“Metabolism and Mechanisms of Renal Cellular Injury Induced by Trichloroethylene”

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Outline of Talk

• Metabolism
  – P450 vs. GST: Species Differences
  – Kidney: Beta-Lyase vs. S-Oxidase
  – Male reproductive system: Implications for toxicity

• DCVC-Induced Renal Toxicity
  – Sex- and species-related differences in acute toxicity: Rat, mouse, human
  – Role of specific GST isoforms in TCE bioactivation
  – Sublethal injury and repair: Rat PT cells
  – Apoptosis, necrosis, and cell proliferation: Human PT cells
  – Role of FMO in DCVC bioactivation: Rats, humans

• In Vivo Evidence for GST Pathway
Role of Individual P450 Enzymes in TCE Bioactivation in Liver and Kidney.

![Diagram showing the role of individual P450 enzymes in TCE bioactivation.](image)
Renal CYP2E1: Rats vs. Humans.

• Rats:
  – Readily detectable
  – Major P450 in PT cells; < 10% of hepatic content

• Humans:
  – No CYP2E1 by pNP hydroxylase activity or Western blot
  – Virtually no detectable P450-dependent metabolism of TCE
Effect of Pyridine on CYP2E1 Expression in Rat Liver and Kidney Microsomes:

![Image showing the effect of Pyridine on CYP2E1 expression in liver and kidney microsomes. The figure includes a gel showing protein bands and a bar graph comparing control and Pyridine-treated samples.](image-url)
Effect of Clofibrate on Expression of CYP2E1 and CYP2C11 in Rat Liver and Kidney Microsomes:

### CYP2E1

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
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<td>Saline</td>
<td>Clof</td>
<td>Saline</td>
<td>Clof</td>
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<td>Liver</td>
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<tr>
<td>Kidney</td>
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### CYP2C11

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<tr>
<td>Kidney</td>
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**Density**

- **Liver Microsomes**
  - Control: [Graph]
  - Clofibrate: [Graph]

- **Kidney Microsomes**
  - Control: [Graph]
  - Clofibrate: [Graph]
CYP2E1 Expression in Mouse Testis and Epididymis:

Lanes 1-3: Testis (10, 25, 50 µg protein);
Lanes 4-6: Epididymis (5, 10, 25 µg protein);
Lane 7: Liver (2 µg protein)
Localization and Distribution of CYP2E1 in Mouse Epididymis and Testis:

a. Epididymis: Epithelial cells (arrow)

b. Testis: Leydig cells (arrow)
Time-Dependent Formation of Chloral from TCE in Incubations of Microsomes from Mouse Testis and Epididymis:

pNP Hydroxylase Activity (pmol/min per mg protein):
Testis = 3.01 ± 0.78; Epididymis = 7.17 ± 1.01.
Microscopic Evidence of Damage to Mouse Epididymis from TCE: 1000 ppm TCE by Inhalation
(6 hr/day x 5 days/week x 4 weeks).

Bar = 25 µm
# TCE and Metabolites in Human Seminal Fluid:

<table>
<thead>
<tr>
<th>Subject</th>
<th>TCE (pg/extract)</th>
<th>CH (pg/extract)</th>
<th>TCOH (pg/extract)</th>
<th>TCA (pg/extract)</th>
<th>DCA (pg/extract)</th>
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<tbody>
<tr>
<td>1</td>
<td>98.8</td>
<td>62.7</td>
<td>16.2</td>
<td>&lt; 100</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>2</td>
<td>1122</td>
<td>510</td>
<td>9.4</td>
<td>&lt; 100</td>
<td>&lt; 100</td>
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<tr>
<td>3</td>
<td>641</td>
<td>1739</td>
<td>10.8</td>
<td>&lt; 100</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>4</td>
<td>5419</td>
<td>69.1</td>
<td>25.5</td>
<td>&lt; 100</td>
<td>13342</td>
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<tr>
<td>5</td>
<td>20.4</td>
<td>108</td>
<td>14.7</td>
<td>&lt; 100</td>
<td>&lt; 100</td>
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<tr>
<td>6</td>
<td>194</td>
<td>119</td>
<td>3.5</td>
<td>&lt; 100</td>
<td>&lt; 100</td>
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<tr>
<td>7</td>
<td>1618</td>
<td>116</td>
<td>2.7</td>
<td>5504</td>
<td>9439</td>
</tr>
<tr>
<td>8</td>
<td>673</td>
<td>61.2</td>
<td>3.2</td>
<td>&lt; 100</td>
<td>&lt; 100</td>
</tr>
</tbody>
</table>
Localization of CYP2E1 in Human Testis and Epididymis.

Testis: Arrow = Leydig cells.  
Epididymis: Arrow = Epithelium.
Localization of CYP2E1 in Monkey Epididymis.

Bar = 200 µm.
Testicular Metabolism and Toxicity of TCE: Conclusions.

- CYP2E1, the major P450 enzyme that metabolizes TCE, is present in testis of mouse, a non-human primate, and humans.
- Activity and expression of CYP2E1 are highest in the epididymis.
- Histopathology observed in epididymis of mice exposed to 1000 ppm inhalation x 6 h/d x 5 d/wk x 4 w.
- Humans exposed occupationally to high levels of TCE exhibit both TCE and its metabolites in seminal fluid.
- Data consistent with role for CYP2E1 in animals and humans in bioactivation of TCE leading to testicular toxicity; likely a fairly high dose needed.
## Relative Rates of TRI Metabolism in Rats and Humans:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Rat</th>
<th>Human</th>
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<tbody>
<tr>
<td>P450</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>GST</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>GGT</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>Beta-Lyase</td>
<td>10</td>
<td>1</td>
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GST Expression in Rat Kidney:

<table>
<thead>
<tr>
<th>Lane #</th>
<th>Sample</th>
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<tbody>
<tr>
<td>1</td>
<td>purified α2-2</td>
</tr>
<tr>
<td>2</td>
<td>rat cortical cells</td>
</tr>
<tr>
<td>3</td>
<td>rPT cells</td>
</tr>
<tr>
<td>4</td>
<td>rDT cells</td>
</tr>
</tbody>
</table>
GST Expression in hPT Cells:

A. GSTA
B. Bar chart showing density for GSTA samples:
   - Sample #:
     - 96-607
     - 97-524
     - 98-626
     - 97-522
     - 98-945

C. GSTP
D. Bar chart showing density for GSTP samples:
   - Sample #:
     - 97-540
     - 98-946
     - 98-627
     - 97-632
     - 98-626
     - 97-654
     - 98-946

E. GSTT
F. Bar chart showing density for GSTT samples:
   - Sample #:
     - 98-505
     - 98-560
     - 98-863
     - 98-240
     - 95-520
     - 98-765
     - 98-607
Comparison of Acute Nephrotoxicity and Hepatotoxicity of TRI, DCVG, and DCVC in Male and Female F344 Rats.

Rat Kidney Cells.

Rat Hepatocytes.

LDH Release (%)
Medium for Primary Cultures of rPT and hPT Cells:

- DMEM:Ham’s F12 (1:1)
- Basic Supplements:
  - NaHCO₃ (20 mM)
  - Hepes (15 mM)
  - Antibiotics (penicillin, streptomycin, amphotericin B; day 0-3 only)
- Growth Factors and Hormones:
  - Insulin
  - Hydrocortisone
  - Transferrin
  - Sodium selenite
  - EGF
  - T₃
Control cells at 24 hr exhibit a generally normal epithelial appearance, although there are some elongated cells and intracellular vesicles. As at 8 and 16 hr, cells treated for 24 hr with 1 μM Sts exhibit extensive cellular debris and little or no recognizable intact cellular structure. Cells treated for 24 hr with DCVC exhibit extensive intracellular vesicularization, elongated morphology, and apoptotic bodies.
Time and Concentration of LDH Release in hPT Cells Exposed to DCVC:

A. Male hPT Cells.

B. Female hPT Cells.
DCVC-Induced Apoptosis in hPT Cells.
Time and Concentration Dependence of DCVC-Induced Changes in hPT Cell Cycle.
Time and Concentration Dependence of DCVC-Induced Changes in Apoptosis and S-Phase hPT Cells.
Effects of DCVC on DNA Synthesis in hPT Cells.
Effect of Inhibitors of Beta-Lyase and S-Oxidase on DCVC-Induced Necrosis and Apoptosis in hPT Cells.
Bioactivation of DCVC: Beta-Lyase vs. FMO.
Morphology of hPT Cells Treated for 24 hr with DCVCS:
DCVCS-Induced Necrosis in hPT Cells:
DCVCS-Induced Apoptosis in hPT Cells:
Role of Beta-Lyase vs. S-Oxidase:

- Beta-Lyase more important in rat kidney.
- S-Oxidase more important in human kidney.
- Apoptosis in hPT cells:
  - DCVC > DCVCS
- Necrosis in hPT cells:
  - DCVCS > DCVC
In Vivo Disposition of TRI Administered to Male and Female Rats by Oral Gavage:

- Male and female F344 rats administered either 2, 5, or 15 mmol/kg TRI in corn oil by oral gavage

- Measured P450- and GST-derived metabolites in blood and urine (24, 48 hr) and in liver and kidney (2, 4, 8, 24, 48 hr)
DCVG in Rat Blood:

A. 2 mmol TRI/kg,

B. 5 mmol TRI/kg,

C. 15 mmol TRI/kg.

Time (hr)
DCVC in Rat Blood:

15 mmol TRI/kg: Male Rat Blood.
DCVG in Rat Liver and Kidney:

A. Female Rat Liver.

B. Female Rat Kidney.
DCVC in Rat Liver:

A. 5 mmol TRI/kg.

B. 15 mmol TRI/kg.
DCVC in Rat Kidney:

5 mmol TRI/kg: Female Rat Kidney.
DCVC in Rat Urine:

A. Male Rat Urine.

B. Female Rat Urine.
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