - In Vivo* Extrapolation → Steady State Blood Concentrations

Human Plasma (6 donor pool) → Plasma Protein Binding
-- IVIVE in a HT Environment --

Modeling *In Vivo* Pharmacokinetics Using *In Vitro* Assays

\[ [\text{Conc}]_{SS} = \frac{\text{Dose Rate} \times \text{Body Weight}}{\text{CL}_{\text{WholeBody}}} \]

\[ \text{CL}_R = \text{F}_{UB} \times \text{GFR} \quad \text{where GFR} \approx 6.7 \text{ L/hr} \]

\[ \text{CL}_H = \frac{\text{F}_{UB} \times Q_L \times \text{CL}_{\text{Int}}}{Q_L + \text{F}_{UB} \times \text{CL}_{\text{Int}}} \quad \text{where } Q_L \approx 90 \text{ L/hr} \]

\[ \text{CL}_{\text{Int}} = \text{HPGL} \times V_L \times \text{CL}_{\text{invitro}} \quad \text{where } \text{HPGL} \approx 137 \text{ million cells/g} \]

\[ V_L \approx 1820 \text{ g} \]

- 100% Oral bioavailability assumed for both $\text{CL}_R$ and $\text{CL}_H$
- Kinetics are assumed to be linear

- $\text{CL}_R$: renal clearance (L/hr)
- $\text{CL}_H$: hepatic clearance (L/hr)
- $\text{CL}_{\text{int}}$: intrinsic clearance (L/hr)
- GFR: glomerular filtration rate (L/hr)
- $\text{F}_{UB}$: fraction unbound in blood
- $Q_L$: hepatic blood flow (L/hr)
- HPGL: hepatocytes per gram liver
- $V_L$: volume of liver (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 AC50 Value

Plasma Concentration

Oral Exposure

Reverse Dosimetry

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

Least Sensitive Assay

Most Sensitive Assay

Rotroff et al., Tox Sci., 2010

Wetmore et al., Tox Sci., 2012
Integrating Human Dosimetry and Exposure with the ToxCast *In Vitro* Assays

Oral Equivalent Dose (mg/kg/day)

Least Sensitive Assay

Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

Most Sensitive Assay

What are humans exposed to?

Chemical

Rotroff et al., *Tox Sci.*, 2010
Wetmore et al., *Tox Sci.*, 2012
Pharmacokinetic Data Across 440 Chemicals Provides Insights into Distributions Across Tested Space

Distribution of Chemical $C_{ss} (\mu M)$

Distribution Information
- Median: 1 $\mu M$
- Upper 90th %ile: 111 $\mu M$
- Upper 95th %ile: 230 $\mu M$

Distribution Summary Statistics
- Median: 5.4
- Lower Quartile: 0.5
- Upper Quartile: 19.2

% Unbound
- Median: 7.83
- Lower Quartile: 0.00
- Upper Quartile: 36.70

Hepatic Clearance ($\mu L/min/10^6$ cells)

Cumulative Percent

Number of Values
# How good are we at predicting *in vivo* $C_{ss}$?

## ToxCast Phase I Chemicals

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

## ToxCast Phase II Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th><em>In vivo</em>-Derived $C_{ss}$ (µM)</th>
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<tbody>
<tr>
<td>Acetaminophen</td>
<td>1.1</td>
<td>0.52</td>
<td>0.57</td>
</tr>
<tr>
<td>2-Chloro-2'-deoxyadenosine</td>
<td>0.28</td>
<td>1.36</td>
<td><strong>0.58</strong></td>
</tr>
<tr>
<td>Coumarin</td>
<td>0.01-0.02</td>
<td>13.63</td>
<td>15.40</td>
</tr>
<tr>
<td>Diphenhydramine HCl</td>
<td>0.11-0.16</td>
<td>3.18</td>
<td>3.57</td>
</tr>
<tr>
<td>6-Propyl-2-thiouracil</td>
<td>1.10</td>
<td>1.58</td>
<td>1.80</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.022</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>0.2-1.8</td>
<td>11.6</td>
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<td>Candoxatril</td>
<td>0.023</td>
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<td>0.14</td>
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<tr>
<td>Flutamide</td>
<td>0.004-0.005</td>
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<td>0.64</td>
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<tr>
<td>PK 11195</td>
<td>0.14</td>
<td>0.58</td>
<td>0.66</td>
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<td>5,5'-Diphenylhydantoin</td>
<td>4.92</td>
<td>1.59</td>
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<td>Triamcinolone</td>
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<td>0.004</td>
<td>0.002</td>
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</tr>
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<td>0.57</td>
<td>0.64</td>
</tr>
<tr>
<td>PFOA</td>
<td>20.12</td>
<td>0.58</td>
<td>0.66</td>
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<td>0.27</td>
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<td>2-10</td>
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</tr>
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#### Note
- ~60% are within 10-fold of *in vivo* $C_{ss}$ values
- ~80% are within 20-fold of *in vivo* $C_{ss}$ values

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*IVIVE Webinar | October 7, 2015*
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

Wambaugh et al., Tox Sci., 2015
Comparing Dosimetry-Adjusted Oral Equivalents against Nominal AC\textsubscript{50} Concentrations

<table>
<thead>
<tr>
<th>CAS #</th>
<th>Chemical</th>
<th>95th %ile</th>
<th>Assay Name (abridged)</th>
<th>AC50 (µM)</th>
<th>Oral Equivalent (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4291-63-8</td>
<td>2-Chloro-2'-deoxyadenosine</td>
<td>2.0713</td>
<td>BSK_SAg_PBMCCytotoxicity</td>
<td>1</td>
<td>0.4828</td>
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<tr>
<td>1806-26-4</td>
<td>4-Octylphenol</td>
<td>1.4109</td>
<td>APR_CellCycleArrest</td>
<td>1</td>
<td>0.7088</td>
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<tr>
<td>57-97-6</td>
<td>7,12-Dimethylbenz(a)anthracene</td>
<td>3.9083</td>
<td>APR_CellCycleArrest</td>
<td>1</td>
<td>0.2559</td>
</tr>
<tr>
<td>148-24-3</td>
<td>8-Hydroxyquinoline</td>
<td>0.0403</td>
<td>APR_p53Act</td>
<td>1</td>
<td>24.8188</td>
</tr>
<tr>
<td>484-17-3</td>
<td>9-Phenanthrol</td>
<td>2.1423</td>
<td>APR_CellLoss</td>
<td>1</td>
<td>0.4668</td>
</tr>
<tr>
<td>57-87-1</td>
<td>7,12-Dimethylbenz(a)anthracene</td>
<td>3.9083</td>
<td>APR_CellCycleArrest</td>
<td>1</td>
<td>0.2559</td>
</tr>
<tr>
<td>120-12-7</td>
<td>Anthracene</td>
<td>0.5800</td>
<td>APR_MitoMembPot</td>
<td>1</td>
<td>1.7241</td>
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<tr>
<td>1912-24-9</td>
<td>Atrazine</td>
<td>0.5998</td>
<td>APR_p53Act</td>
<td>1</td>
<td>1.6672</td>
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<tr>
<td>55285-14-8</td>
<td>Carbasulfan</td>
<td>0.0056</td>
<td>NVS_ENZ_rAChE</td>
<td>1</td>
<td>177.2814</td>
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<tr>
<td>7173-51-5</td>
<td>Didecyl dimethyl ammonium chloride</td>
<td>3.3686</td>
<td>APR_CellLoss</td>
<td>1</td>
<td>0.2969</td>
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<tr>
<td>76-87-9</td>
<td>Fentin hydroxide</td>
<td>318.039</td>
<td>APR_CellLoss</td>
<td>1</td>
<td>0.0001</td>
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<tr>
<td>99-76-3</td>
<td>Methylparaben</td>
<td>0.1768</td>
<td>APR_CellCycleArrest</td>
<td>1</td>
<td>5.6561</td>
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<tr>
<td>50-65-7</td>
<td>Niclosamide</td>
<td>0.3073</td>
<td>APR_MitoMass</td>
<td>1</td>
<td>3.2544</td>
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<tr>
<td>50-65-7</td>
<td>Niclosamide</td>
<td>0.3073</td>
<td>APR_NuclearSize</td>
<td>1</td>
<td>3.2544</td>
</tr>
<tr>
<td>50-65-7</td>
<td>Niclosamide</td>
<td>0.3073</td>
<td>APR_OxidativeStress</td>
<td>1</td>
<td>3.2544</td>
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<tr>
<td>26530-20-1</td>
<td>Ochthinone</td>
<td>0.6864</td>
<td>APR_MitoticArrest</td>
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<td>1.4569</td>
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<tr>
<td>57-83-0</td>
<td>Progesterone</td>
<td>0.2007</td>
<td>APR_MitoMembPot</td>
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<tr>
<td>83-79-4</td>
<td>Rotenone</td>
<td>0.3131</td>
<td>APR_MitoticArrest</td>
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<td>3.1941</td>
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<td>79902-63-9</td>
<td>Simvastatin</td>
<td>0.6379</td>
<td>APR_CellCycleArrest</td>
<td>1</td>
<td>1.5677</td>
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<tr>
<td>79902-63-9</td>
<td>Simvastatin</td>
<td>0.6379</td>
<td>APR_MitoMass</td>
<td>1</td>
<td>1.5677</td>
</tr>
<tr>
<td>156052-68-5</td>
<td>Zoxamide</td>
<td>168.1532</td>
<td>APR_CellCycleArrest</td>
<td>1</td>
<td>0.0059</td>
</tr>
<tr>
<td>156052-68-5</td>
<td>Zoxamide</td>
<td>168.1532</td>
<td>APR_MitoMass</td>
<td>1</td>
<td>0.0059</td>
</tr>
</tbody>
</table>

Same AC\textsubscript{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

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

\textsuperscript{a} MHE: Maximum Human Exposure

\textsuperscript{b} AER: Acceptable Exposure Ratio

\textsuperscript{c} AC\textsubscript{50}: Concentration that inhibits 50% of the maximum observed effect
Comparing *In Vitro* ToxCast-derived Points of Departure Against *In Vivo* Rodent LELs

- Rat Liver Metabolism
- Rat Plasma Protein Binding
- Computational IVIVE Model
- Blood Concentrations at Steady State
- ~600 *In Vitro* ToxCast Assays
- ToxCast AC$_{50}$ Value
- Plasma Concentration
- Oral Exposure
- Reverse Dosimetry
- Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

Wetmore *et al.*, *Tox Sci.*, 2013
Comparing *In Vitro* ToxCast-derived Points of Departure Against *In Vivo* Rodent LELs

**In Vivo** Low Effect Level from ToxRefDB (mg/kg/day)

Minimum *In Vitro* Rat Oral Equivalent Dose (mg/kg/day)

Subset of 59 Chemicals from ToxCast Phase I

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)
- $C_{\text{max}}$ vs. $C_{\text{ss}}$
- 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: $C_{\text{int}}$ 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 \( Cl_{in\,vitro} \) → SimCYP → General Population

- \( Plasma\,C_{ss} \neq Plasma\,C_{ss} \) General Population
Population-based *In Vitro-In Vivo* Extrapolation

Primary Hepatocytes

Hepatic Clearance
\[ Cl_{\text{in vitro}} \]

Plasma \( C_{ss} \)

General Population

Plasma \( C_{ss} \) for:
- Neonates
- Asians
- Northern Europeans
- Children
- And so on...

Intrinsic Clearance Rates

- CYP1A2
- CYP3A4
- ClrCYP1A2
- ClrCYP3A4

- CYP2E1
- CYP3A5
- UGT1A1
- ClrCYP2E1
- ClrCYP3A5
- ClrUGT1A1

- CYP2C9
- CYP2B6
- ClrCYP2C9
- ClrCYP2B6

- UGT2B7
- ClrUGT2B7

- CYP2C8
- CYP2C19
- ClrCYP2C8
- ClrCYP2C19

- UGT1A4
- ClrUGT1A4

- CYP2D6
- ClrCYP2D6

- CYP3A5
- ClrCYP3A5

- CYP2C19
- ClrCYP2C19

- UGT1A1
- ClrUGT1A1

- CYP2B6
- ClrCYP2B6

- UGT2B7
- ClrUGT2B7

- CYP2E1
- ClrCYP2E1

- CYP2C9
- ClrCYP2C9

- UGT1A4
- ClrUGT1A4

- CYP2D6
- ClrCYP2D6

- CYP3A5
- ClrCYP3A5

- CYP2C19
- ClrCYP2C19

- UGT1A1
- ClrUGT1A1

- CYP2B6
- ClrCYP2B6

- UGT2B7
- ClrUGT2B7

- CYP2E1
- ClrCYP2E1

- CYP2C9
- ClrCYP2C9

- UGT1A4
- ClrUGT1A4

- CYP2D6
- ClrCYP2D6

- CYP3A5
- ClrCYP3A5

- CYP2C19
- ClrCYP2C19

- UGT1A1
- ClrUGT1A1

- CYP2B6
- ClrCYP2B6
Integrating High-Throughput Pharmacokinetics with the ToxCast In Vitro Assays

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

Population-Based IVIVE Model

Plasma Concentration

Reverse Dosimetry

~600 In Vitro ToxCast Assays

ToxCast AC_{50} Value

Oral Exposure

Steady State Plasma Concentrations for Different Subpopulations

~600 In Vitro ToxCast Assays

Recombinant Enzyme Metabolism

Human Plasma Protein Binding

Population:  A         B        C

Least Sensitive Assay

Most Sensitive Assay

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

Wetmore et al., 2014, Toxicol.Sci, 142(1):210-14
Integrating High-Throughput Pharmacokinetics with the ToxCast In Vitro Assays

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

**Carbaryl**

HK$_{AF}$ = 11.4

**Upper 95th percentile $C_{ss}$**

**Lifestage or Subpopulation**

(Age (yr) or Ethnic)

**HK$_{AF}$**: human toxicokinetic adjustment factor
Comparison of $C_{ss}$ Values Derived Across Multiple Lifestages and Subpopulations

# Estimated Chemical-Specific Toxicokinetic Adjustment Factors

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Median $C_{ss}$ for Healthy Population</th>
<th>95th Percentile $C_{ss}$ for Most Sensitive</th>
<th>Most Sensitive</th>
<th>Estimated $HK_{AF}$</th>
<th>% Contribution of Isozyme Differences to Average $HK_{AF}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetochlor</td>
<td>0.026</td>
<td>0.15</td>
<td>Neonatal</td>
<td>6.7</td>
<td>86</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>0.099</td>
<td>0.66</td>
<td>Neonatal</td>
<td>6.7</td>
<td>86</td>
</tr>
<tr>
<td>Bensulide</td>
<td>0.241</td>
<td>0.97</td>
<td>Neonatal</td>
<td>4.0</td>
<td>79</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>0.043</td>
<td>0.49</td>
<td>Neonatal</td>
<td>11.4</td>
<td>87</td>
</tr>
<tr>
<td>Difenoconazole</td>
<td>0.201</td>
<td>0.49</td>
<td>Renal Insufficiency</td>
<td>3.5</td>
<td>99</td>
</tr>
<tr>
<td>Fludioxonil</td>
<td>0.38</td>
<td>4.37</td>
<td>Neonatal</td>
<td>11.5</td>
<td>87</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.029</td>
<td>0.14</td>
<td>Neonatal</td>
<td>4.9</td>
<td>83</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.001</td>
<td>0.009</td>
<td>Neonatal</td>
<td>6.5</td>
<td>90</td>
</tr>
<tr>
<td>Tebupirimfos</td>
<td>0.107</td>
<td>0.38</td>
<td>Renal Insufficiency</td>
<td>3.5</td>
<td>15</td>
</tr>
</tbody>
</table>
Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations
Utility in a Tiered Testing Approach

Tier 1 Testing

- *In Vitro Assays for Bioactivity*
  - Weak, Non-Specific Interacting Chemicals
    - Define First Order Margin-of-Exposure
  - Potent, Specific Interacting Chemicals
    - Define Tentative Mode-of-Action
  - Human *In Vitro* Pharmacokinetic Assays and IVIVE Modeling
    - Conservative First Order Human Exposure Characterization

MOE >100 to >1000

STOP

Tier 2 Testing

- Short-term Rodent Transcriptomic Studies
  - Define Second Order Margin-of-Exposure
- Confirm *In Vivo* Mode-of-Action and Human Relevance
  - Refined Pharmacokinetic Estimates
  - Refined Second Order Human Exposure Characterization

MOE >100 to >1000

STOP

STOP

Tier 3 Testing

[Standard Tox Studies]

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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References

**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