Rodent Lung Tumors in NTP Studies

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Overview

• NTP studies with increased lung tumor incidence
• Examples
  – Styrene, Naphthalene, Coumarin
  – Ethylbenzene, Cumene
• Potential predisposing factors in pulmonary carcinogenesis
Summary of Lung Lesions in NTP Studies

- Common non-neoplastic lesions
  - Hyperplasia, inflammation, infiltrate, hemorrhage
- Alveolar/Bronchiolar adenomas/carcinomas are the most common lung tumors in mouse and rat
- NTP calls for carcinogenicity
  - Clear Evidence (CE)
  - Some Evidence (SE)
  - Equivocal Evidence (EE)
  - No Evidence (NE)

Dixon et al., Toxicologic Pathology, 36: 428-439, 2008
## Background Lung Tumors
*(NTP Historical controls, 2013)*

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Male Rat</th>
<th>Female Rat</th>
<th>Male Mouse</th>
<th>Female Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB Adenoma</td>
<td>2.4</td>
<td>1.3</td>
<td>15.3</td>
<td>5.7</td>
</tr>
<tr>
<td>AB Carcinoma</td>
<td>1.3</td>
<td>0.1</td>
<td>13.9</td>
<td>4.0</td>
</tr>
<tr>
<td>AB Ad/Carc</td>
<td>3.6</td>
<td>1.4</td>
<td>27.7</td>
<td>9.5</td>
</tr>
</tbody>
</table>

*Rat (n=700/sex) = F344/NTac; Mouse (n=950/sex) = B6C3F1; All numbers are in percentages (%)*

- No incidence of spontaneous cystic keratinizing epithelioma and squamous cell carcinoma in controls
  - Only seen with treatment
Chemically Induced Lung Tumors
(67/580 NTP studies where same chemical was tested in rats and mice)

<table>
<thead>
<tr>
<th>Animal Species and Sex</th>
<th>Carcinogenic including all organs (CE and SE) n=67</th>
<th>Carcinogenic including all organs (including EE) n=67</th>
<th>Studies with Lung tumor response (CE and SE) n=67</th>
<th>Studies with Lung tumor response (including EE) n=67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Male</td>
<td>69% (46)</td>
<td>81% (54)</td>
<td>21% (14)</td>
<td>24% (16)</td>
</tr>
<tr>
<td>Rat Female</td>
<td>70% (47)</td>
<td>84% (56)</td>
<td>21% (14)</td>
<td>24% (16)</td>
</tr>
<tr>
<td>Mouse Male</td>
<td>63% (42)</td>
<td>78% (52)</td>
<td>51% (34)</td>
<td>60% (40)</td>
</tr>
<tr>
<td>Mouse Female</td>
<td>76% (51)</td>
<td>82% (55)</td>
<td>60% (40)</td>
<td>64% (43)</td>
</tr>
</tbody>
</table>

• Tumors in multiple sites - 87% (58/67)
• Positive lung tumor response
  – Both Rats and Mice: 21% (14/67)
<table>
<thead>
<tr>
<th>TR #</th>
<th>Chemical</th>
<th>Ames</th>
<th>Route</th>
<th>Male Rat</th>
<th>Female Rat</th>
<th>Male Mouse</th>
<th>Female Mouse</th>
<th>Multiple sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR-185</td>
<td>Styrene</td>
<td>-</td>
<td>Gavage</td>
<td>NE</td>
<td>NE</td>
<td>(EE)</td>
<td>NE</td>
<td>No</td>
</tr>
<tr>
<td>TR-410</td>
<td>Naphthalene</td>
<td>-</td>
<td>Inhal’n</td>
<td>CE</td>
<td>CE</td>
<td>NE</td>
<td>(SE)</td>
<td>No</td>
</tr>
<tr>
<td>TR-422</td>
<td>Coumarin</td>
<td>+</td>
<td>Gavage</td>
<td>SE</td>
<td>EE</td>
<td>(SE)</td>
<td>(CE)</td>
<td>Yes</td>
</tr>
<tr>
<td>TR-466</td>
<td>Ethylbenzene</td>
<td>-</td>
<td>Inhal’n</td>
<td>CE</td>
<td>SE</td>
<td>(SE)</td>
<td>SE</td>
<td>Yes</td>
</tr>
<tr>
<td>TR-542</td>
<td>Cumene</td>
<td>-</td>
<td>Inhal’n</td>
<td>CE</td>
<td>SE</td>
<td>(CE)</td>
<td>(CE)</td>
<td>Yes</td>
</tr>
<tr>
<td>TR-534</td>
<td>Divinylbenzene</td>
<td>-</td>
<td>Inhal’n</td>
<td>EE</td>
<td>NE</td>
<td>NE</td>
<td>(EE)</td>
<td>Yes</td>
</tr>
<tr>
<td>TR-370</td>
<td>Benzofuran</td>
<td>-</td>
<td>Gavage</td>
<td>NE</td>
<td>SE</td>
<td>(CE)</td>
<td>(CE)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Cruzan et al., 2009

NTP Data on Select Chemicals

Cruzan et al., 2009
Styrene (NCI, 1979)

- Positive cytogenetic effects (SCE, ChrAb, micronucleus) in human lymphocytes and mammalian cells in vitro
- Mice were exposed (300, 150 mg/kg) for 78 weeks and unexposed for 13 weeks before euthanasia
- Mouse
  - Male: Alveolar/Bronchiolar Adenoma/Carcinoma (A/B Ad/Carc)

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Naphthalene (NTP, 1992, 2000)

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<tbody>
<tr>
<td>TR-410,</td>
<td>Naphthalene</td>
<td>-</td>
<td>Inhal’n</td>
<td>CE</td>
<td>CE</td>
<td>NE</td>
<td>(SE)</td>
<td>No</td>
</tr>
<tr>
<td>TR-500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Positive cytogenetic effects (SCE, ChrAb)
- Mouse
  - Female: Alveolar/Bronchiolar Adenoma/Carcinoma
- Rat
  - Male and female: Olfactory neuroblastoma and nasal epithelial adenoma (NTP 2000)
Coumarin (NTP, 1993)

- Positive cytogenetic effects (SCE, ChrAb)
- Mouse
  - Male and Female: Alveolar/Bronchiolar Adenoma/Carcinoma
  - Male and Female: Forestomach SCC
  - Female: Hepatocellular adenoma/carcinoma
- Rat
  - Male: Renal tubule adenoma

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<td>+</td>
<td>Gavage</td>
<td>SE</td>
<td>EE</td>
<td>(SE)</td>
<td>(CE)</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Ethylbenzene (NTP, 1999)

- Negative for genotoxicity
- Mouse
  - Male: Alveolar/Bronchiolar Adenoma/Carcinoma
  - Female: Hepatocellular adenoma/carcinoma
- Rat
  - Male and Female: Renal tubule adenoma
Cumene (NTP, 2009)

- Negative for genotoxicity
- Mouse
  - Male and Female: Alveolar/Bronchiolar Adenoma/Carcinoma
  - Female: Hepatocellular Adenoma/Carcinoma
- Rat
  - Male and Female: Nose, respiratory epithelium adenoma
  - Male: Renal tubule adenoma

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<th>Ames</th>
<th>Route</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
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</thead>
<tbody>
<tr>
<td>TR-542</td>
<td>Cumene</td>
<td>-</td>
<td>Inhal’n</td>
<td>CE</td>
<td>SE</td>
<td>(CE)</td>
<td>(CE)</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Potential Predisposing Factors in Pulmonary Carcinogenesis

- Cytotoxicity
  - degeneration, necrosis, inflammation

- Cell proliferation
  - Hyperplasia

- Mutations in cancer genes

- Target sites in early time points
  - Terminal bronchioles, alveolar duct, alveoli

- Target cells for tumorigenesis
  - Type II cells
  - Clara (club) cells
Styrene, 13-week Mouse Gavage Study
Loss of Clara (club) cells

Control

H & E

Treated

CC10
Ethylbenzene, 13-week Mouse Inhalation Study

- No pulmonary histologic lesions were noted
- Clara (club) cells unaffected as demonstrated by CC10 stain
Cumene, 13-week Mouse Inhalation Study

- No histologic lesions in the nose or lung
- Clara (club) cells unaffected as demonstrated by CC10 stain
Ethylbenzene-induced Mouse Lung Tumors: Type II Cell Phenotype (+ve for SPC and –ve for CC10)
Cumene-induced Mouse Lung Tumors:
Type II Cell Phenotype (+ve for SPC and –ve for CC10)
Mutational Analysis of Mouse Lung Tumors: Cumene Study

<table>
<thead>
<tr>
<th>Cumene (ppm)</th>
<th>n</th>
<th>Kras</th>
<th>Tp53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Historical controls</td>
<td>117</td>
<td>33 (28%)</td>
<td>No data</td>
</tr>
<tr>
<td>Chamber Controls</td>
<td>7</td>
<td>1 (14%)</td>
<td>0</td>
</tr>
<tr>
<td>125</td>
<td>4</td>
<td>1 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>13</td>
<td>10 (77%)</td>
<td>5 (38%)</td>
</tr>
<tr>
<td>500</td>
<td>18</td>
<td>17 (94%)</td>
<td>11 (61%)</td>
</tr>
<tr>
<td>1,000</td>
<td>17</td>
<td>17 (100%)</td>
<td>11 (65%)</td>
</tr>
<tr>
<td>Cumene Total</td>
<td>52</td>
<td>45 (87%)</td>
<td>27 (52%)</td>
</tr>
</tbody>
</table>

- Predominant *Kras* mutations (Cumene vs Spontaneous)
  - Codon 12 (GTT): G to T transversions (21% vs 0.008%)

- Predominant *Tp53* mutations
  - Exon 5: 89% vs 0%
  - *Tp53* protein expression (IHC): 56% vs 14%
Summary and Discussion

• Lung tumor incidences in NTP studies are higher in the mouse than in the rat
• Mouse lung tumors are usually associated with tumors in multiple sites, with some exceptions like Styrene and Naphthalene
• Structurally similar compounds may cause lung tumors through different mechanisms
  – Mechanism: cytotoxicity vs genotoxicity
  – Cellular target: Type II cells vs Clara cells vs ???
  – Tumor type: Type II predominantly but possible Clara cells
  – Distribution: Lung only vs multiple organ sites
  – Comparison to human disease (morphological & molecular)
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• Ms. Kylie Brockenfelt
• Ms. Emily Singletary

US EPA
• Dr. Charles Wood
## Variation in Clara cell numbers across species

### TABLE 2. Comparison of numerical density and percentage of Clara cells in the bronchiolar epithelial population of adults.

<table>
<thead>
<tr>
<th>Species</th>
<th>Bronchiolar epithelium density (#/mm²)</th>
<th>Clara cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Density (#/mm²)</td>
</tr>
<tr>
<td>Mouse</td>
<td>9,759 ± 1,700</td>
<td>8,730 ± 1,966</td>
</tr>
<tr>
<td>Hamster</td>
<td>14,238 ± 2,794</td>
<td>8,248 ± 2,106</td>
</tr>
<tr>
<td>Rat (Sprague-Dawley)</td>
<td>18,813 ± 2,722</td>
<td>14,028 ± 2,918</td>
</tr>
<tr>
<td>Rat (Fisher 344)</td>
<td>17,070 ± 791</td>
<td>4,336 ± 201</td>
</tr>
<tr>
<td>Rabbit</td>
<td>15,073 ± 706</td>
<td>9,261 ± 434</td>
</tr>
<tr>
<td>Cat</td>
<td>19,532 ± 383</td>
<td>19,532 ± 383</td>
</tr>
<tr>
<td>Bonnet monkey</td>
<td>9,565 ± 304</td>
<td>8,800 ± 280</td>
</tr>
</tbody>
</table>
Concordance of Rat and Mouse lung tumor incidence

• Positive lung tumor incidence response in both Rats and Mice: 21% (14/67)

• Studies with lung tumors - Rat only: 6 chemicals
  – Diphenhydramine HCl; Dimethyl hydrogen phosphite; Talc; 1,2-Epoxybutane; Nickel subsulfide; Gallium arsenide)

• Studies with lung tumors - Mouse only: 7 chemicals
  – Styrene; Trifluralin; Ozone; Dimethyl terephthalate; Estradiol mustard; N-Methylolacrylamide; bis (2-chloro-1-methylethyl) ether)
Styrene-7,8-oxide (SO)

- Indirect exposure via styrene (~90% metabolized)
- SO genotoxic (Ames and Cytogenetic assays)
- SO by gavage induced forestomach Squamous cell carcinomas in mice and rats; Hepatocellular carcinomas in male mice – *No lung tumors* (Lijinsky, 1986)
- A metabolite of 4-vinylphenol, a minor (0.1%) styrene urinary metabolite is potent pneumo- and hepato-toxicant (10x Styrene and 5x SO)
- Inhibitors of Cyp2E1, 2F1 and other Cyps reduced toxicity (Carlson, 2002)
Divinyl Benzene

• Negative for mutagenicity and clastogenicity

• Mouse - ABA/ABC
  – Female: 6/50, 12/50, 8/50, 13/49
  – Male: 16/49, 10/49, 8/49, 8/49

• No tumors in male rats only hyperplasia and metaplasia in nose and lung

• Male rat:
  – Kidney: renal tubule carcinoma 0/50, 0/49, 2/50, 3/49)
  – Brain: oligodendroglioma or astrocytoma 0/49, 1/50, 3/50, 0/50
Benzofuran

• Male mouse:
  – ABA/ABCA: 10/49, 9/39, 19/4
  – HCA\textsubscript{Ad}/HCC/HBA: 12/49, 31/39, 40/48

• Female mouse:
  – ABA/ABCA: 2/50, 9/48, 14/47
  – HCA\textsubscript{Ad}: 1/50, 9/48, 21/47
  – Forestomach tumors: 2/50, 9/50, 5/50

• Rat, Female- Renal tubular cell adenoma