Species Difference in Response and Cell of Origin

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Anatomy and Airway Cell types vary by Species

The following vary position in the tracheo-bronchiolar airway tree:

cell types

susceptibility to injury

local dose (route of exposure)

capability to repair
Comparison of Epithelial Composition in Conducting Airways of Mice and Rhesus Monkeys

**Mouse**

- Proximal Bronchus
  - Basal cells
  - Ciliated cells
  - Clara cells
  - Goblet cells

- Midlevel Airway
  - Basal cells
  - Ciliated cells
  - Clara cells
  - Goblet cells

- Terminal Bronchiole
  - Basal cells
  - Ciliated cells
  - Clara cells
  - Goblet cells

**Monkey**

- Proximal Bronchus
  - Basal cells
  - Ciliated cells
  - Clara cells
  - Goblet cells

- Midlevel Airway
  - Basal cells
  - Ciliated cells
  - Clara cells
  - Goblet cells

- Respiratory Bronchiole
  - Basal cells
  - Ciliated cells
  - Clara cells
  - Goblet cells
Naphthalene
Naphthalene is toxic to Club (Clara) cells regardless of route of exposure

Images from Van Winkle et al 1999
## Species and Site Selective Toxicity of Naphthalene in Adult Animals- 24 hrs post exposure

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Trachea</th>
<th>Bronchiole</th>
<th>Parenchyma</th>
<th>Olfactory</th>
<th>Nasal</th>
<th>Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>50</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LD$_{50}$=380 mg/kg</td>
<td>200</td>
<td>+</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>++</td>
<td>++++</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>inhalation</td>
<td>2-5 ppm</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 ppm</td>
<td>+++</td>
<td>++</td>
<td></td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LD$_{50}$=1600 mg/kg</td>
<td>800</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Current OSHA exposures are 10 ppm TWA, 15 ppm STEL

Plopper et al., 1992; 1993; West et al, 2001; Lee et al., 2005; Dodd et al, 2012.
Acute Naphthalene and the Cycle of Injury and Repair

DIFFERENTIATION → STEADY STATE → + NA → CLARA CELL INJURY

MIGRATION

PROLIFERATION

CILIATED CELL SQUAMATION

Cell Proliferation following Acute i.p. NA Exposure

Graph showing BrdU Positive Nuclei per Length of Basal Lamina over time after treatment in days.

Images showing BrdU distribution at 1 day and 2 days post-treatment.

References:
Van Winkle et al AJP: Lung 1995
Stripp et al AJP: Lung 1995
Lawson et al Am J Pathol 2002
Female Mice are more susceptible than Male mice to NA toxicity

Control  Treated

Male

Female

200 mg/kg ip from Van Winkle et al 2002 AJP: Lung p L1122-34
Neonatal mice are more susceptible than adult mice to NA toxicity
25 mg/kg ip

Fanucchi et al TAAP 1997 144(1):96-104
Repeated Inhalation or Injection of Naphthalene causes “Tolerance”

Tolerance is resistance to a high challenge dose following a week or more of exposure to repeated doses well below the LD50

- NA i.p. tolerance *Lakritz et al 1996; O'Brien et al 1989*
- NA inhalation tolerance *West, Van Winkle et al 2003*
- incomplete tolerance i.p. in females *Sutherland et al 2012*
- tolerance is due to induction of gamma GCS *West et al 2002*
A property intrinsic to the airway epithelium makes it “tolerant”

Morphology of Epithelium in NA Tolerance (inhaled NA)

Tolerant + challenge

Nodules in tolerant mice

Other info re: Mode of Action

- Glutathione depletion occurs early, before tox
- P450 required
- Protein binding of reactive metabolites
- Naphthalene epoxide and downstream metabolites are toxic to Clara cells (Chichester et al studies)
- CYP2F2 contributes to mouse lung Clara cell toxicity- lessons from the knockout mouse
- Female mice are more susceptible than male mice to acute toxicity
Ethylbenzene
Ethylbenzene

• Information concerning the carcinogenicity of ethylbenzene in animals comes from an NTP-sponsored bioassay in male and female rats and mice exposed to 0, 75, 250, or 750 ppm ethylbenzene for up to 2 years (NTP 1999).

• NTP (1999) concluded that ethylbenzene showed some evidence of carcinogenic activity in male mice based on increased incidence of alveolar/bronchiolar neoplasms (NTP 1999).

• Lung: alveolar/bronchiolar adenoma (5/50, 9/50, 10/50, 16/50); alveolar/bronchiolar adenoma or carcinoma (7/50, 10/50, 15/50, 19/50)
Evaluation of Potential Modes of Action of Inhaled Ethylbenzene in Rats and Mice

<table>
<thead>
<tr>
<th>Exposure (ppm)</th>
<th>Males</th>
<th>Females</th>
<th>Mice in the Four-week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>75</td>
<td>750</td>
</tr>
<tr>
<td>Relative liver weight</td>
<td>6.07</td>
<td>5.88</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>(0.46)</td>
<td>(0.29)</td>
<td>(0.44)*</td>
</tr>
<tr>
<td>Relative lung weight</td>
<td>0.706</td>
<td>0.724</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>(0.045)</td>
<td>(0.047)</td>
<td>(0.051)</td>
</tr>
<tr>
<td>Liver S-phase DNA synthesis-LI%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrilobular</td>
<td>1.89</td>
<td>2.77</td>
<td>23.11</td>
</tr>
<tr>
<td></td>
<td>(1.58)</td>
<td>(2.06)</td>
<td>(11.45)*</td>
</tr>
<tr>
<td>Midzonal</td>
<td>1.87</td>
<td>4.26</td>
<td>11.00</td>
</tr>
<tr>
<td></td>
<td>(1.71)</td>
<td>(2.25)</td>
<td>(7.05)*</td>
</tr>
<tr>
<td>Periportal</td>
<td>1.05</td>
<td>2.14</td>
<td>2.82</td>
</tr>
<tr>
<td></td>
<td>(1.05)</td>
<td>(1.77)</td>
<td>(2.20)</td>
</tr>
<tr>
<td>Lung S-phase DNA synthesis-LI%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small airways</td>
<td>3.47</td>
<td>NA</td>
<td>9.73</td>
</tr>
<tr>
<td></td>
<td>(1.85)</td>
<td>(5.80)*</td>
<td>(3.89)</td>
</tr>
<tr>
<td>Alveoli</td>
<td>5.63</td>
<td>NA</td>
<td>7.80</td>
</tr>
<tr>
<td></td>
<td>(4.08)</td>
<td>(4.51)</td>
<td>(3.96)</td>
</tr>
</tbody>
</table>

Styrene
24 mos Styrene Oxide vapor in Male/Female Mice

Cruzan et al 2002 Reg Toxicol and Pharm 35, 308-319
Is the Club (Clara) cell a target?

(a) Control (H and E)

(b) Styrene- 160 ppm 104 wks

Styrene- 104 wks anti CC10

Lung cell fractions enriched for CC have enhanced styrene metabolism—but is it the target?

### TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>% Clara</th>
<th>% Type II</th>
<th>R enantiomer</th>
<th>S enantiomer</th>
<th>R/S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.3 ± 3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.5 ± 4.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.4 ± 4.1</td>
<td>6.9 ± 2.2</td>
<td>3.62 ± 1.09</td>
<td></td>
</tr>
<tr>
<td>55.8 ± 8.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.3 ± 27.7</td>
<td>23.0 ± 8.2</td>
<td>3.98 ± 0.75</td>
<td></td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.8 ± 3.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.3 ± 4.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.7 ± 1.1</td>
<td>8.0 ± 2.6</td>
<td>0.47 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>37.3 ± 9.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.0 ± 1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.2 ± 3.6</td>
<td>11.0 ± 3.2</td>
<td>1.02 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

*Note.* R and S enantiomer values in pmols/10⁶ cells/min.
<sup>a</sup> Calculated on basis of total number of nucleated cells.
<sup>b</sup> Percent is mean ± SE for 4 experiments.
<sup>c</sup> Percent is mean ± SE for 3 experiments.

Decrease in labelling index of terminal bronchioles of Cyp2F2 null mice exposed to either styrene or styrene oxide for 5 days (Cruzan et al 2012) compared to styrene exposed WT indicates involvement of CYP2F2 in toxicity. Note that dosing was ip.
CYP2E1-null and Cyp2F2-null mice
LDH in BALF - is it CYP2F?

Mice were given 6nmol/kg styrene ip
BALF was assessed for LDH activity
(Shen, S et al Chem Res Toxicol 2013
Dec 19. Epub)

Increased protein covalent binding in cells with increased cyp2E

What is the role of the liver?

Table 2
Toxicity of styrene in wild-type and hepatic cytochrome P450 reductase knockout mice.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>BALF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Cells</td>
<td>N</td>
</tr>
<tr>
<td>WT</td>
<td>Control</td>
<td>7</td>
<td>32 ± 9$^f$</td>
<td>7</td>
</tr>
<tr>
<td>WT</td>
<td>Styrene$^a$</td>
<td>9</td>
<td>633 ± 97$^g$</td>
<td>9</td>
</tr>
<tr>
<td>KO</td>
<td>Control</td>
<td>6</td>
<td>43 ± 11$^f$</td>
<td>6</td>
</tr>
<tr>
<td>KO</td>
<td>Styrene$^a$</td>
<td>8</td>
<td>61 ± 15$^f$</td>
<td>8</td>
</tr>
</tbody>
</table>

Within each column values with different superscripts (f, g) are significantly different ($p < 0.05$).

$^a$ 600 mg/kg ip 24 h prior to sacrifice.

$^b$ Cells per microliter.
Summary Questions:

• Is there clear morphologic evidence of club (Clara) cell cytotoxicity?
  – Naphthalene- yes
  – Styrene – not in vivo, some evidence from in vitro biochemical studies with isolated cells
  – Ethylbenzene - no

• Is there a clear temporal distinction between cytotoxicity (from EM or histopath) and proliferation in terminal bronchiolar epithelial cells?
  – Naphthalene- yes, acutely. Not clear that these are separate under conditions of repeated exposure and likely overlaps.
  – Styrene – no, cytox not well defined on a cellular basis in intact tissue
  – Ethylbenzene – no, cytox not well defined on a cellular basis in intact tissue

• Are there species differences in response in the lung?
  – Naphthalene- yes for both cytotoxicity and tumors in lungs of mice (female) and not rats
  – Styrene – tumors in mice but not rat lungs. Cytox unclear
  – Ethylbenzene- tumors in mice (male) but not rat lungs. Cytox unclear