Human Metabolism of New and Emerging Pesticides

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*We deeply regret the untimely death of Dr Rose on May 23rd, 2006.
Chemical Toxicity: A Cascade of Events

Exposure and Absorption at Portals of Entry

Distribution to Organs and Tissues

Excretion

Metabolism to More Toxic Metabolites

Metabolism to Conjugation Products

Metabolism to Less Toxic Metabolites

Redistribution to Organs and Tissues

Interaction with Macromolecules (Protein, DNA, RNA, Receptors, etc)

Turnover and Repair

Toxic Effects
Genetic, Carcinogenic, Reproductive, Neurotoxic, Etc.

All of the above may be used in human health and/or environmental risk analysis
Human Health Risk Analysis: A Summary

- Dose-Response Assessment
- Hazard Assessment
- Exposure Assessment

Risk Characterization

Risk Assessment

Risk Analysis

Risk Management

Risk Communication
Significance of Human Metabolic Studies

1. Metabolism is one of the key events leading to toxicity or detoxication.
2. Significant species differences in metabolism may result in differential toxicity.
3. Identification of metabolic pathways and the enzymes involved will enable better risk assessment.
4. Only human studies will be of value in the identification of populations at risk.
5. Pesticide exposure potentially can result in significant interactions with both endogenous and exogenous chemicals.
Human Metabolism of Pesticides

- Chlorpyrifos, Fonofos, Phorate
- Carbaryl, Carbofuran
- Fipronil
- Endosulfan
- Permethrin
- Pesticide - Pesticide Interactions
- Pesticide – Hormonal Interactions
- Pesticide Induction Studies
Chlorpyrifos Metabolites

Chlorpyrifos

\[ \text{CYP} \rightarrow \begin{bmatrix} \text{S} & \text{O} \\ \text{O} & \text{P} & \text{O} & \text{S} \end{bmatrix} \]

Chlorpyrifos-oxon

3,5,6-trichloro-2-pyridinol

Diethyl phosphorodithioate
Metabolic Activity of Human CYP Isoforms Towards Chlorpyrifos

![Graph showing metabolic activity of human CYP isoforms towards Chlorpyrifos. The x-axis represents CYP isoforms (1A2, 2B6, 2C9, 2C19, 3A4), and the y-axis represents nmol/nmol CYP/min. The graph compares Oxon and TCP activity.]
## Chlorpyrifos Metabolism by Individual Human Liver Microsomes

<table>
<thead>
<tr>
<th>CYP</th>
<th>Desulfuration</th>
<th>Dearylation</th>
<th>Desulfuration/Dearylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG6</td>
<td>0.09</td>
<td>0.16</td>
<td>0.56</td>
</tr>
<tr>
<td>HG23</td>
<td>0.16</td>
<td>0.31</td>
<td>0.50</td>
</tr>
<tr>
<td>HG42</td>
<td>0.74</td>
<td>0.67</td>
<td>1.10</td>
</tr>
<tr>
<td>HG43</td>
<td>0.08</td>
<td>0.61</td>
<td>0.13</td>
</tr>
<tr>
<td>HG112</td>
<td>0.67</td>
<td>0.91</td>
<td>0.74</td>
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</tbody>
</table>
Metabolism of Chlorpyrifos by Polymorphic CYP3A4

![Graph showing metabolism of Chlorpyrifos by different polymorphisms of CYP3A4.](image-url)
Chlorpyrifos: Summary of Results

Chlorpyrifos is metabolized to both primary products by five human CYP isoforms.

CYP2B6 is most active in forming the toxic metabolite.

CYP2C19 is most active in forming the degradation product.

Rough predictions of metabolic activity can be made based on individual phenotypes.
Carbaryl Metabolism

Carbaryl

4-Hydroxycarbaryl

5-Hydroxycarbaryl

Carbaryl Methylol
Carbaryl Metabolism is Primarily due to CYP2B6, 1A2, and 3A4

![Graph showing the nmol metabolite/nmol P450/min for various enzymes, including 5-hydroxy carbaryl, 4-hydroxy carbaryl, and carbaryl methylol.](image)
## Carbaryl Metabolism in Individual Human Liver Microsomes

<table>
<thead>
<tr>
<th>Individuals</th>
<th>5-OH</th>
<th>4-OH</th>
<th>Methylol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG6</td>
<td>0.02</td>
<td>0.40</td>
<td>0.17</td>
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<tr>
<td>HG23</td>
<td>0.03</td>
<td>0.48</td>
<td>0.21</td>
</tr>
<tr>
<td>HG42</td>
<td>0.04</td>
<td>0.48</td>
<td>0.93</td>
</tr>
<tr>
<td>HG43</td>
<td>0.03</td>
<td>0.31</td>
<td>0.18</td>
</tr>
<tr>
<td>HG112</td>
<td>0.05</td>
<td>0.63</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Carbofuran Metabolism

Carbofuran $\xrightarrow{\text{CYP}}$ 3-Hydroxy Carbofuran

3-Keto Carbofuran $\xrightarrow{\text{CYP}}$ 3-Keto-7-Phenol

Non-enzymatic

3-Keto-7-Phenol $\xleftarrow{\text{Non-enzymatic}}$
Permethrin

Esterase

Phenoxybenzyl alcohol

Alcohol dehydrogenase

Phenoxybenzaldehyde

Phenoxybenzoic acid
Metabolic studies of chlorpyrifos and carbaryl suggest that xenobiotics metabolized by CYP2B6 may be perturbed by exposure to chlorpyrifos and related pesticides.
Chlorpyrifos Inhibition of Carbaryl Metabolism *In Vitro*

**Graph:**
- **Y-axis:** nmoles carbaryl/mg protein/min
- **X-axis:** [Chlorpyrifos] (uM)

- **Bars:**
  - 5-hydroxycarbaryl
  - 4-hydroxycarbaryl
  - Carbaryl methylol

- **Legend:**
  - 5-hydroxycarbaryl
  - 4-hydroxycarbaryl
  - Carbaryl methylol

- **Data Points:**
  - 0 uM: 0.2 nmoles carbaryl/mg protein/min
  - 1.25 uM: 0.18 nmoles carbaryl/mg protein/min
  - 2.5 uM: 0.15 nmoles carbaryl/mg protein/min
  - 12.5 uM: 0.12 nmoles carbaryl/mg protein/min
  - 125 uM: 0.05 nmoles carbaryl/mg protein/min

- **Significance:**
  - * indicates statistical significance.
Is permethrin metabolism significantly inhibited by other pesticides?

- First metabolic step is ester cleavage to form phenoxybenzyl alcohol. Will typical esterase inhibitors inhibit permethrin metabolism?

- Yes. Potent and complete inhibition by chlorpyrifos oxon. Less potent inhibition by carbaryl.
What About Endogenous Substrates?

Because chlorpyrifos is metabolized by CYP3A4 which is a primary isoform for both testosterone and estradiol metabolism, is there metabolic interaction between chlorpyrifos and human steroid hormones?
Pesticide Effects on In Vitro Testosterone Metabolism

- $6\beta$-hydroxytestosterone accounts for approximately 86% of all testosterone metabolites in human liver microsomes.

- CYPs 3A4 and 3A5 account for most of the $6\beta$-hydroxytestosterone formed.

- Preincubation of CYP3A4 with chlorpyrifos (2 µM) resulted in 98% inhibition of major testosterone metabolites (100 µM testosterone substrate concentration).
Effects of Pesticides and Other Chemicals on Estradiol Metabolism in Human Liver Microsomes

% Control Activity

- Control
- Chlorpyrifos
- Fonofos
- Phorate
- DEET
- Fipronil
- Imidacloprid
- Deltamethrin
- Permethrin
- Carbofuran
- Carbaryl
- Naphthalene
- Pyridostigmine bromide
Effects of Pesticides and Other Chemicals on Estradiol Metabolism by CYP1A2
Effects of Pesticides and Other Chemicals on Estradiol Metabolism by CYP3A4
Human Hepatocyte Induction by Pesticides and DEET

[Bar chart showing induction levels for various pesticides and DEET concentrations]
Samples 183-246
AhR G1661A Snp
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