Mechanistic evidence integration case study: using ten key characteristics of carcinogens and a systematic review approach for antimony trioxide (Sb2O3) cancer hazard identification

Amy Wang1, Joanne Trgovcich3, Kristine L. Witt1, Andrew Ewens3, Jessica Geter4, Sanford Garner3, Gloria Jahnke1, Stephanie L. Smith-Roe1, Ruth Lunn1

1NTP, National Institute of Environmental Health Sciences (NIEHS), 2ICF, 3ILS, 4Formerly ILS

**Goal**
Apply an approach that uses ten key characteristics of carcinogens (KCs) 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 for

**Pub Med**

**SCOPUS**

Web of Sciences

on 10 key characteristics of carcinogens (KCs)

| 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 immunomodulation |
| 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 transparent
- more transparent

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

NTP Report on Carcinogens (RoC) 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. Aims to answer a specific question (without perceived emphasis), we had voluminous possible carcinogenic mechanisms. Evaluating a substance’s broad and quantitative mechanistic information. It may include: guidelines for mechanistic evidence synthesis and descriptors and criteria to determine level of evidence for mechanistic information.

**Mechanistic information evaluation method**

1. **Search and screen literature**
   - Number of references providing primary information in each KC

2. **Study evaluation**
   - Number of references in each KC is influenced by research history and interest, and is not necessarily proportional to a KC’s contribution of 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)

**KC2 Sb2O3 is highly reactive to sulphydryl groups (thiols), especially vicinal thiol groups**

**KC3 Sb2O3 interacts with**
- Antioxidants (e.g., GSH)
- Redox enzymes

**KC4 Sb2O3 directly inhibits**
- Peptide (e.g., GSH)
- Redox enzymes

**KC5 Sb2O3 is a genotoxic**
- Any DNA damage (eukaryotes)
- Chromosomal alterations
- Micronucleus induction
- Sister chromatid exchange
- Any mutation (prekaryotes)
- Any mutation (erythropoiesis)

**KC6 Sb2O3 is a potent antioxidant**
- Any DNA damage (prekaryotes)
- Chromosomal alterations
- Micronucleus induction
- Sister chromatid exchange
- Any mutation (prekaryotes)
- Any mutation (erythropoiesis)

**KC7 Sb2O3 is a potent antioxidant**
- Any DNA damage (eukaryotes)
- Chromosomal alterations
- Micronucleus induction
- Sister chromatid exchange
- Any mutation (prekaryotes)
- Any mutation (erythropoiesis)

**KC8 Sb2O3 is a potent antioxidant**
- Any DNA damage (eukaryotes)
- Chromosomal alterations
- Micronucleus induction
- Sister chromatid exchange
- Any mutation (prekaryotes)
- Any mutation (erythropoiesis)

**KC9 Sb2O3 is a potent antioxidant**
- Any DNA damage (eukaryotes)
- Chromosomal alterations
- Micronucleus induction
- Sister chromatid exchange
- Any mutation (prekaryotes)
- Any mutation (erythropoiesis)

**KC10 Sb2O3 is a potent antioxidant**
- Any DNA damage (eukaryotes)
- Chromosomal alterations
- Micronucleus induction
- Sister chromatid exchange
- Any mutation (prekaryotes)
- Any mutation (erythropoiesis)

**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**