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
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.

from Ginsberg et al., 2005, Environ. Health Persp., 113, 1243-49.
The Impact of Population Variability on Risk Assessment

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

\[
ISEF = \frac{CI_{int, HLM} (\text{uL/min/mg protn})}{CI_{int, rCYP} (\text{uL/min*pmol P450}) \times \text{HLM CYP abundance (pmol P450/mg protn})}
\]

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

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

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

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

**Azoxystrobin**

- CYP3A4
- CYP3A5
- CYP2C19
- CYP2C9

**Fludioxonil**

- CYP2C9
- CYP2C19
- CYP1A2
- CYP3A4
- CYP2E1

No UGT metabolism detected
Recombinant Isozyme Clearance Rates (2)

**Carbaryl**

- Ln Concentration (µM) vs. Time (min)
- CYP2E1, CYP2C9, CYP1A2, CYP2C19, CYP2B6

**Difenoconazole**

- Ln Concentration (µM) vs. Time (min)
- 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>In vivo 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>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
Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations
Matching Oral Equivalent Doses and Exposure Estimates for Subpopulations

**Bensulide**

**Tebupirimfos**

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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