Incorporating Population Variability and Sensitive Subpopulations into Dosimetry for High-Throughput Toxicity Testing

Computational Toxicology Communities of Practice
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The Hamner Institutes for Health Sciences
Setting the Stage…
Incorporating Dosimetry with High-Throughput Screening Data
Setting the Stage…
Using Reverse Dosimetry to Estimate Population-Based Oral Equivalent Doses

1 mg/kg/day Oral Exposure

Metabolic and binding parameters

Assay X (e.g., ACE inhibition)

ToxCast AC<sub>50</sub> Value

Plasma Concentration

Oral Exposure

Upper 95<sup>th</sup> Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

Oral Dose Required to Achieve Steady State Plasma Concentrations Equivalent to AC<sub>50</sub>

\[
\frac{\text{Oral Equivalent (mg/kg/day)}}{\text{ToxCast AC}_{50} \text{ (uM)}} = \frac{1 \text{ mg/kg/day}}{\text{Upper 95}^{\text{th}} \text{ Percentile Css (uM)}}
\]
Incorporating Human Dosimetry and Exposure into High-Throughput

*In Vitro* Toxicity Screening


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Integration of Dosimetry, Exposure, and High-Throughput Screening Data in Chemical Toxicity Assessment


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Dosimetry and Exposure Strategy
Limited to General Population
The Impact of Population Variability on Risk Assessment

Clearance differences span across multiple juvenile subpopulations...

... and geriatric subpopulations.

Clearance results for full database (27 substrates).


from Ginsberg et al., 2005, Environ. Health Persp., 113, 1243-49.
Sole reliance on pharmacokinetic data for a “generic” population could lead to a significant underestimation of risk to a susceptible subpopulation.

Population-based *In Vitro*-to-*In Vivo* Extrapolation
Incorporating Recombinant Phase I and II Enzyme Data into IVIVE Modeling

Scaling rCYP Data to HLM using intersystem extrapolation factors

\[
ISEF = \frac{CI_{\text{int, HLM}} \text{ (uL / min / mg protn)}}{CI_{\text{int, rCYP}} \times \text{HLM CYP abundance}}
\]

\[
CI_{\text{int}} = \text{intrinsic clearance}
\]

\[
\text{HLM} = \text{human liver microsomes}
\]

\[
r\text{CYP} = \text{recombinant CYP isoform}
\]

Hepatic CYP Isozyme Abundance in Healthy Adults (% of Total)
Integrating High-Throughput Pharmacokinetics with the ToxCast *In Vitro* Assays (1)
Integrating High-Throughput Pharmacokinetics with the ToxCast In Vitro Assays (2)
## Experimental Design

<table>
<thead>
<tr>
<th>Test System:</th>
<th>BD Supersomes.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymes:</strong></td>
<td>13 CYPs, 5 UGTs, 2 controls, 1 human liver microsome pool.</td>
</tr>
<tr>
<td><strong>Positive Controls:</strong></td>
<td>Suitable substrate for each enzyme, in duplicate.</td>
</tr>
<tr>
<td><strong>Chemicals:</strong></td>
<td>9</td>
</tr>
<tr>
<td><strong>Negative Controls:</strong></td>
<td>Enzymes lacking cofactors &amp; metabolically inactive supersomes.</td>
</tr>
<tr>
<td><strong>Time Points:</strong></td>
<td>60 minute time course; 0 min, 5 min, 15 min, 30 min, 60 min.</td>
</tr>
<tr>
<td><strong>Concentrations:</strong></td>
<td>1 µM &amp; 10 µM, in triplicate.</td>
</tr>
</tbody>
</table>
Recombinant Isozyme Clearance Rates (1)

Azoxystrobin

Ln Concentration (µM)

Time (min)

Fludioxonil

Ln Concentration (µM)

Time (min)

No UGT metabolism detected

UGT1A1

UGT1A9

CYP2C9

CYP2C19

CYP1A2

CYP3A4

CYP2E1

CYP2C9

CYP2C19

CYP1A2

CYP3A4

CYP2E1

CYP3A4

CYP3A5

CYP2C19

CYP2E1

CYP2C9

CYP2C19

CYP1A2

CYP3A4

CYP2E1
Recombinant Isozyme Clearance Rates (2)

**Carbaryl**

- CYP2E1
- CYP2C9
- CYP1A2
- CYP2C19
- CYP2B6

**Difenoconazole**

- CYP3A4
- CYP3A5
- CYP2C19
- CYP2C8
- CYP2C9
- CYP1A2
- CYP2C18

No UGT metabolism detected
Combining Isozyme Clearance and Abundance Data to Determine Fraction Metabolized

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>No. Chemicals % fm &gt; 5%</th>
<th>% fm Range</th>
<th>Chemicals with % fm &gt; 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>3</td>
<td>0.4 - 91.4</td>
<td>Bensulide, Carbaryl, Fludioxonil</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>6</td>
<td>2.1-63.1</td>
<td>Azoxystrobin, Bensulide, Carbaryl, Difenoconazole, Haloperidol, Tebupirimfos</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>7</td>
<td>1.0-80.2</td>
<td>Acetochlor, Azoxystrobin, Bensulide, Difenoconazole, Haloperidol, Lovastatin Tebupirimfos</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>2</td>
<td>1.4-6.4</td>
<td>Lovastatin, Tebupirimfos</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>2</td>
<td>2.6-19.3</td>
<td>Haloperidol, Tebupirimfos</td>
</tr>
<tr>
<td>UGT1A4</td>
<td>3</td>
<td>0.1-12.1</td>
<td>Difenoconazole, Haloperidol, Lovastatin</td>
</tr>
</tbody>
</table>
Comparison of $C_{ss}$ Values Derived Across Multiple Subpopulations (1)
Comparison of $C_{ss}$ Values Derived Across Multiple Subpopulations (2)
Agreement between *In Vivo* and IVIVE-derived $C_{ss}$ Values using Recombinant CYP-based Clearance Rates

<table>
<thead>
<tr>
<th>Chemical</th>
<th><em>In vivo</em> PK $C_{ss}$ (µM)</th>
<th>IVIVE $C_{ss}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>0.030</td>
<td>0.046</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.090-0.126</td>
<td>0.029</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.004-0.009</td>
<td>0.001</td>
</tr>
</tbody>
</table>
## Estimated Chemical-Specific Toxicokinetic Adjustment Factors

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Median $C_{ss}$ for Healthy Population</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; 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

- **Azoxystrobin**
- **Fludioxonil**
Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations
Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations

Bensulide

Tebupirimfos

Oral Equivalent Dose or Estimated Exposure (mg/kg/day)

Subpopulation (Age (yr) or Ethnic)

Below x-axis (at 3.5E-05 mg/kg/d)
Conclusions

- Demonstrates the feasibility of measuring isozyme-specific clearance rates and using them to capture population variability for industrial chemicals.

- IVIVE-derived $C_{ss}$ values were in good agreement for $C_{ss}$ values derived from *in vivo* data.

- The pharmacokinetic variability observed when comparing general to the most sensitive population spanned a range of 3 to 11.5-fold.

- The extent of this variability was determined primarily by a chemical’s overall clearance rate.

- Subpopulation-based pharmacodynamic differences will also contribute to the variable susceptibilities that may be observed following chemical exposure.
Key Points

• First comprehensive attempt to combine physiologic and PK differences to quantitate variability anticipated between age, ethnic and disease-based populations.

• While the chemical-specific TK adjustment factors routinely exceeded the default 3.2-fold UF assigned for TK-based variability, the adjustment factors for these chemicals were typically within 10-fold (max AF = 11.5).
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