Mechanistic and Other Relevant Data

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Scientific judgment considering all relevant information

- Antimony(III) trioxide

- Also consider compounds containing Sb(III)
  - E.g., antimony(III) trichloride, antimony potassium tartrate

- In aqueous solution, Sb(III) compounds form

\[ \text{Sb(OH)}_2^+ \quad \text{Sb(OH)}_3 \quad \text{Sb(OH)}_4^- \]
Questions

• Does the available mechanistic data provide supporting evidence for the cancer effects observed in experimental animals?
  – What are the major biological effects contributing to the carcinogenicity of antimony trioxide?

• Is there compelling data indicating that the agent acts through mechanisms which do not operate in humans?
10 Characteristics of Human Carcinogens

- Electrophilic
- Genotoxic
- DNA repair down
- Epigenetic alteration
- Oxidative stress

- Chronic inflammation
- Immune response up
- Receptor-mediated effects
- Cell immortalization

- Cell proliferation, death, or alter nutrient supply
Strong Evidence of 5 Characteristics for Sb\textsuperscript{III}

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Strong Evidence of 5 Characteristics for Sb\textsuperscript{III}

Sb\textsuperscript{III}\textsubscript{2}O\textsubscript{3}

Electrophilic

↑Oxidative stress

Genotoxic

Other compounds containing Sb\textsuperscript{III} also
Antimony Compounds Are Electrophilic

- Antimony compounds can interact with proteins and nucleic acids
- Sb(III) is highly reactive to sulfhydryl groups (thiols)
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- Sb(III) is highly reactive to sulfhydryl groups (thiols), especially vicinal thiol groups

FAD: riboflavin. GSSG: oxidized glutathione. NADPH: reduced from of nicotinamide adenine dinucleotide phosphate. NADP+: nicotinamide adenine dinucleotide phosphate.
Antimony Compounds Are Electrophilic

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Glutathione (GSH)

FAD: riboflavin. GSSG: oxidized glutathione. NADPH: reduced from of nicotinamide adenine dinucleotide phosphate. NADP+: nicotinamide adenine dinucleotide phosphate.

- Many enzymes involved in the redox process and DNA binding (e.g., DNA repair) have thiol or vicinal thiol groups
Sb\textsuperscript{III} compounds, including Sb\textsubscript{2}O\textsubscript{3}, decrease antioxidants (e.g., reduced form of glutathione, GSH).
**Sb\(\text{III}\) Compound**

**Oxidative Stress and Damage**

- \(\text{Sb}^{\text{III}}\) compounds, including \(\text{Sb}_2\text{O}_3\), decrease antioxidants (e.g., reduced form of glutathione, GSH)

- \(\text{Sb}^{\text{III}}\) compounds directly inhibit redox enzymes

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**Diagram:**

- Antimony(III) compound

  - \(\downarrow\) Mitochondrial membrane potential
  - \(\uparrow\) ROS
  - \(\downarrow\) GSH
  - \(\uparrow\) DNA damage
  - Glutathione S-transferase
  - Glutathione reductase
  - MRP1 efflux pump

- \(\text{Sb}^{\text{III}}(\text{GSH})_3\)
• Effects likely via interaction with thiol groups of protein (enzymes) and peptide (GSH)
### Sb\textsuperscript{III}_2O\textsubscript{3} Causes DNA Damage

<table>
<thead>
<tr>
<th></th>
<th>Sb\textsuperscript{III}_2O\textsubscript{3}</th>
<th>\textit{In vitro}</th>
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<tbody>
<tr>
<td>Any DNA damage (prokaryotes)</td>
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In the lung of mice after 12 months of inhalation exposure

+ positive
- negative
**Sb\text{III}_2O_3** Is Clastogenic

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<tr>
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</tr>
<tr>
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*in human leucocytes: chromosomal damage (excluding gaps)*

+ positive  
- negative

\textsuperscript{a} Negative in rats; uncertain in mice due to severe study limitations.  
\textsuperscript{b} Correction from public comment version monograph
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**Note:**
- a Negative in rats; uncertain in mice due to severe study limitations.
- b Correction from public comment version monograph

- in Chinese hamster V79 cells
- in mature erythrocytes of mice after 12 months of inhalation exposure
Sb\textsubscript{III}_2O\textsubscript{3} Is Clastogenic

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In human lymphocytes and Chinese hamster V79 cells

\begin{itemize}
  \item [+] positive
  \item [-] negative
\end{itemize}
### Sb$_{III}$_2O$_{3}$ Does Not Cause Base-Substitution or Frame shift

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### Sb\textsuperscript{III}Cl\textsubscript{3} and Sb\textsuperscript{III}\textsubscript{2}O\textsubscript{3} Have Similar Genotoxicity

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<th>Test</th>
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* mutations seen in Sb\textsubscript{2}O\textsubscript{3}-induced lung tumors
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- DNA repair
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Other compounds containing Sb\textsuperscript{III} also

\[ \text{Sb}^{\text{III}}_2\text{O}_3 \]
Sb\textsuperscript{III} Compound Can Inhibit DNA Repair

- Sb\textsuperscript{III}Cl\textsubscript{3} \textit{in vitro} inhibits repair of various types of DNA damage in lesion-specific manner
  - Nucleotide excision repair (NER) pathway, non-homologous end-joining repair (NHEJ) and homologous recombination (HR) repair pathways were affected

Nuclear excision repair

RPA: replication protein A.  XPA: xeroderma pigmentosum complementation group A.  XPC: xeroderma pigmentosum complementation group C.  XPE: xeroderma pigmentosum complementation group E.
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Nuclear excision repair

- Whether Sb\textsuperscript{III}\textsubscript{2}O\textsubscript{3} inhibits DNA repair is inconclusive
  - Only available study was on unscheduled DNA synthesis, an insensitive indicator of repair, and result was negative

RPA: replication protein A.  XPA: xeroderma pigmentosum complementation group A.  XPC: xeroderma pigmentosum complementation group C.  XPE: xeroderma pigmentosum complementation group E.
Sb(III)-prevented decrease in epidermal growth factor receptor can preserve proliferation potential

- Skin is a cancer site in mice exposed to Sb$_{2}$O$_3$
- Sb$_{2}$O$_3$ increased Egfr mutation in the alveolar/bronchiolar tumors of mice and rats
  - Egfr mutation was not seen in non-tumor lung tissue or in spontaneous lung tumors
**Summary**

- **Electrophilicity**
  - Affinity to vicinal thiol groups

Interact with:
- Peptides (e.g., GSH)
- Proteins/enzymes (including zinc finger)

**Increase oxidative stress**

- Decrease DNA damage repair capacity

**Genotoxicity**
- DNA damage
- Chromosomal aberrations
- Sister chromatid exchange

Cause receptor-mediated effects
- e.g., Prevent cell differentiation → Preserve proliferation potential

Direct evidence from $\mathrm{Sb}^{\text{III}}_2\mathrm{O}_3$

Direct evidence from compounds containing $\mathrm{Sb}^{\text{III}}$
Does the available mechanistic data provide supporting evidence for the cancer effects observed in experimental animals?

Yes, mechanistic information is supportive.

What are the major biological effects contributing to the carcinogenicity of antimony trioxide?

- Some effects were seen in
  - cells at cancer sites
  - human cells

Is there compelling data indicating that the agent acts through mechanisms which do not operate in humans?

No.
Clarification Questions?
Mechanistic and Other Relevant Data

- Comment on whether the mechanistic data and other relevant data (Section 6: Mechanistic and Other Relevant Data, and Appendix E) presented in the cancer evaluation component antimony trioxide are clear, technically correct, and objectively presented.

- Comment on whether the mechanistic and other relevant data (Section 6 and Appendix E) are relevant for evaluating the biological plausibility of carcinogenic effects of antimony trioxide in humans.
  - Provide any scientific criticisms of the NTP’s synthesis of these data for assessing effects of antimony trioxide.
  - Identify any information that should be added or deleted.