A case study using a systematic review approach for cancer hazard identification that incorporates the 10 key characteristics (KCs) of carcinogens

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Goal

Apply an approach that uses ten key characteristics of carcinogens to evaluate mechanistic information of substances' carcinogenicity to identify human cancer hazard

Background

Evaluating a substance's broad and voluminous possible carcinogenic mechanism is challenging. To search mechanistic information unbiasedly (without perceived emphasis), we had developed search strings[1] for

PubMed   Scopus   Clarivate Analytics = Web of Sciences

on 10 key characteristics (KCs) of carcinogens[2]

	The ability of a substance to
KC1	act as an electrophile either directly or after metabolic activation
KC2	be genotoxic
KC3	alter DNA repair or cause genomic instability
KC4	induce epigenetic alterations
KC5	induce oxidative stress
KC6	induce chronic inflammation
KC7	be immunosuppressive
KC8	modulate receptor-mediated effects
KC9	cause immortalization
KC10	alter cell proliferation, cell death, or nutrient supply

Systematic review is an approach that aims to answer a specific question while minimizing bias. Advantages of systematic review (vs. descriptive literature review):
- useful for handling inconsistent results.
- use a pre-determined protocol (e.g., search terms, inclusion and exclusion criteria)
- consistent evaluation of study quality (e.g., risk of bias, study utility)
- more comprehensive
- more transparent

## Sb₂O₃ as a case study in NTP Report on Carcinogens (RoC)

NTP Report on Carcinogens (RoC)[3] is mandated by Congress to identify cancer hazards for people living in the US
- Overall evaluation is based on human cancer studies, animal cancer studies, and mechanistic and other relevant information

Listing	Listing criteria
Known to be human carcinogen	Sufficient evidence of cancer in humans (including mechanistic information in humans)
Reasonably anticipated to be human carcinogen	Limited evidence of cancer in humans, OR
	Substance belong to a class whose member is listed in RoC, OR
	Convincing mechanistic information the substance would likely cause cancer in humans

- RoC is used by the public and various agencies for decision making

Antimony trioxide is used in making flame retardants, polyethylene terephthalate (PET) plastics, specialty glass, and paints
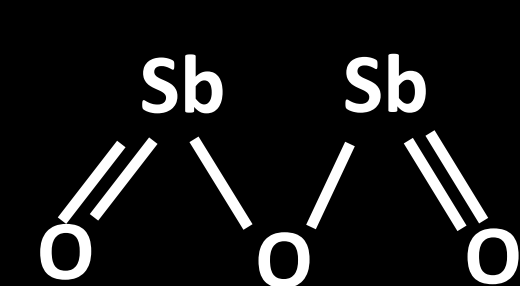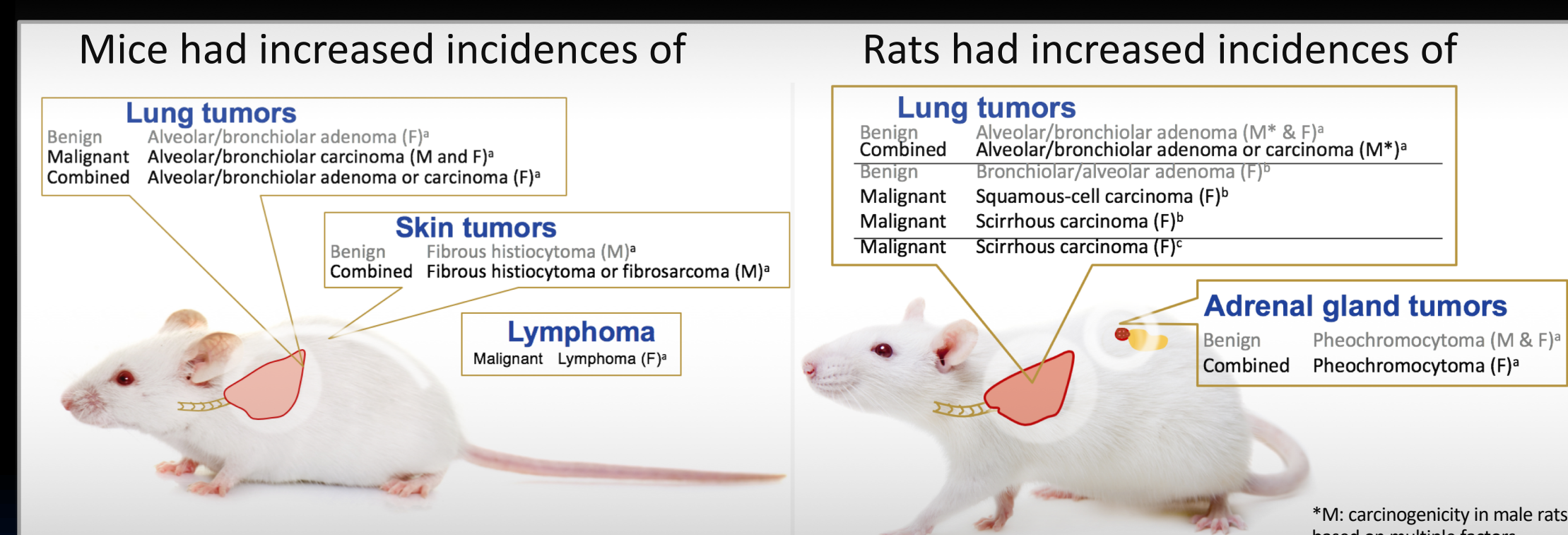
Occupational exposure is the main source of exposure

- Other forms of Sb at high temperature could turn into Sb₂O₃
- People can be exposed to Sb₂O₃ from breathing contaminated air (e.g., house dust from wear and tear of flame-retardant treated textiles, traffic pollution)
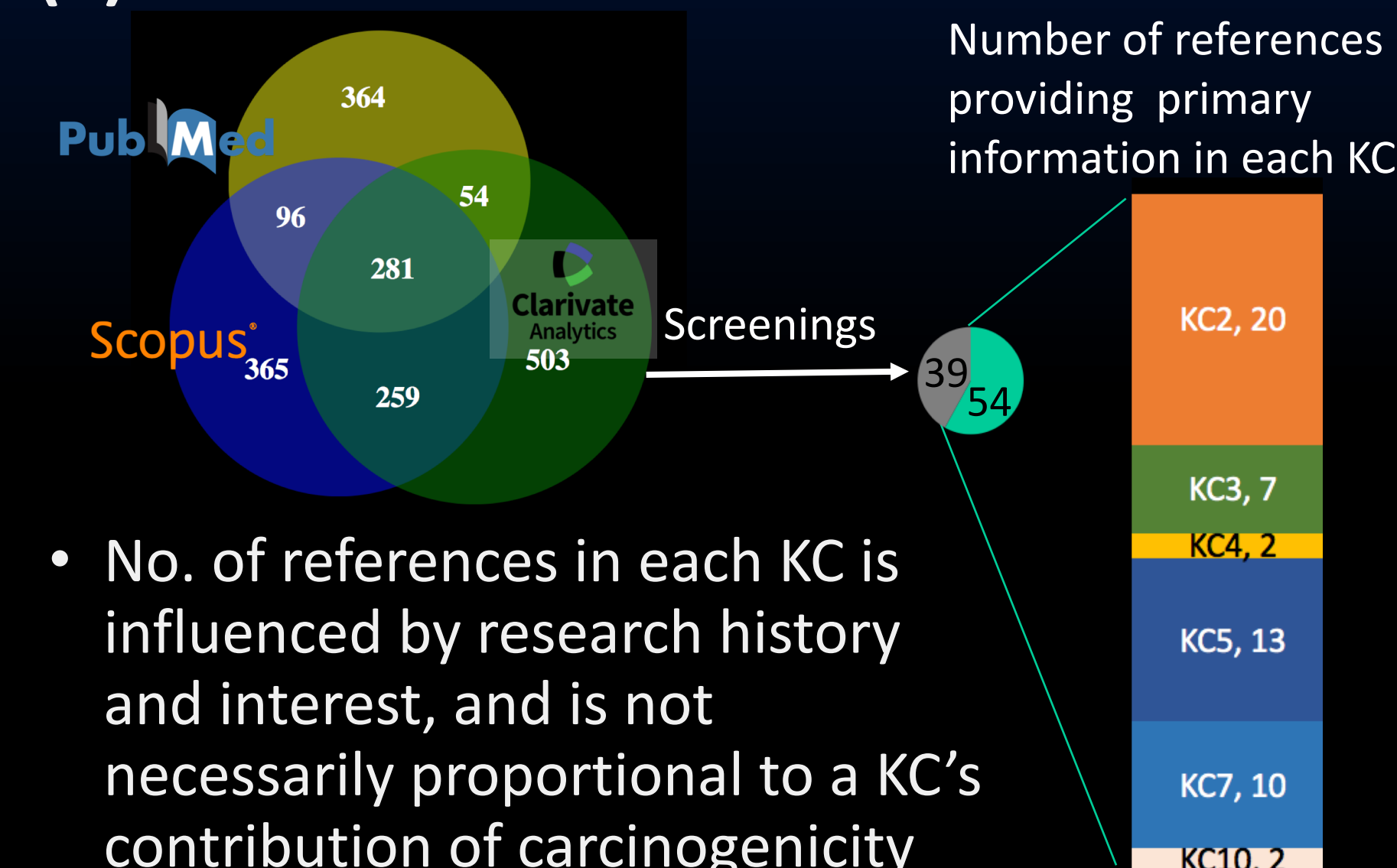
- Level of evidence of cancer in humans is inadequate
- Level of evidence of cancer in experimental animal is sufficient

Mice had increased incidences of
Lung tumors
Benign Alveolar/bronchiolar adenoma (F)[a]
Malignant Alveolar/bronchiolar adenoma (M and F)[a]
Combined Alveolar/bronchiolar adenoma (M*)[a]
Skin tumors
Benign Fibrous histiocytoma (M)[a]
Combined Fibrous histiocytoma or fibrosarcoma (M*)[a]
Lymphoma
Malignant Lymphoma (F)[a]

Rats had increased incidences of
Lung tumors
Benign Alveolar/bronchiolar adenoma (M* & F)[a]
Combined Alveolar/bronchiolar adenoma or carcinoma (M*)[a]
Benign Squamous-cell carcinoma (F)[a]
Malignant Scirrhous carcinoma (F)[a]
Malignant Scirrhous carcinoma (F)[a]
Adrenal gland tumors
Benign Pheochromocytoma (M & F)[a]
Combined Pheochromocytoma (F)[a]

*M: carcinogenicity in male rats based on multiple factors

[a]NTP 2017 TR-590   [b]Groth et al. 1986   [c]Watt 1983
[d]Newton et al. 1994 Rat study reported no increase in tumors

Mechanistic information evaluation method

(1) Search and screen literature

PubMed 364, 96, 54, 281, Scopus 365, 259, Clarivate Analytics 503

Screenings → 39/54

Number of references providing primary information in each KC

KC2, 20
KC3, 7
KC4, 2
KC5, 13
KC7, 10
KC10, 2

- No. of references in each KC is influenced by research history and interest, and is not necessarily proportional to a KC's contribution of carcinogenicity

(2) Study evaluation

For genotoxicity studies, as an example, we considered
- Substance: identity, purity, solubility, etc.
- Study design and report: model system (e.g., humans, animals, in vivo, in vitro, biochemical, in silico), exposure route, directness (of measurement to interested events), sensitivity (e.g., detection method, group size), etc.
- Study utility to inform carcinogenicity

(3) Synthesis of mechanistic information by KCs
All relevant data, including literature, Tox21, and omic data, are considered.
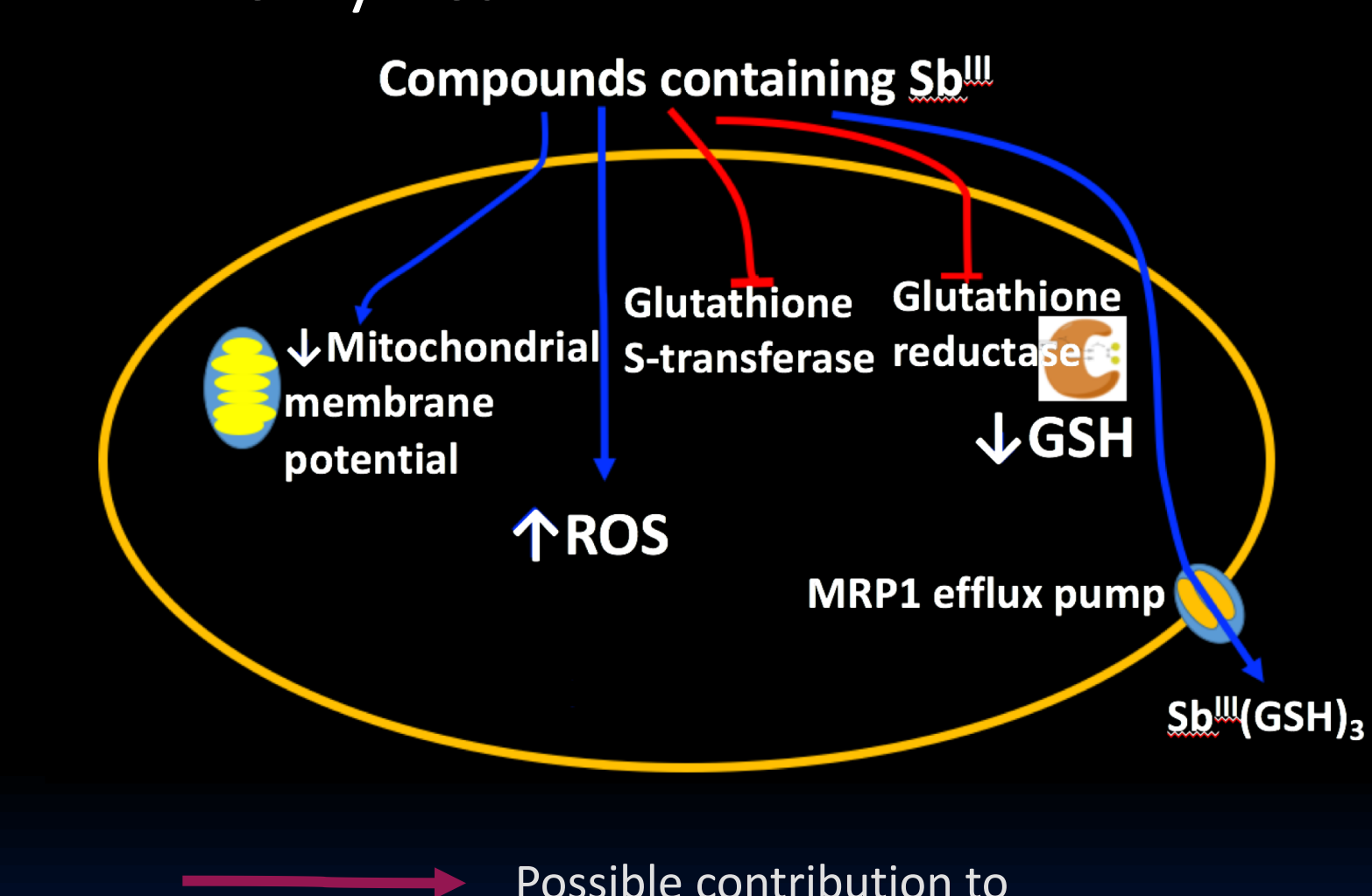
Results

KC1 Sb₂O₃ interacts with
- Peptides (e.g., GSH)
- Proteins/ enzymes (including zinc finger)

Sb[III] is highly reactive to sulfhydryl groups (thiols), especially vicinal thiol groups
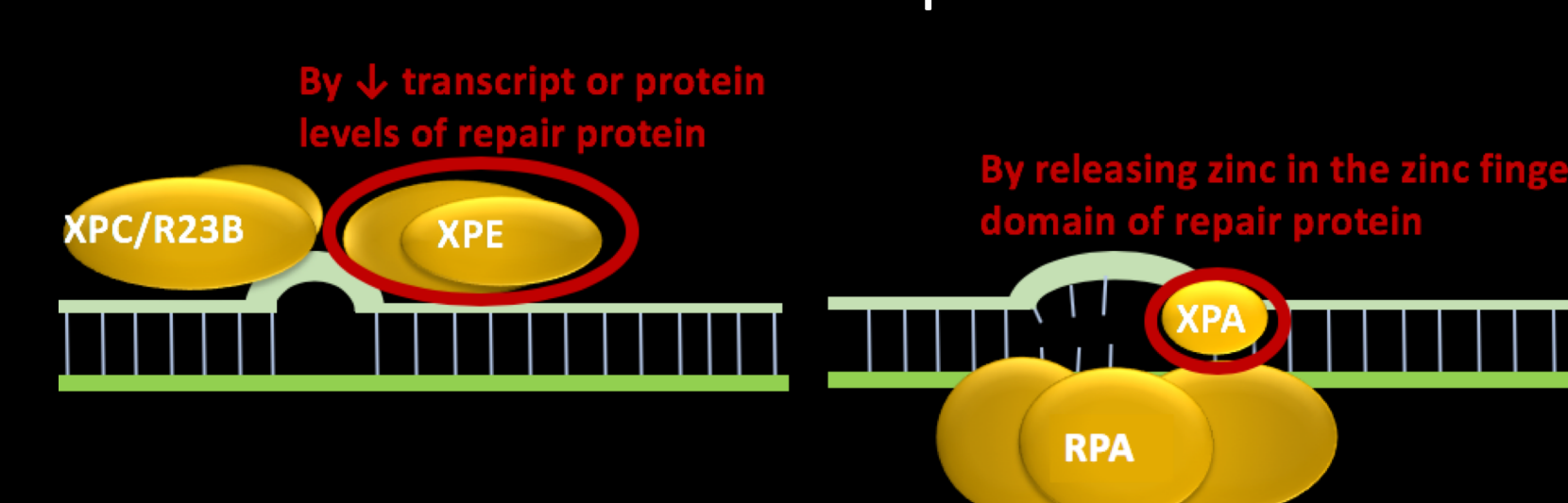
KC5 Sb₂O₃ ↓ antioxidants (e.g., GSH)
- Sb[III] compounds directly inhibit redox enzymes

Compounds containing Sb[III]
↓Mitochondrial membrane potential
Glutathione S-transferase
Glutathione reductase
↓GSH
↑ROS
MRP1 efflux pump
Sb[III](GSH)₃

Possible contribution to

KC8 Antimony(III) potassium tartrate prevents decrease in epidermal growth factor receptor (EGFR) → prevent cell differentiation → could preserve proliferation potential

KC3 Sb₂Cl₃ interferes with nucleotide excision repair

By ↓ transcript or protein levels of repair protein
XPC/R23B XPB
By releasing zinc in the zinc finger domain of repair protein
XPA
RPA

KC2 Sb₂O₃

	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)	-	-*

+ positive - negative
[a] Negative in rats; uncertain in mice due to severe study limitations.
[b] Correction from public comment version monograph
* mutations seen in Sb₂O₃-induced lung tumors

Summary

KC10, KC1, KC2, KC3, KC4, KC5, KC6, KC7, KC8, KC9, KC10

Sb₂O₃

O₂⁻

- ▇ Direct evidence from Sb₂O₃
- ▇ Direct evidence from inorganic compounds containing Sb[III]
- ▇ No direct evidence suggesting a major role of Sb₂O₃ carcinogenicity

- Based on sufficient animal evidence and supporting mechanistic information NTP recommends Sb₂O₃ be listed as reasonably anticipated to be a human carcinogen [4]
- KCs provides an unbiased approach for searching possible mechanisms

Next steps

Develop a more structured framework for mechanistic information evaluation. It may include
- guiding questions for study quality evaluation
- guidelines for mechanistic evidence synthesis
- descriptors and criteria to determine level of evidence for mechanistic information

References
1. RoC handbook, including search strings https://ntp.niehs.nih.gov/pubhealth/roc/handbook/index.html
2. Smith MT et al 2016. Environ. Health Perspect. 124:713-21
3. NTP Report on Carcinogens https://ntp.niehs.nih.gov/pubhealth/roc/index.html
4. A final decision for listing in the RoC has not been made.
Antimony Trioxide RoC Monograph https://ntp.niehs.nih.gov/pubhealth/roc/listings/antimonyt/index.html