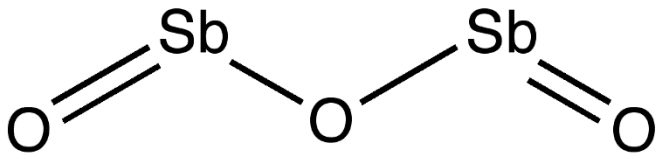
Mechanistic and Other Relevant Data

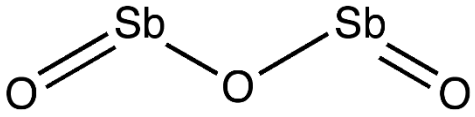


Amy Wang, PhD
Office of the Report on Carcinogens
National Institute of Environmental Health Sciences

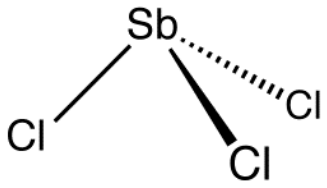
January 24, 2018

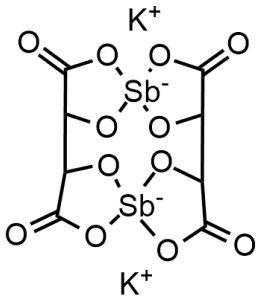
Scientific judgment considering all relevant information

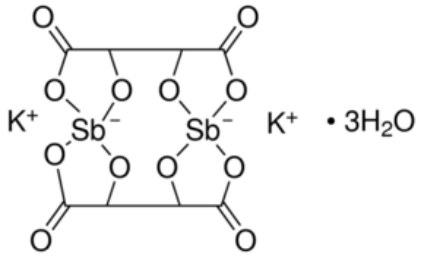
- **Antimony(III) trioxide**



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







- **In aqueous solution, Sb(III) compounds form**

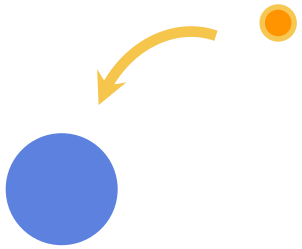
$Sb(OH)_2^+$ $Sb(OH)_3$ $Sb(OH)_4^-$

Questions

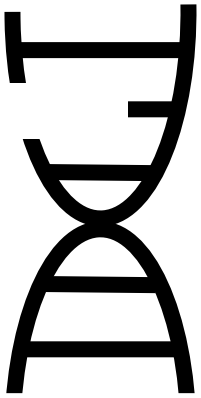
- **Does the available mechanistic data provide supporting evidence for the cancer effects observed in experimental animals?**
  - **What are the major biological effects contributing to the carcinogenicity of antimony trioxide?**
- **Is there compelling data indicating that the agent acts through mechanisms which do not operate in humans?**

10 Characteristics of Human Carcinogens

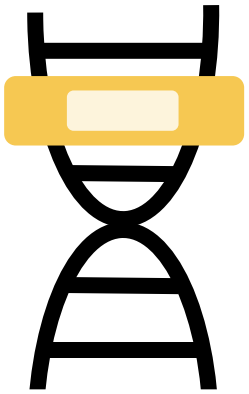
Electrophilic



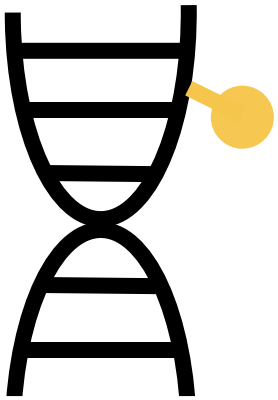
Genotoxic



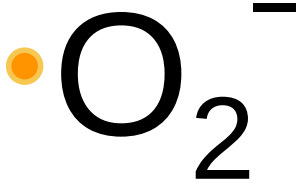
⬇DNA repair



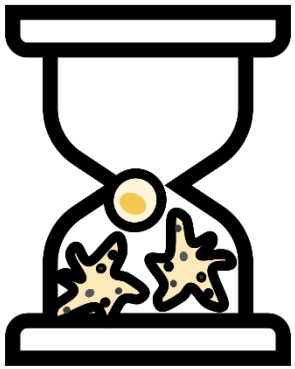
➡Epigenetic alteration



⬆Oxidative stress

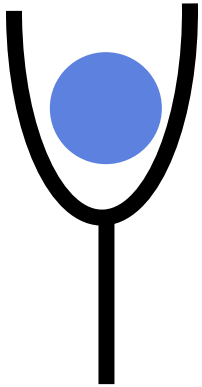


➡Chronic inflammation



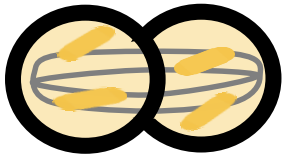
⬆⬇Immune response

➡ Receptor-mediated effects

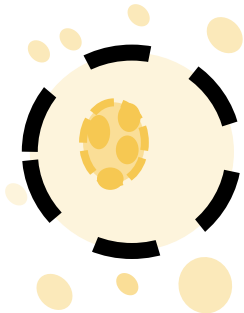


➡Cell immortalization

⬆Cell proliferation,



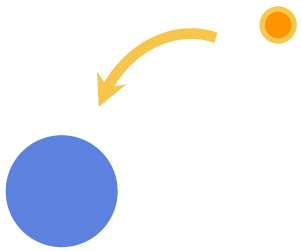
⬇cell death,



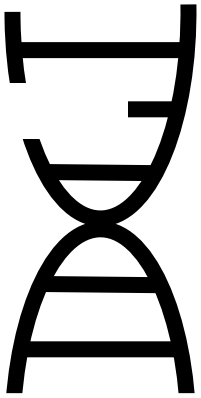
Or alter nutrient supply

Strong Evidence of 5 Characteristics for Sb^{III}

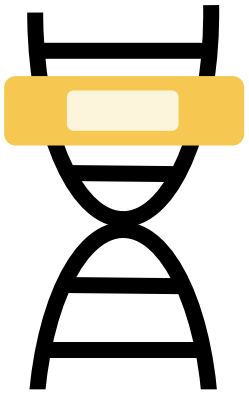
Electrophilic



Genotoxic

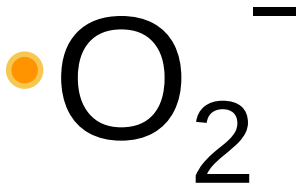


⬇ DNA repair



➡ Epigenetic alteration

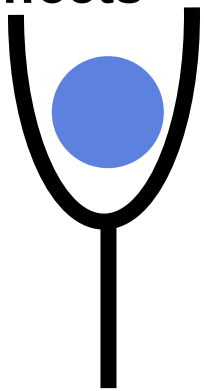
⬆ Oxidative stress



➡ Chronic inflammation

⬆⬇ Immune response

➡ Receptor-mediated effects



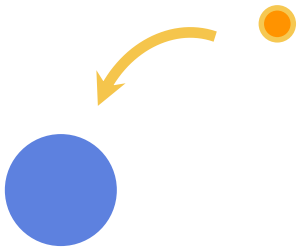
➡ Cell immortalization

⬆ Cell proliferation, ⬇ cell death, or alter nutrient supply

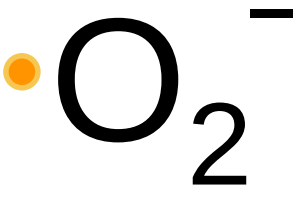
Strong Evidence of 5 Characteristics for Sb^{III}

$Sb^{III}_2O_3$

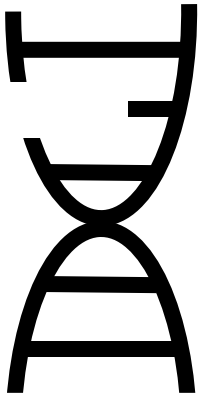
Electrophilic



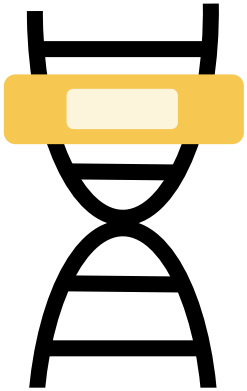
↑Oxidative stress



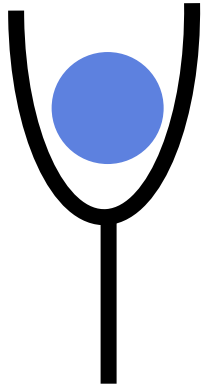
Genotoxic



↓DNA repair



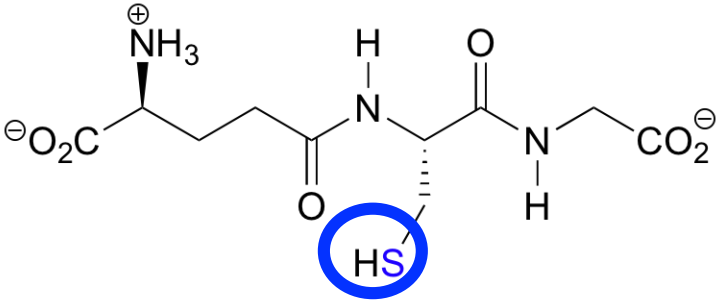
➡ Receptor-mediated effects



Other compounds containing Sb^{III} also

Antimony Compounds Are Electrophilic

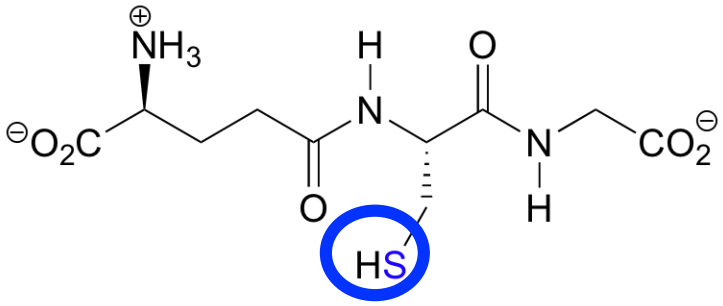
- **Antimony compounds can interact with proteins and nucleic acids**
- **Sb(III) is highly reactive to sulfhydryl groups (thiols)**



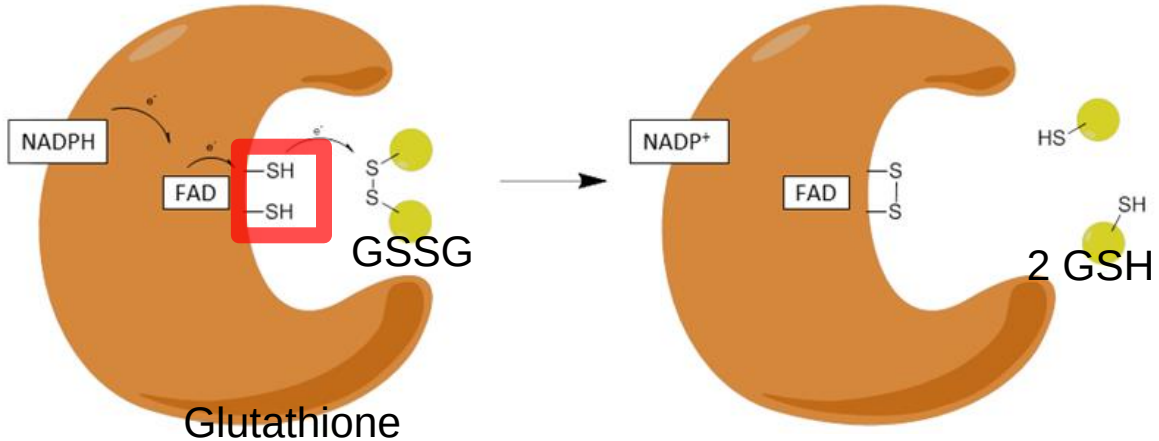
Glutathione (GSH)

Antimony Compounds Are Electrophilic

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



Glutathione (GSH)



Glutathione reductase

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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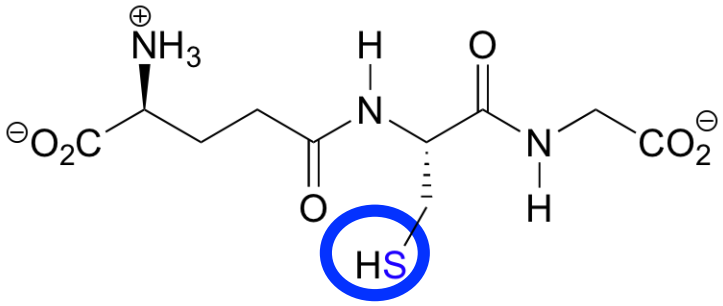
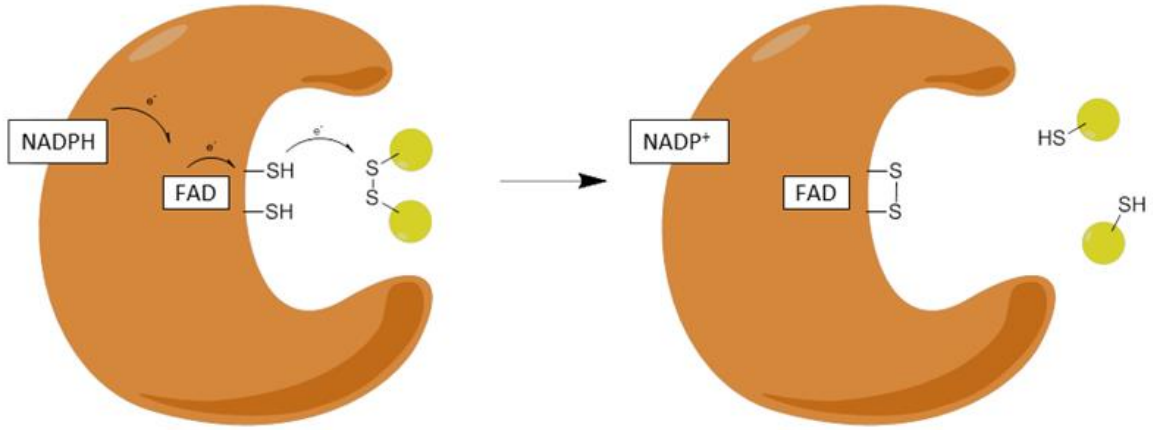


Glutathione (GSH)



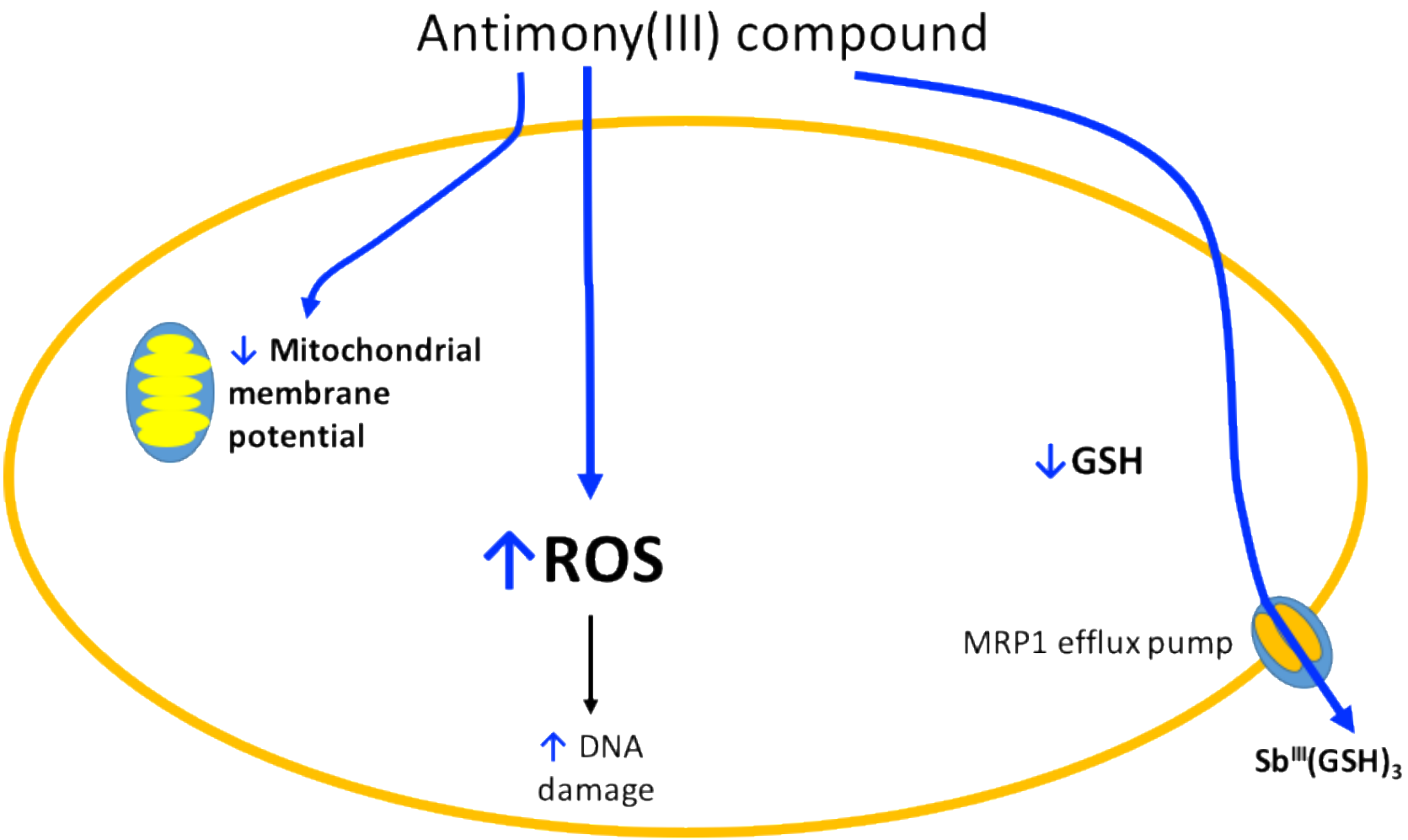
reductase

- **Many enzymes involved in the redox process and DNA binding (e.g., DNA repair) have thiol or vicinal thiol groups**

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

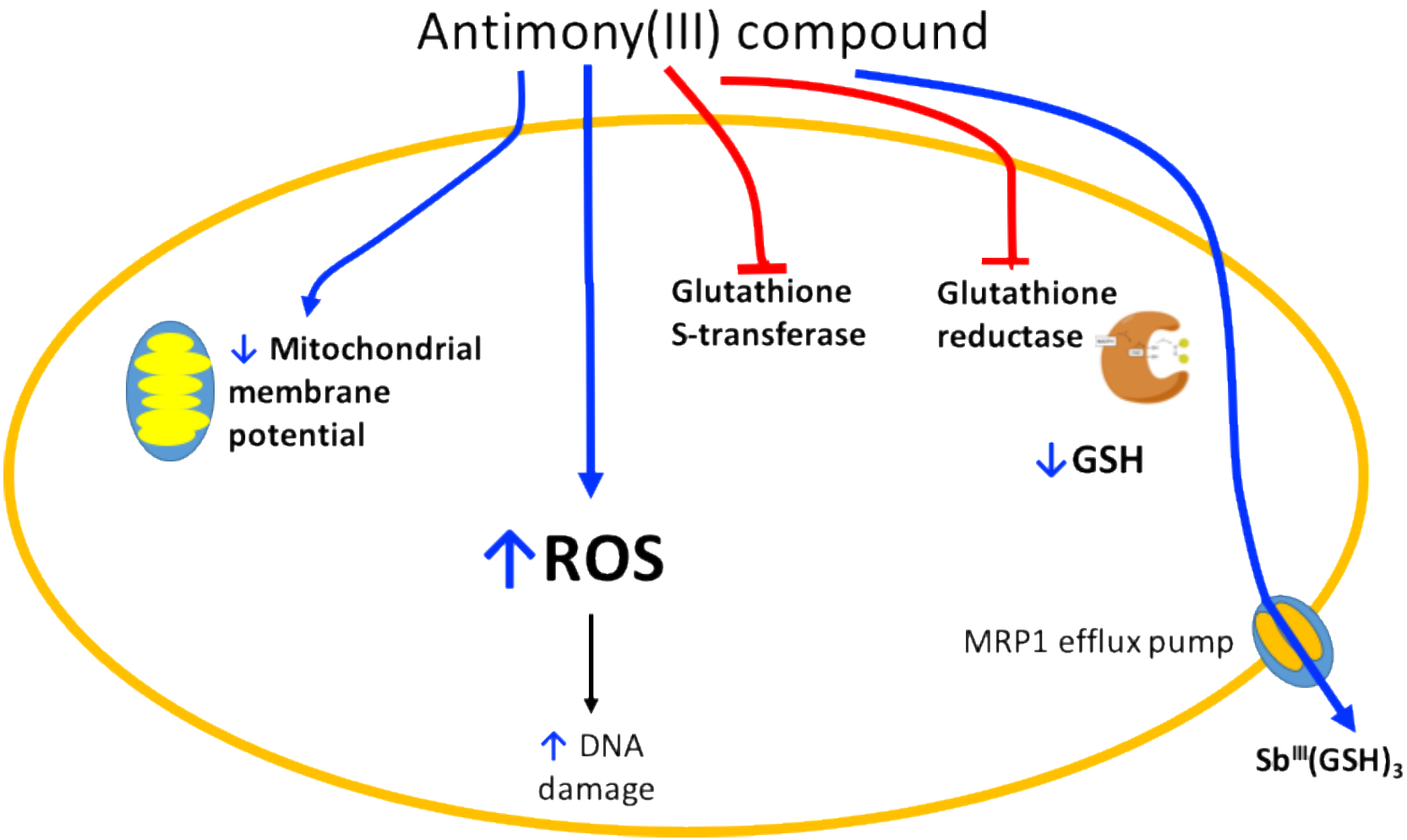
Sb^{III} Compound ↑ Oxidative Stress and Damage

- **Sb^{III} compounds, including Sb_2O_3, decrease antioxidants (e.g., reduced form of glutathione, GSH)**



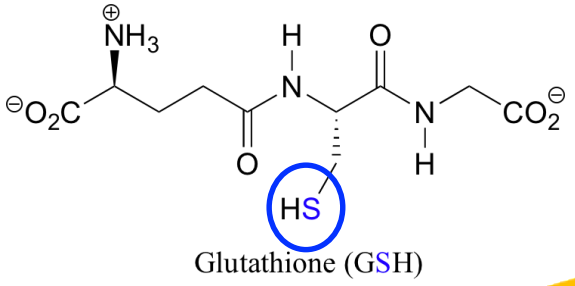
Sb^{III} Compound ↑ Oxidative Stress and Damage

- Sb^{III} compounds, including Sb_2O_3, decrease antioxidants (e.g., reduced form of glutathione, GSH)
- **Sb^{III} compounds directly inhibit redox enzymes**



Sb^{III} Compound ↑ Oxidative Stress and Damage

- **Effects likely via interaction with thiol groups of protein (enzymes) and peptide (GSH)**



$Sb^{III}_2O_3$ Causes DNA Damage

	$Sb^{III}_2O_3$	
	In vitro	*In vivo*
Any DNA damage (prokaryotes)	+	+
Any DNA damage (eukaryotes)	+	+

In the lung of mice after 12 months of inhalation exposure

+ positive

- negative

$Sb^{III}_2O_3$ Is Clastogenic

	$Sb^{III}_2O_3$	
	In vitro	*In vivo*
Any DNA damage (prokaryotes)	+	+
Any DNA damage (eukaryotes)	+	+
Chromosomal aberrations	+	-[a]
Micronucleus induction	+[b]	+
Sister chromatid exchange	+	No data

in human leucocytes: chromosomal damage (excluding gaps)

\+ positive

\- negative

[a] Negative in rats; uncertain in mice due to severe study limitations.

[b] Correction from public comment version monograph

$Sb^{III}_2O_3$ Is Clastogenic

	$Sb^{III}_2O_3$	
	In vitro	*In vivo*
Any DNA damage (prokaryotes)	+	+
Any DNA damage (eukaryotes)	+	+
Chromosomal aberrations	+	-[a]
Micronucleus induction	+[b]	+
Sister chromatid exchange	+	No data

in Chinese hamster V79 cells

in mature erythrocytes of mice after 12 months of inhalation exposure

\+ positive

\- negative

[a] Negative in rats; uncertain in mice due to severe study limitations.

[b] Correction from public comment version monograph

$Sb^{III}_2O_3$ Is Clastogenic

	$Sb^{III}_2O_3$	
	In vitro	*In vivo*
Any DNA damage (prokaryotes)	+	+
Any DNA damage (eukaryotes)	+	+
Chromosomal aberrations	+	-[a]
Micronucleus induction	+[b]	+
Sister chromatid exchange	+	No data

in human lymphocytes and Chinese hamster V79 cells

\+ positive

\- negative

[a] Negative in rats; uncertain in mice due to severe study limitations.

[b] Correction from public comment version monograph

$Sb^{III}_2O_3$ Does Not Cause Base-Substitution or Frame shift

	$Sb^{III}_2O_3$	
	In vitro	*In vivo*
Any DNA damage (prokaryotes)	+	+
Any DNA damage (eukaryotes)	+	+
Chromosomal aberrations	+	-[a]
Micronucleus induction	+[b]	+
Sister chromatid exchange	+	No data
Any mutation (prokaryotes)	-	No data
Any mutation (eukaryotes)	-	No data*

\+ positive

\- negative

[a] Negative in rats; uncertain in mice due to severe study limitations.

[b] Correction from public comment version monograph

$Sb^{III}Cl_3$ and $Sb^{III}_2O_3$ Have Similar Genotoxicity

	$Sb^{III}_2O_3$		$Sb^{III}Cl_3$	
	In vitro	*In vivo*	*In vitro*	*In vivo*
Any DNA damage (prokaryotes)	+	+	+	No data
Any DNA damage (eukaryotes)	+	+	+	No data
Chromosomal aberrations	+	-[a]	No data	No data
Micronucleus induction	+[b]	+	+	+
Sister chromatid exchange	+	No data	+	No data
Any mutation (prokaryotes)	-	No data	-	No data
Any mutation (eukaryotes)	-	-	No data	No data

+ positive

- negative

[a] Negative in rats; uncertain in mice due to severe study limitations.

[b] Correction from public comment version monograph

* mutations seen in Sb_2O_3-induced lung tumors

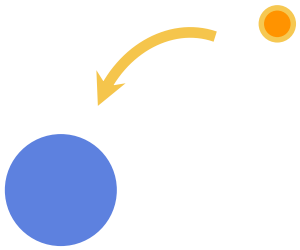
Strong Evidence of 5 Characteristics for Sb^{III}

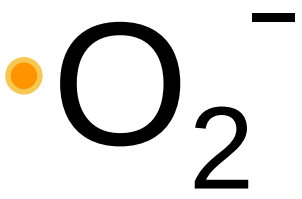
Electrophilic

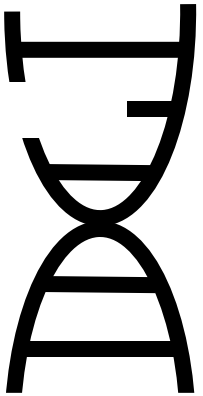
↑Oxidative stress

Genotoxic

$Sb^{III}_2O_3$

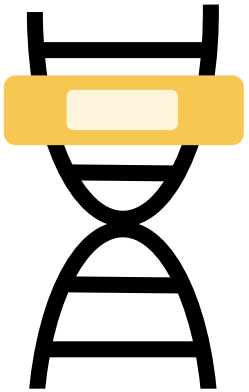




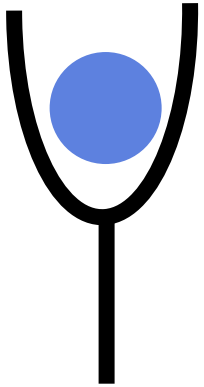


Other compounds containing Sb^{III} also

↓DNA repair



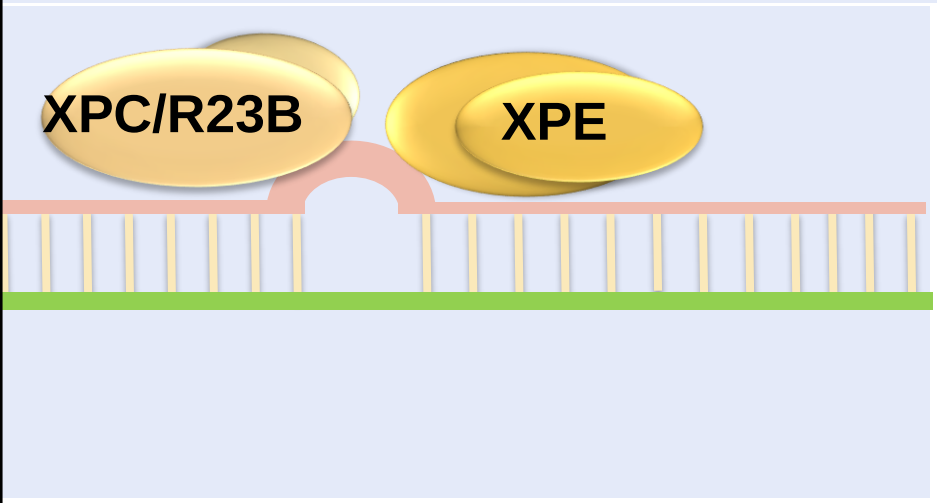
→ Receptor-mediated effects

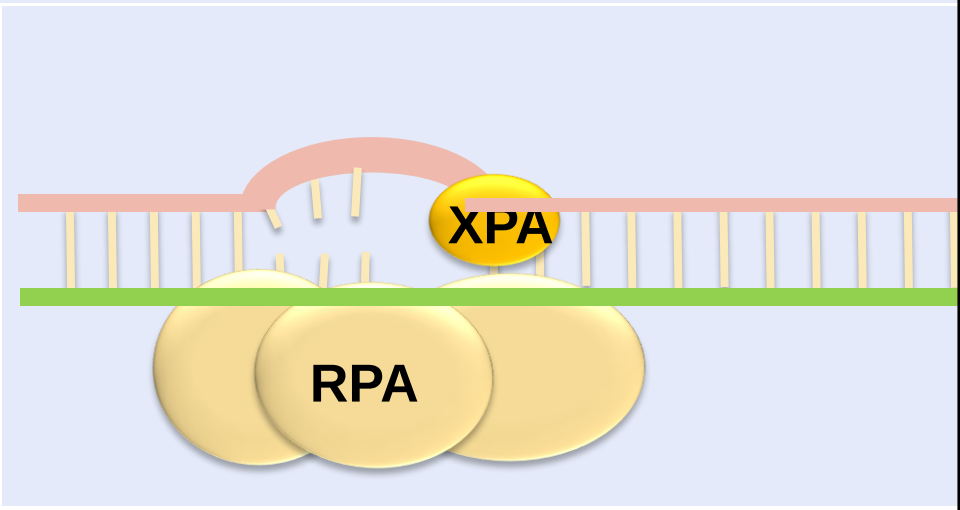


Sb^{III} Compound Can Inhibit DNA Repair

- **$Sb^{III}Cl_3$ *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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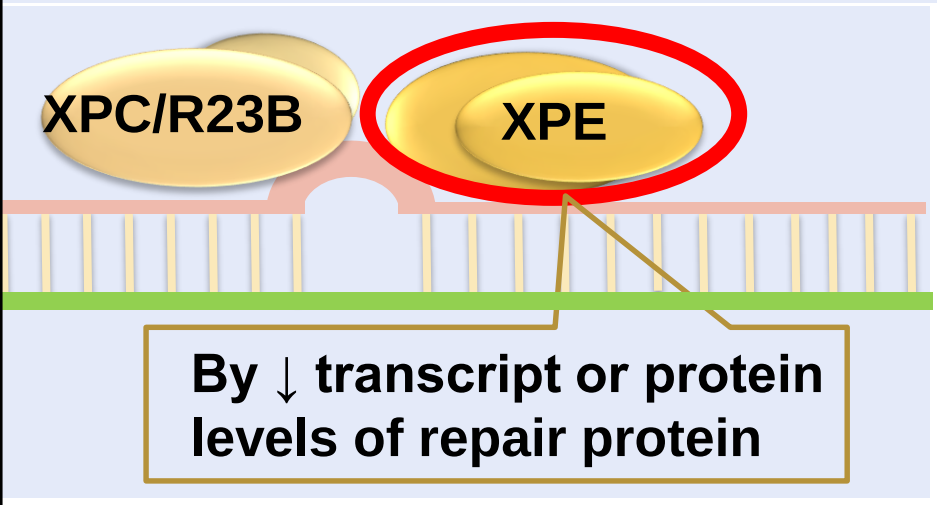

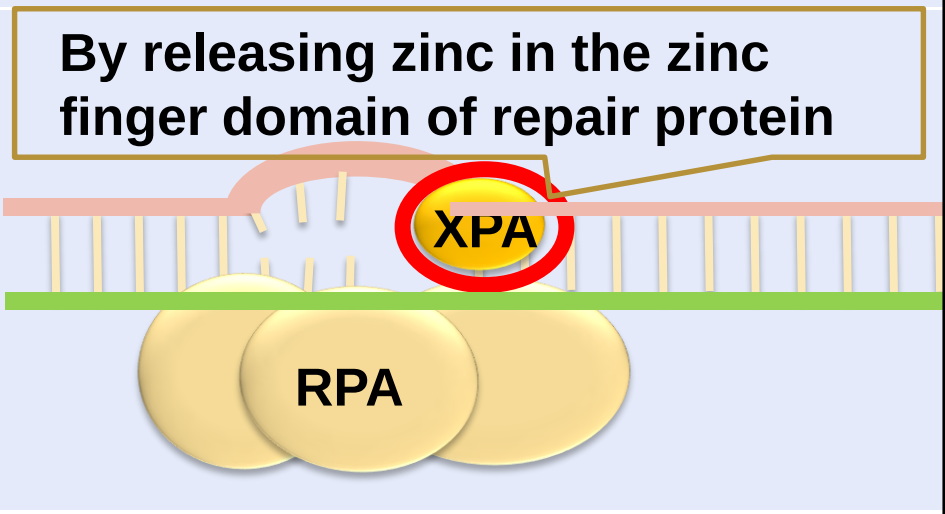

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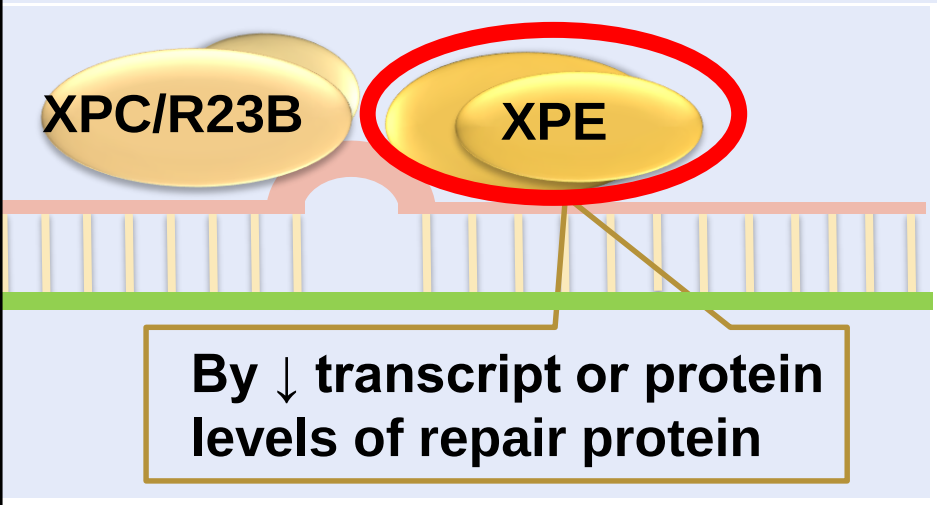

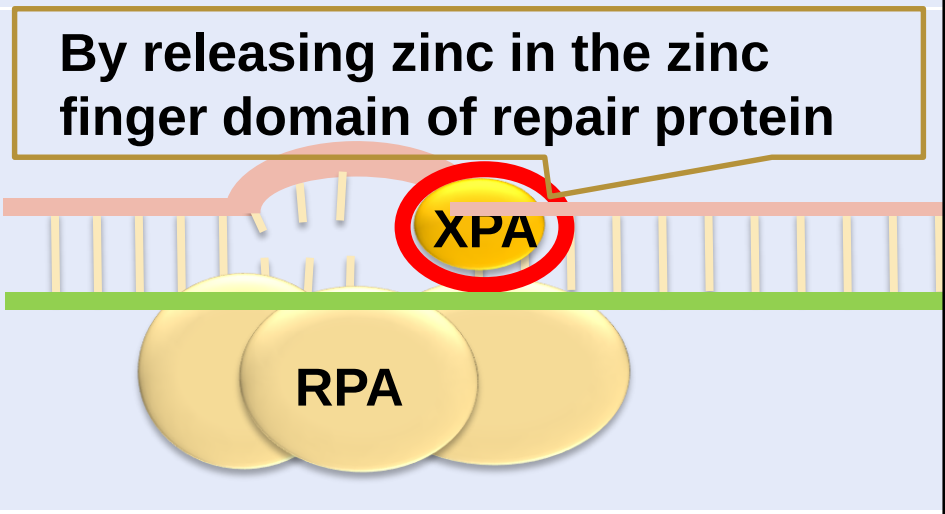

- **Whether $Sb^{III}_2O_3$ inhibits DNA repair is inconclusive**
  - **Only available study was on unscheduled DNA synthesis, an insensitive indicator of repair, and result was negative**

RPA: replication protein A. XPA: xeroderma pigmentosum complementation group A.
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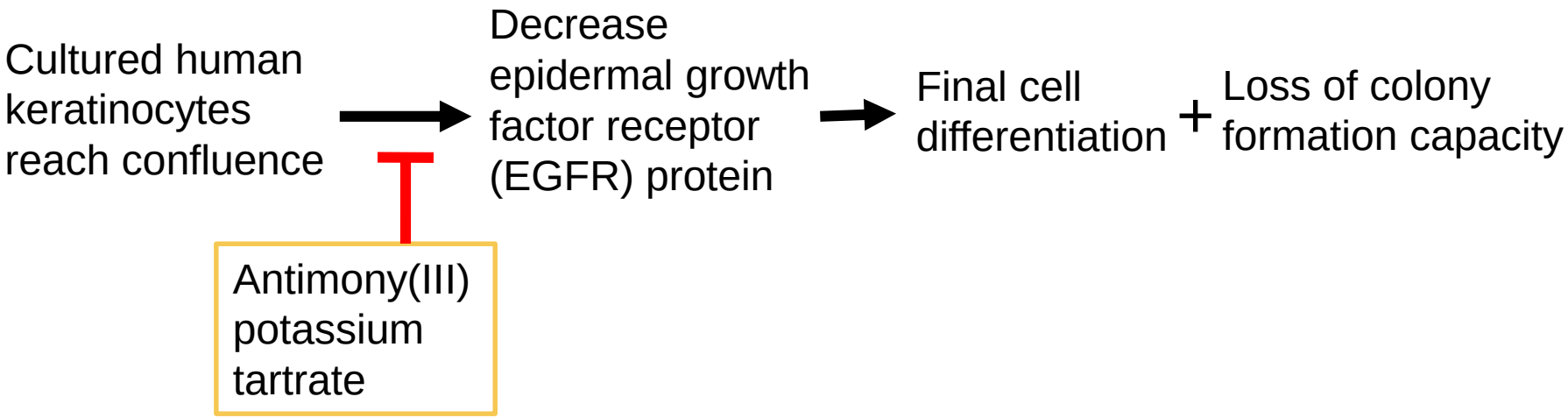
Receptor-Mediated Effects

Sb(III)-prevented decrease in epidermal growth factor receptor can preserve proliferation potential



- **Skin is a cancer site in mice exposed to $Sb^{III}_2O_3$**
- **$Sb^{III}_2O_3$ 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 O_2^-

Decrease DNA damage repair capacity

Cause receptor-mediated effects

e.g., Prevent cell differentiation → Preserve proliferation potential

Genotoxicity
- DNA damage
- Chromosomal aberrations
- Sister chromatid exchange

Direct evidence from $Sb^{III}_2O_3$

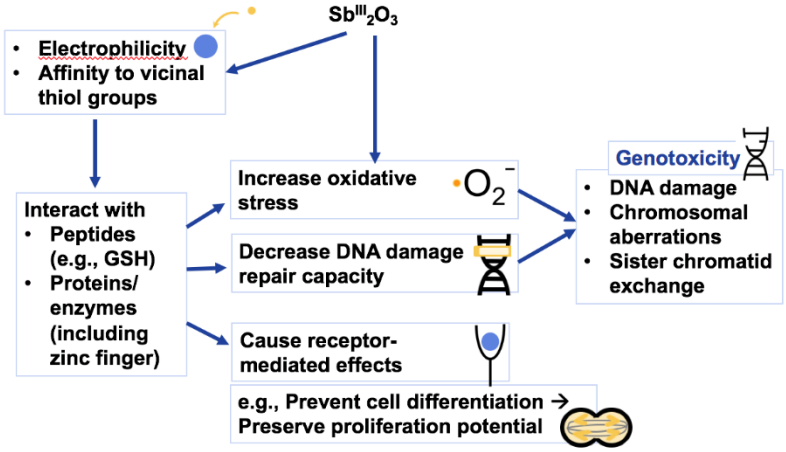
Direct evidence from compounds containing Sb^{III}

Answering Questions

- **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.**