Cancer Studies in Experimental Animals

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One of the RoC listing criteria for reasonably anticipated to be a human carcinogen

- Sufficient evidence of carcinogenicity from studies in experimental animals
  - Increased incidence of malignant and/or
    - a combination of malignant and benign tumors
      - (1) in multiple species
      - or at multiple tissues sites
      - (2) by multiple routes of exposure, or
      - (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset
Questions

• What is the level of evidence (sufficient or inadequate) for the carcinogenicity of antimony trioxide in animal studies?

• What are the methodological strengths and limitations of the studies?

• At what tissue sites were tumors observed?

• What role does lung overload play in causing any observed rat lung tumors?
Outline

• Studies included
• Study quality assessment
• Findings
• Questions to reviewers
Five inhalation studies meet inclusion criteria

Mouse
- B6C3F1/N
  - Duration: 52 weeks
  - NTP 2017

Rat
- Wistar Han
  - Duration: 104 weeks
  - NTP 2017
- F344
  - Exposure: 52 weeks
  - Post-exposure observation: 52 weeks
  - Newton et al. 1994
- (Fisher) CDF (female only)
  - Duration: 104 weeks
  - Watt 1983
- Wistar
  - Duration: 78 weeks
  - Groth et al. 1986
Study qualities (potential bias and sensitivity) were assessed consistently using standard questions.

For questions, see Handbook for Preparing RoC Monograph.
Study qualities were assessed consistently

Each study was given one level of overall utility in assessing carcinogenicity

<table>
<thead>
<tr>
<th>Study design</th>
<th>Exposure</th>
<th>Outcome</th>
<th>Analysis &amp; reporting</th>
<th>Overall utility</th>
</tr>
</thead>
<tbody>
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<td>+++ High</td>
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<td>++ Moderate</td>
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<td></td>
<td></td>
<td></td>
<td>0 Inadequate</td>
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</tbody>
</table>
All studies have some level of utility

<table>
<thead>
<tr>
<th>Animal/Randomization</th>
<th>Concurrent controls</th>
<th>Animal model</th>
<th>Statistical power</th>
<th>Chemical characterization</th>
<th>Dosing regimen</th>
<th>Exposure duration</th>
<th>Outcome methodology</th>
<th>Adequacy of study duration</th>
<th>Consideration of confounding</th>
<th>Reporting and statistics</th>
<th>Tumor combining</th>
<th>Overall utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
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<tr>
<td>Newton et al. 1994</td>
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<td>++</td>
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<tr>
<td>Watt 1983</td>
<td>NR</td>
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<td>NR</td>
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<td>++</td>
<td>NR</td>
<td>+</td>
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</tbody>
</table>
Mice Had Increased Incidences of

**Skin tumors**
- Benign: Fibrous histiocytoma (M)
- Combined: Fibrous histiocytoma or fibrosarcoma (M)

**Lung tumors**
- Benign: Alveolar/bronchiolar adenoma (F)
- Malignant: Alveolar/bronchiolar carcinoma (M and F)
- Combined: Alveolar/bronchiolar adenoma or carcinoma (F)

**Lymphoma**
- Malignant: Lymphoma (F)
Mice Had Increased Incidences of Lung tumors

<table>
<thead>
<tr>
<th>Type</th>
<th>Tumor Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>Alveolar/bronchiolar adenoma (F)</td>
</tr>
<tr>
<td>Malignant</td>
<td>Alveolar/bronchiolar carcinoma (M and F)</td>
</tr>
<tr>
<td>Combined</td>
<td>Alveolar/bronchiolar adenoma or carcinoma (F)</td>
</tr>
</tbody>
</table>

**Graphs:**
- **Lung: malignant**
  - Male and Female Incidence vs. Exposure Concentration
- **Lung: combined**
  - Female Incidence vs. Exposure Concentration
## Mice Lung Tumors

<table>
<thead>
<tr>
<th>Antimony trioxide concentration</th>
<th>3 mg/m³</th>
<th>10 mg/m³</th>
<th>30 mg/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary overload</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Preneoplastic</td>
<td>F, M</td>
<td>F, M</td>
<td>F, M</td>
</tr>
<tr>
<td>Benign</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Malignant</td>
<td>F, M</td>
<td>F, M</td>
<td>F, M</td>
</tr>
<tr>
<td>Combined</td>
<td>F</td>
<td>F</td>
<td>F</td>
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</tbody>
</table>

- Overload –
  - may be from poorly soluble, low intrinsic toxicity particles

- Overload alone does not lead to lung tumors in mice

- Tumors increased at 3 mg/m³ (i.e., below overload threshold)

- Genotoxicity seen in lung (increased DNA damage) and in blood (increased micronucleus)

⇒ Antimony trioxide has some intrinsic toxicity
Mice Had Increased Incidences of Skin tumors

- Benign Fibrous histiocytoma (M)
- Combined Fibrous histiocytoma or fibrosarcoma (M)

Skin: combined

Incidence (among 50 mice)

Exposure Concentration (mg/m³)
Mice Had Increased Incidences of Lymphoma

All organs (lymphoma): malignant

Incidence (among 50 mice)

Exposure Concentration (mg/m³)

Female

Lymphoma
Malignant Lymphoma (F)
One of the RoC listing criteria for reasonably anticipated to be a human carcinogen

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### Lung tumors

<table>
<thead>
<tr>
<th>Type</th>
<th>Tumor Type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>Alveolar/bronchiolar adenoma (M* &amp; F)</td>
<td>NTP 2017</td>
</tr>
<tr>
<td>Combined</td>
<td>Alveolar/bronchiolar adenoma or carcinoma (M*)</td>
<td>NTP 2017</td>
</tr>
<tr>
<td>Benign</td>
<td>Bronchiolar/alveolar adenoma (F)</td>
<td>Groth et al. 1986</td>
</tr>
<tr>
<td>Malignant</td>
<td>Squamous-cell carcinoma (F)</td>
<td>Watt 1983</td>
</tr>
<tr>
<td>Malignant</td>
<td>Scirrhous carcinoma (F)</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>Scirrhous carcinoma (F)</td>
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</tr>
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</table>

### Adrenal gland tumors

<table>
<thead>
<tr>
<th>Type</th>
<th>Tumor Type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>Pheochromocytoma (M &amp; F)</td>
<td>Newton et al. 1994</td>
</tr>
<tr>
<td>Combined</td>
<td>Pheochromocytoma (F)</td>
<td>NTP 2017</td>
</tr>
</tbody>
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Newton et al. 1994 reported no increase in tumors.

*M: carcinogenicity in male rats based on multiple factors (see following slides)
Rats had increased incidences of lung tumors:

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<thead>
<tr>
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<tbody>
<tr>
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Adrenal gland tumors:

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<th>Tumor Type</th>
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<tbody>
<tr>
<td>Benign</td>
<td>Pheochromocytoma (M &amp; F)</td>
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<tr>
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<td>Pheochromocytoma (F)</td>
<td>NTP 2017</td>
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Newton et al. 1994 reported no increase in tumors.

*M: carcinogenicity in male rats based on multiple factors (see following slides)
Factors considered in NTP 2017 rat study

- Incidences of alveolar/bronchiolar adenoma exceed current and historical controls

- Alveolar/bronchiolar carcinoma seen in 2/50 male rats at 10 mg/m³
  - Rare tumor: 0/299 in NTP historical control, 2/731 at RCC, 1/1217 at Charles River (total: 3/2247, or 0.13%)

- Adenoma can progress to carcinoma

- Lung tumors in mice

- Some intrinsic toxicity of antimony trioxide: genotoxicity in mice

→ Antimony trioxide has lung carcinogenicity in rats, even though the increase in incidence was not statistically significant

→ Lung overload alone does not explain the lung tumors in rats
Pheochromocytoma of the Adrenal Medulla

Treatment (antimony trioxide) effect

Adrenal medulla

Hypoxia

Catecholamines (epinephrine and norepinephrine)

Rat Adrenal

Normal

Hyperplasia

Pheochromocytoma
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or at multiple tissues sites

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(3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset
Increased incidences in malignant tumors or combined tumors (benign or malignant) in two species at multiple tissue sites

<table>
<thead>
<tr>
<th>Tissue sites</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malignant</td>
<td>Combined</td>
</tr>
<tr>
<td>Lung</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>–</td>
<td>F</td>
</tr>
<tr>
<td>Skin</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Whole body (lymphoma)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
What is the level of evidence (sufficient, limited, or inadequate) for the carcinogenicity of antimony trioxide in animal studies?

- NTP proposes “sufficient” level of evidence (multiple species, multiple tissue sites)
Questions

- What is the level of evidence (sufficient, limited, or inadequate) for the carcinogenicity of antimony trioxide in animal studies?
  - Propose “sufficient”

- What are the methodological strengths and limitations of the studies?
Questions

- What is the level of evidence (sufficient, limited, or inadequate) for the carcinogenicity of antimony trioxide in animal studies?
  - Sufficient (multiple species, multiple tissue sites)

- What are the methodological strengths and limitations of the studies?

- At what tissue sites were tumors observed?
  - Lung, skin, whole body (lymphoma), and adrenal gland
Questions

• What is the level of evidence (sufficient or inadequate) for the carcinogenicity of antimony trioxide in animal studies?
  – Sufficient (multiple species, multiple tissue sites)

• What are the methodological strengths and limitations of the studies?

• At what tissue sites were tumors observed?
  – Lung, adrenal gland, skin, and lymphoma (whole body)

• What role does lung overload play in causing observed rat lung tumors?
  – Lung tumors are not completely explained by overload (e.g., intrinsic toxicity)
Cancer Studies in Experimental Animals

- Comment on whether the scientific information from cancer studies in experimental animals for antimony trioxide is clear, technically correct, and objectively presented.
  - Identify any information that should be added or deleted.

- Comment on whether the approach and assessment of the utility of the animal carcinogenicity studies (study quality and sensitivity to detect an effect) for informing the cancer evaluation is systematic, transparent, objective, and clearly presented (Sections 5.2, Appendix D).

- Provide any scientific criticisms of NTP’s cancer assessment of the experimental animal studies of exposure to antimony trioxide and how findings from the scientific evidence across studies were synthesized (Section 5.3).