National Toxicology Program
U.S. Department of Health and Human Services

Draft Report on Carcinogens Monograph on Antimony Trioxide: Appendices

Peer-Review Draft

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Appendix A: Literature Search Strategy

Introduction

The objective of the literature search approach is to identify published literature that is relevant for evaluating the potential carcinogenicity of antimony trioxide (https://ntp.niehs.nih.gov/ntp/about_ntp/bsc/2016/december/meetingmaterials/draftantimonytrioxide_508.pdf). The literature search strategy was used to identify publications in the following areas:

- Properties and human exposure (focusing on the U.S. population)
- Disposition (ADME) and toxicokinetics
- Human cancer studies
- Studies of cancer in experimental Animals
- Mechanistic data and other relevant effects
  - Genetic and related effects
  - Mechanistic considerations

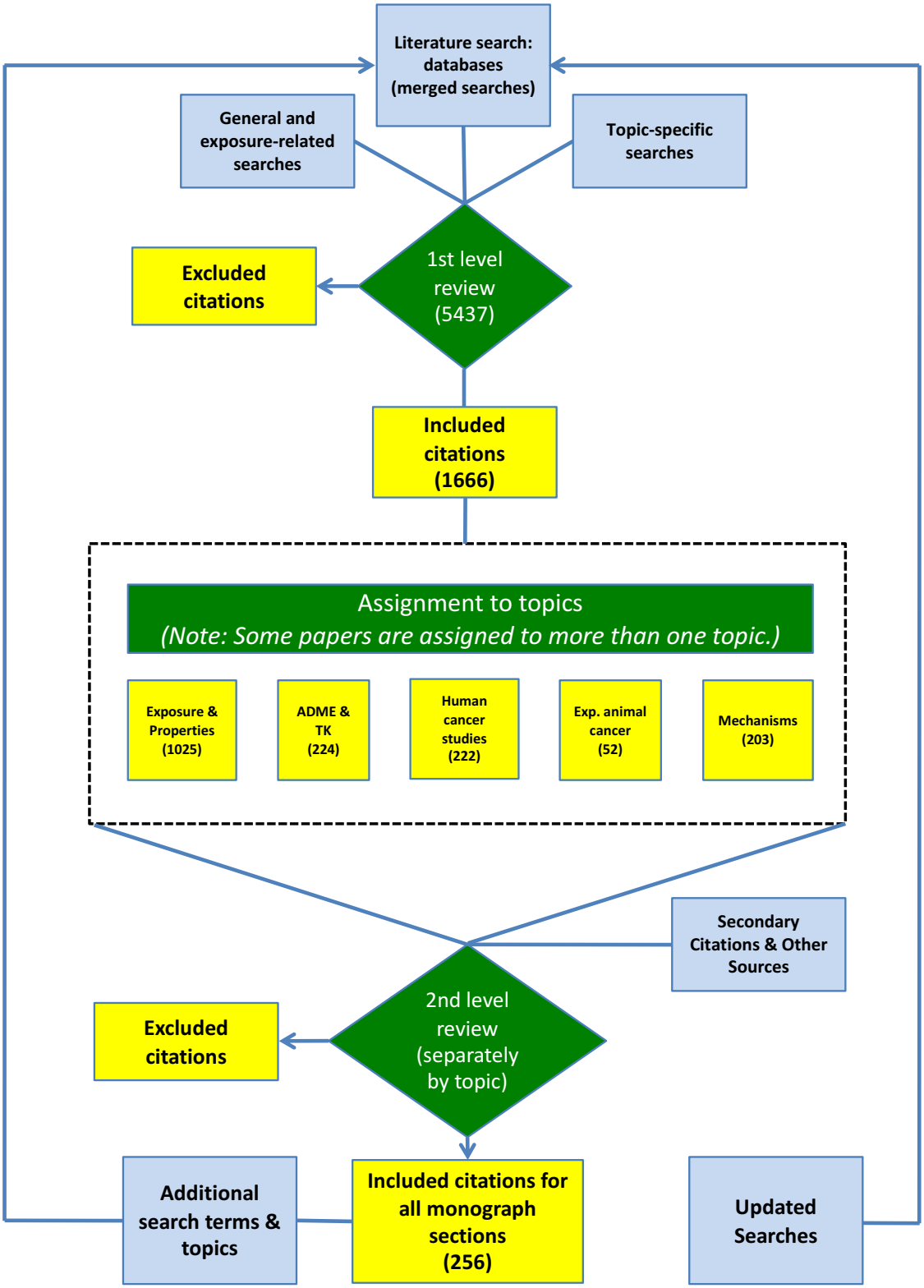
A.1 General approach

Database searching encompasses selecting databases and search terms and conducting the searches. Searches of several citation databases are generally conducted using search terms for antimony, combined with search terms for cancer and/or specific topics, including epidemiological and mechanistic studies. A critical step in the process involves consultation with an information specialist to develop relevant search terms. These terms are used to search bibliographic databases. Table A-1 highlights the general concepts searched and databases consulted. To review all the terms used, please refer to the full search strings in Antimony: RoC Protocol (https://ntp.niehs.nih.gov/ntp/roc/protocols/antimonytrioxide_508.pdf).

Table A-1. Major topics searched

Topic	Search Method	Databases searched
Exposure	Antimony String **AND** occur*[tiab]	PubMed
Human Studies	Antimony String **AND** ORoC Epidemiological (Human) Studies Search **AND** ORoC Cancer Search	PubMed, Scopus, Web of Science
Animal Studies	Antimony String **AND** Experimental Animals Studies Search **AND** ORoC Cancer Search	PubMed, Scopus, Web of Science
Mechanism and Genotoxicity	Antimony String **AND** ORoC Characteristics of Carcinogens Search	PubMed, Scopus, Web of Science

Figure A-1. Literature search strategy and review



A.2 Search strategies

Relevant literature is identified using search terms, data sources, and strategies as discussed below.

General data search: This search covers a broad range of general data sources for information relevant to many or all of the wide range of monograph topics pertaining to antimony.

Exposure-related data search: This search covers a broad range of potential sources for exposure-related information and physical-chemical properties.

Database searches in PubMed, Scopus, and Web of Science: The majority of the primary literature used to draft the antimony monograph was identified from searches of these three extensive databases available through the NIEHS Library. Searches for antimony were combined with the search terms for each of the monograph topics listed above to create the specific literature searches.

Searches for human cancer studies are somewhat unique because they involve the identification of search terms for exposure scenarios that might result in exposure of people to antimony. For antimony, these exposure-related search terms were based on uses of antimony identified from the EPA's TRI database and the Chemical Data Report rule website.

QUOSA library of occupational case-control studies search of the QUOSA-based library of more than 6,000 occupational case-control studies, approximately 95% of which are currently available as searchable full-text pdfs, was conducted using the term "antimony."

Secondary sources: Citations identified from authoritative reviews or from primary references located by literature search, together with publications citing key papers identified using the Web of Science, "Cited Reference Search," were also added.

A.3 Exclusion of treatment for leishmaniasis from human cancer searches

The use of antimony for the treatment of leishmaniasis is considered an intentional medical exposure and out of the scope of this monograph. The large corpus of literature related to leishmaniasis treatment was excluded when identifying human studies. Unlike other parts of the monograph, in which leishmaniasis related content was excluded via search terms, the mechanisms section literature search did not exclude leishmaniasis via the use of search terms. The studies on the *Leishmania* parasite itself were excluded at levels 1 and 2 by reviewers, and studies on the host or cells not infected by leishmaniasis were included for information related to mechanism.

A.4 Updating the literature search

The literature searches will be updated prior to submitting the draft monograph for peer review and prior to finalizing the monograph. Monthly search alerts for antimony searches were created in PubMed, Scopus, and Web of Science, and the results of these searches from the closing date of the initial search will be downloaded for review.

A.5 Review of citations using web-based systematic review software

Citations retrieved from literature searches were uploaded to web-based systematic review software and screened using inclusion and exclusion criteria. Multi-level reviews of the literature

were conducted, with initial reviews (Level 1) based on titles and abstracts only to identify citations that could be excluded and to assign the included literature to one or more monograph topics; subsequent reviews (Level 2) for literature assigned to the various monograph topics (Exposure, ADME & TK, Human cancer studies, etc.) were based on full-text (i.e., PDFs) of the papers and were carried out by the writer and scientific reviewer for each monograph section. Two reviewers, at least one of whom is a member of the ORoC at NIEHS, participated at each level of review.

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Appendix B: ADME Tables

Table B-1. Antimony(III) trioxide levels[a] (μg/g) in red blood cells during a 1-year chronic inhalation exposure (6 mo and 12 mo samples) and a 1-year observation period (6 mo and 12 mo samples) in Fischer 344 male and female rats

Group	6 mo	12 mo	18 mo (6 mo obs)	24 mo (12 mo obs)
Males				
I- Control	ND	ND	0.17 ± 0.39	ND
II- 0.055 mg/m^3	0.53 ± 0.31	1.09 ± 0.21	0.86 ± 0.68	ND
III- 0.51 mg/m^3	5.07 ± 0.29	7.55 ± 0.60	3.93 ± 0.25	2.53 ± 0.27
IV- 4.5 mg/m^3	34.5 ± 3.8	70.7 ± 6.3	38.6 ± 4.8	30.5 ± 7.5
Females				
I- Control	ND	ND	ND	ND
II- 0.055 mg/m^3	0.74 ± 0.06	1.48 ± 0.10	0.81 ± 0.30	ND
III- 0.51 mg/m^3	5.69 ± 0.62	9.94 ± 1.32	6.53 ± 0.90	3.39 ± 0.28
IV- 4.5 mg/m^3	75.6 ± 8.4	121 ± 10.6	74.6 ± 18.3	36.6 ± 15.5

Source: Newton *et al.* (1994).
Mo = month; ND = not detected (lowest limit of detection = 0.02 μg of antimony/mL, i.e., 0.024 μg of antimony(III) trioxide/mL).
[a]Total antimony in red blood cells was reported as total antimony(III) trioxide using the relationship 1 mole Sb_2O_3 = 1.197 mole Sb_2.

Table B-2. Blood antimony concentrations (μg/g blood) in female rats and mice exposed to antimony trioxide (N = 5 except where indicated)

	Day 61	Day 124	Day 269	Day 369	Day 551
Female Mice					
Controls	0.001 ± 0.000	0.001 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
3 mg/m^3	0.043 ± 0.002**	0.058 ± 0.001**	0.053 ± 0.006**	0.052 ± 0.003**	0.061 ± 0.010**
10 mg/m^3	0.083 ± 0.002**	0.089 ± 0.002**	0.091 ± 0.002**	0.088 ± 0.003**	0.087 ± 0.004**
30 mg/m^3	0.141 ± 0.003**	0.148 ± 0.005**	0.163 ± 0.008** [a]	0.137 ± 0.007**	0.163 ± 0.006** [a]
Female Rats					
Controls	0.139 ± 0.012	0.050 ± 0.002	0.077 ± 0.002	0.084 ± 0.008	0.066 ± 0.005
3 mg/m^3	7.352 ± 0.375**	16.135 ± 0.995**	39.590 ± 3.915**	50.917 ± 2.296**	63.297 ± 3.906**
10 mg/m^3	18.079 ± 0.793**	40.350 ± 1.543**	88.833 ± 2.210**	102.083 ± 2.738**	149.192 ± 8.472**[a]
30 mg/m^3	43.574 ± 1.741**	96.082 ± 3.940**	175.437 ± 6.471**	200.239 ± 10.302**	231.934 ± 8.681**

Source: NTP (2016c).
**Significantly different ($P < 0.01$) from the chamber control group by Shirley's test.
[a]N = 4.

Table B-3. Tissue distribution of antimony (μg antimony/g tissue) in rats after oral exposure to antimony(III) trioxide by gavage or in the diet

Tissue	Controls (M/F)[a]	1000 mg/kg Sb_2O_3 suspension p.o. for 1 day (M/F)[a]	1000 mg/kg Sb_2O_3 suspension p.o. for 14 days (M/F)[a]	2% Sb_2O_3 in diet* for 49 days[b]	2% Sb_2O_3 in diet* for 8 months[c]
Thyroid	0.098/0.195	1.507/2.103	2.639/2.280	88.9	156
Adrenal	NR	NR	NR	67.8	NR
Lung	0.004/0.002	0.041/0.061	0.746/0.882	14	3.7
Spleen	0.010/0.032	0.197/0.113	1.485/1.386	18.9	8.1
Heart	0.004/0.003	0.042/0.041	0.643/0.356	7.6	5.1
Kidney	0.003/0.002	0.012/0.023	0.323/0.261	6.7	6.0
Liver	0.004/0.003	0.041/0.064	0.823/0.675	8.9	15.5
Bone marrow	0.080/0.142	1.192/1.996	2.486/3.517	NR	NR
Bone or femur	0.019/0.010	0.048/0.032	0.254/0.265	NR	2.5
Muscle	0.003/0.003	0.005/0.005	0.039/0.044	NR	0.3
Whole blood	0.003/0.003	0.708/0.640	8.278/6.886	NR	NR

Sources: [a] TNO Quality of Life 2005 as cited by EU 2008; [b] Westrick 1953; [c] Gross *et al.* 1955 as cited by EU 2008.
NR = not reported.
*Based on consumption of 5 g of food per day per 100 g body weight (Johns Hopkins University 2017), rats exposed to 2% Sb_2O_3 in the diet or by gavage at 1,000 mg/kg body weight would be exposed to approximately 0.1 g per 100 g body weight.

Appendix C: Human Studies Tables

Table C-1. Evaluation of selection bias in human cancer studies.

Study	Selection bias
Jones 1994	*Rating*: ++; *Direction*: ↓ *Rationale*: Only an external analysis was conducted. Although the impact of healthy worker survivor effect (HWSE) is mitigated by stratification by time-since-exposure, HWSE is still possible and may bias results toward the null.
Schnorr *et al.* 1995	*Rating*: ++; ↓ *Rationale*: Only an external analysis was conducted. HWSE was not accounted for in this analysis, which may result in an underestimating of the risk estimates.
Jones *et al.* 2007	*Rating*: ++; ↓ *Rationale*: Missing death information for 5.7% of untraced individuals would slightly bias results if they experienced the outcome. HWSE was not accounted for in the analyses, however, the impact of the smelter closing during follow-up would reduce the residual survival advantage.
Wingren and Axelson 1993	*Rating*: +++; ↔ *Rationale*: Cases and controls were selected from the same parishes. No evidence suggests that the selection of subjects was related to both antimony exposure and disease.

↑ = Results bias away from the null; ↓ = Results bias toward the null; ↔ = Unknown direction of bias

Table C-2. Evaluation of exposure assessment methods in human cancer studies.

Study	Exposure assessment rating
Jones 1994	*Rating*: ++/+++; *Direction*: ↔ *Rationale*: Exposure assessment methods have decent sensitivity and specificity, leading to reliable classification with respect to ever-exposure to antimony and exposure duration. Antimony exposure is assumed based on job description at smelter site.
Schnorr *et al.* 1995	*Rating*: ++; ↓ *Rationale*: Exposure was reliably characterized as ever-exposure to antimony and duration of antimony exposure, but not with respect to concentration of exposure. Based on the reported environmental sampling data, antimony air exposure varied by plant location and year sampled; however, exposure is not captured at the individual level due to lack of information on job duties, and therefore, may be subject to misclassification.
Jones *et al.* 2007	*Rating*: ++; ↓ *Rationale*: Given the modeling efforts used to account for the uncertainty in early air contamination levels, and because air sampling concentrations are likely an underestimate of true individual antimony exposure, exposure levels and timing may not represent true antimony concentrations and worker exposure prior to 1972. Authors mention changes in plant processes before NIOSH collected exposure estimates. The 3 scenarios for back-extrapolation (1. twice as high air concentrations in 1937, 2. average concentration from 1937 to 1972, and 3. a doubling in concentrations from 1937-1960 then a decrease to 1972 levels) are assumptions based on little empirical data.
Wingren and Axelson 1993	*Rating*: +; ↑ *Rationale*: Exposure to antimony was based on reported job title at death. Those classified as unexposed who may have worked in a glass producing facility or had other antimony occupational exposure over a lifetime may have misclassified exposure. Reported level of antimony used by surveyed glass working facilities may not represent individual-level

Study	Exposure assessment rating
	exposure to employees. Facility surveys of antimony use was taken at one time point; unknown if antimony use patterns were consistent.

↑ = Results bias away from the null; ↓ = Results bias toward the null; ↔ = Unknown direction of bias

Table C-3. Evaluation of outcome assessment in human cancer studies.

Study	Outcome assessment rating
Jones 1994	*Rating*: +++; *Direction*: ↔ *Rationale*: Outcome methods distinguish between diseased and non-diseased subjects. Follow-up and diagnoses are conducted independent of exposure status.
Schnorr *et al.* 1995	*Rating*: +++; ↔ *Rationale*: Outcome methods distinguish between diseased and non-diseased subjects. Follow-up and diagnoses are conducted independent of exposure status.
Jones *et al.* 2007	*Rating*: +++; ↔ *Rationale*: Outcome methods distinguish between diseased and non-diseased subjects. Follow-up and diagnoses are conducted independent of exposure status.
Wingren and Axelson 1993	*Rating*: ++; ↑ *Rationale*: Outcome methods distinguish between diseased and non-diseased subjects. Occupational title (i.e. exposure status) was collected from the death and burial register, which noted mortality status. Given the lack of information on the blinding status, diagnostic bias cannot be ruled out. If coder identified diseased subjects as being exposed, it would bias the results away from the null.

↑ = Results bias away from the null; ↓ = Results bias toward the null; ↔ = Unknown direction of bias

Table C-4. Evaluation of study sensitivity in human cancer studies.

Study	Sensitivity rating
Jones 1994	*Rating*: ++; *Direction*: ↔ *Rationale*: Study has few exposed cases but a substantial duration of exposure with a long range for follow-up. Stratification by exposure duration and years increase sensitivity.
Schnorr *et al.* 1995	*Rating*: ++; ↔ *Rationale*: Study had a small-to-moderate number of exposed cases. There was adequate duration for follow-up, with a substantial duration of exposure. Duration and ever-exposure were measured, but not the range of antimony concentrations.
Jones *et al.* 2007	*Rating*: +; ↔ *Rationale*: Adequate number of potentially-exposed subjects but a small number of exposed cases. Exposure characterized by job-exposure matrix and detailed work histories. Exposure was modeled with a substantial range and level of exposure with adequate duration for latency. However, exposure was not at an individual level and exposure was extrapolated based on assumptions.
Wingren and Axelson 1993	*Rating*: +; ↔ *Rationale*: Study captures variability in antimony use by parish where cases and controls died. However, given the unknown number of exposed subjects, exposed cases, the unknown number of controls, and the unknown individual-level exposure to antimony in glass workers, this study has poor sensitivity.

↑ = Results bias away from the null; ↓ = Results bias toward the null; ↔ = Unknown direction of bias

Table C-5. Evaluation of potential for confounding bias for human cancer studies.

Study	Confounding rating
Jones 1994	*Rating*: +; *Direction*: ↑ *Rationale*: No control for smoking or occupational co-exposures in statistical analysis. Likely co-exposure to arsenic and PAHs (lung carcinogens) based on smelting source materials. Smoking not controlled for, despite high prevalence in the study population.
Schnorr *et al.* 1995	*Rating*: +++; ↔ *Rationale*: No control for smoking or occupational co-exposures in statistical analysis. Confounding from occupational co-exposures to arsenic and lead are minimal based on source information and environmental testing. Smoking prevalence rates were assumed to be low in this particular population.
Jones *et al.* 2007	*Rating*: ++; ↑ *Rationale*: No attempt to statistically account for measured occupational co-exposures in analysis. High level of correlation between antinomy, lead, and arsenic air concentrations suggests likely occupational co-exposure. Minimal concern for smoking, but not controlled for in analysis.
Wingren and Axelson 1993	*Rating*: +; ↑ *Rationale*: Smoking and occupational co-exposures lead and asbestos were not statistically controlled for in analysis. Lead and antimony use patterns were highly correlated, and lead was associated with an increased risk of stomach cancer mortality in this population; therefore, risk of confounding bias is high.

↑ = Results bias away from the null; ↓= Results bias toward the null; ↔ = Unknown direction of bias

Table C-6. Evaluation of analysis and selective reporting for human cancer studies.

Study	Analysis rating	Reporting rating
Jones 1994	*Rating*: +++ *Rationale*: The study used relevant data and appropriate assumptions and methods of analysis.	*Rating*: +++ *Rationale*: No evidence that reporting of the data or analyses were limited to only a subset of data that were collected.
Schnorr *et al.* 1995	*Rating*: ++ *Rationale*: 95% CI are generally favorable to 90% CI regardless of *a priori* outcome status.	*Rating*: +++ *Rationale*: No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.
Jones *et al.* 2007	*Rating*: ++ *Rationale*: 95% CI are generally favorable to 90% CI regardless of *a priori* outcome status.	*Rating*: +++ *Rationale*: No evidence that reporting of the data or analyses were limited to only a subset of the data that were collected.
Wingren and Axelson 1993	*Rating*: ++ *Rationale*: 95% CI are generally favorable to 90% CI regardless of *a priori* outcome status.	*Rating*: ++ *Rationale*: It is unknown whether reporting was done on only a subset on data. Sample size for cases, controls, and exposure groups were not reported.

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Appendix D: Animal Study Quality Tables

Table D-1. Kanisawa and Schroeder (1969) study of male and female (combined) mice exposed to antimony potassium tartrate in drinking water for the lifespan of the animals

Utility question	Rating	Rationale
Study design		
Randomization	NR	Not reported.
Controls	+++	Concurrent control group, exposed to doubly deionized water with added essential trace elements, had the same number of animals as the exposure group did.
Historical data		No
Animal model	+++	Both sexes of random bred mice were used, giving a high level of external validity.
Statistical power	+++	A large number (54) of mice per sex per group were used.
Exposure		
Chemical characterization	NR	No chemical characterization was reported, not even purity.
Dosing regimen	+	The maximally tolerated dose level was not reached, because the treated group did not show decreased body weight compared to the control group. The dose might not have been high enough to detect neoplastic effects.
Exposure duration	+++	Mice were exposed for their lifetimes.
Dose-response	+	Only one concentration was tested and no rational for the dose selection was reported. Dose response relationships cannot be evaluated due to only one dose level.
Outcome		
Pathology	++	Only gross lesions were microscopically evaluated.
Consistency between groups	++	The treated and control groups were treated the same while mice were alive. The examination of organs/tissues varied, because only gross lesions (not all major organs) were examined microscopically.
Study duration	+++	The study duration was lifetime, up to the animals' natural death.
Confounding		
Confounding	+++	Testing substance purity and supplier were unknown. Exposure to antimony via other sources (feed, housing) was negligible because the feed was antimony free and metal exposure via housing was minimized.
Reporting and analysis		
Reporting data and statistics	++	Although tumor incidents were not statistically analyzed in the study, the data were reported and enabled us to conduct statistical analysis. Statistical methods were described as "numerical data were treated by Chi-squire analysis and by Student's t test", but the reported probability in tables did not specify the result was from which method.
Combining lesions	++	Tumor incidence was reported for two sexes combined only, instead of male and female separately. Site specific (lung, liver, mammary gland, and other) information was limited to tumor incidence, with no subtype. Tumors were also grouped by origin (epithelial, non-epithelial) along with being benign or malignant. Overall information did not allow detecting of specific tumor type increase in either sex.

Overall utility: +. Due to many limitations, including only one tested concentration (below maximally tolerated dose), unknown test substance purity, tumor incidences only reported in combined sexes with no histologic information, and lack of site specific information (except incidences of three sites in sexes combined), this study is of low utility. Data lack sufficient details to allow us determine whether any specific type of tumor had increased.

Table D-2. NTP (2017) study of male rats exposed to antimony trioxide by inhalation for 105 weeks

Utility question	Rating	Rationale
Study design		
Randomization	+++	Animals were randomly assigned to groups.
Controls	+++	Concurrent chamber control was used. Data was also compared with historical control.
Historical data	Yes	
Animal model	+++	Standard model.
Statistical power	+++	A large number, 50/sex/group, of animals were used.
Exposure		
Chemical characterization	+++	The chemical and exposure chamber were well characterized, showing high purity, stability, and homogeneity. Concentration inside the exposure chamber was measured in real-time and alarmed if readings were not within limits of acceptable concentrations. Aerosol size, measured monthly, was also consistently less than 4 um (for male rats, MMAD = 1-1.4 um, GSD 1.8-2.2; for female rats, MMAD = 0.9 - 1.5 um, GSD = 1.7 - 2.1). Stability in the generation and exposure system was tested before the test, during the test (at 4 weeks for rats), and the end of 2 year study.
Dosing regimen	NR	Consistent and very close to target concentrations. The highest exposure level was near maximally tolerated levels as evidenced by the trend of decreased survival and a significant decrease in body weight. Neoplasms were significantly increased.
Exposure duration	+++	Exposure duration was near life-span
Dose-response	+++	Three dose levels spanning a range of 30 fold were used.
Outcome		
Pathology	+++	Detailed and covering all tissues. Full necropsy with histological exam of all major organs was conducted and verified by an independent quality control pathologist.
Consistency between groups	+++	Nothing was reported to suggest that animals from different groups were treated differently.
Study duration	+++	Near life-span study duration was used.
Confounding		
Confounding	+++	No concerns of confounding were reported.
Reporting and analysis		
Reporting data and statistics	+++	Statistical analysis was clearly reported.
Combining lesions	+++	No indication of concern. Detailed groupings were provided.

Overall utility: +++. There were no concerns of confounding as the chemical was pure, stable, the exposure was well characterized, and all groups were treated the same. The study had a high level of sensitivity to detect neoplasms as it used large numbers of both sexes of rats, exposed at three dose levels, which reached the maximally tolerated level, for a near life-span duration. However, the strain of rat used was new to NTP and so little historical control data existed compared to other strains. Complete necropsies with histological examination of most organs was performed, so the ability to detect neoplasms was high.

Table D-3. NTP (2017) study of female rats exposed to antimony trioxide by inhalation for 105 weeks

Utility question	Rating	Rationale
Study design		

Utility question	Rating	Rationale
Randomization	+++	Animals were randomly assigned to groups
Controls	+++	Concurrent chamber control was used. Data was also compared with historical control.
Historical data	No	
Animal model	+++	Standard model.
Statistical power	+++	A large number, 50/sex/group, of animals were used.
Exposure		
Chemical characterization	+++	The chemical and exposure chamber were well characterized, showing high purity, stability, and homogeneity. Concentration inside the exposure chamber was measured in real-time and alarmed if readings were not within limits of acceptable concentrations. Aerosol size, measured monthly, was also consistently less than 4 um (for male rats, MMAD = 1-1.4 um, GSD 1.8-2.2; for female rats, MMAD = 0.9 - 1.5 um, GSD = 1.7 - 2.1). Stability in the generation and exposure system was tested before the test, during the test (at 4 weeks for rats), and the end of 2 year study.
Dosing regimen	NR	Consistent, and very close to target, concentrations. The highest exposure level was near maximally tolerated levels as evidenced by the trend of decreased survival and a significant decrease in body weight. Neoplasms were significantly increased.
Exposure duration	+++	Exposure duration was near life-span.
Dose-response	+++	Three dose levels spanning a range of 30 fold were used.
Outcome		
Pathology	+++	Detailed and covering all tissues. Full necropsy with histological exam of all major organs was conducted and verified by an independent quality control pathologist.
Consistency between groups	+++	Nothing was reported to suggest that animals from different groups were treated differently.
Study duration	+++	Near life-span study duration was used.
Confounding		
Confounding	+++	No concerns of confounding were reported.
Reporting and analysis		
Reporting data and statistics	+++	Statistical analysis was clearly reported.
Combining lesions	+++	No indication of concern. Detailed groupings were provided.

Overall utility: +++. There were no concerns of confounding as the chemical was pure, stable, the exposure was well characterized, and all groups were treated the same. The study had a high level of sensitivity to detect neoplasms as it used large numbers of both sexes of rats, exposed at three dose levels, which reached the maximally tolerated level, for a near life-span duration. However, the strain of rat used was new to NTP and so little historical control data existed compared to other strains. Complete necropsies with histological examination of most organs was performed, so the ability to detect neoplasms was high.

Table D-4. NTP (2017) study of male mice exposed to antimony trioxide by inhalation for 105 weeks

Utility question	Rating	Rationale
Study design		
Randomization	+++	Animals were randomly assigned to groups.
Controls	+++	Concurrent chamber control was used. Data was also compared with historical control.
Historical data	Yes	
Animal model	+++	Standard model.
Statistical power	+++	A large number, 50/sex/group, of animals were used.
Exposure		
Chemical characterization	+++	The chemical and exposure chamber were well characterized, showing high purity, stability, and homogeneity. Concentration inside the exposure chamber was measured in real-time and alarmed if readings were not within limits of acceptable concentrations. Aerosol size, measured monthly, was also consistently less than 4 um (for male rats, MMAD = 1-1.4 um, GSD 1.8-2.2; for female rats, MMAD = 0.9 - 1.5 um, GSD = 1.7 - 2.1). Stability in the generation and exposure system was tested before the test, during the test (at 4 weeks for rats), and the end of 2-year study.
Dosing regimen	NR	Consistent, and very close to target, concentrations. The highest exposure level was near maximally tolerated levels as evidenced by the trend of decreased survival and a significant decrease in body weight. Neoplasms were significantly increased.
Exposure duration	+++	Exposure duration was near life-span.
Dose-response	+++	Three dose levels spanning a range of 30 fold were used.
Outcome		
Pathology	+++	Detailed and covering all tissues. Full necropsy with histological exam of all major organs was conducted and verified by an independent quality control pathologist.
Consistency between groups	+++	Nothing was reported to suggest that animals from different groups were treated differently.
Study duration	+++	Near life-span study duration was used.
Confounding		
Confounding	+++	No concerns of confounding were reported.
Reporting and analysis		
Reporting data and statistics	+++	Statistical analysis was clearly reported.
Combining lesions	+++	No indication of concern. Detailed groupings were provided.

Overall utility: +++. There were no concerns of confounding as the chemical was pure, stable, the exposure was well characterized, and all groups were treated the same. The study had a high level of sensitivity to detect neoplasms as it used large numbers of both sexes of rats, exposed at three dose levels, which reached the maximally tolerated level, for a near life-span duration. However, the strain of rat used was new to NTP and so little historical control data existed compared to other strains. Complete necropsies with histological examination of most organs was performed, so the ability to detect neoplasms was high.

Table D-5. NTP (2017) study of female mice exposed to antimony trioxide by inhalation for 105 weeks

Utility question	Rating	Rationale
Study design		
Randomization	+++	Animals were randomly assigned to groups.
Controls	+++	Concurrent chamber control was used. Data were also compared with historical control.
Historical data	No	
Animal model	+++	Standard model.
Statistical power	+++	A large number, 50/sex/group, of animals were used.
Exposure		
Chemical characterization	+++	The chemical and exposure chamber were well characterized, showing high purity, stability, and homogeneity. Concentration inside the exposure chamber was measured in real-time and alarmed if readings were not within limits of acceptable concentrations. Aerosol size, measured monthly, was also consistently less than 4 um (for male rats, MMAD = 1-1.4 um, GSD 1.8-2.2; for female rats, MMAD = 0.9 - 1.5 um, GSD = 1.7 - 2.1). Stability in the generation and exposure system was tested before the test, during the test (at 4 weeks for rats), and the end of 2 year study.
Dosing regimen	NR	Consistent, and very close to target, concentrations. The highest exposure level was near maximally tolerated levels as evidenced by the trend of decreased survival and a significant decrease in body weight. Neoplasms were significantly increased.
Exposure duration	+++	Exposure duration was near life-span.
Dose-response	+++	Three dose levels spanning a range of 30 folds were used.
Outcome		
Pathology	+++	Detailed and covering all tissues. Full necropsy with histological exam of all major organs was conducted and verified by an independent quality control pathologist.
Consistency between groups	+++	Nothing was reported to suggest that animals from different groups were treated differently.
Study duration	+++	Near life-span study duration was used.
Confounding		
Confounding	+++	No concerns of confounding were reported.
Reporting and analysis		
Reporting data and statistics	+++	Statistical analysis was clearly reported.
Combining lesions	+++	No indication of concern. Detailed groupings were provided.

Overall utility: +++. There were no concerns of confounding as the chemical was pure, stable, the exposure was well characterized, and all groups were treated the same. The study had a high level of sensitivity to detect neoplasms as it used large numbers of both sexes of rats, exposed at three dose levels, which reached the maximally tolerated level, for a near life-span duration. However, the strain of rat used was new to NTP and so little historical control data existed compared to other strains. Complete necropsies with histological examination of most organs was performed, so the ability to detect neoplasms was high.

Table D-6. Groth *et al.* (1986) study of male rats exposed to antimony trioxide by inhalation for 53 weeks followed by post-exposure observation for 71 to 73 weeks

Utility question	Rating	Rationale
Study design		
Randomization	NR	Not reported.
Controls	+++	Concurrent untreated chamber controls (filtered air) were used.
Historical data	No	
Animal model	+++	Male and female inbred rats were used.
Statistical power	+++	A large number of rats (90/sex/group) were used.
Exposure		
Chemical characterization	+	The low purity (80%) and contamination by carcinogens (arsenic and lead) and others (tin, cesium aluminum, and bromine) was reported. The aerosol concentrations didn't reach target levels of 50 mg/m^3 until after 5 months of adjustment and modifications on the exposure equipment. MMAD of aerosol of 2.80 um was fine, but the GSD was not reported. Aerosol size appeared only measured once at 6 month of exposure.
Dosing regimen	++	The exposure level was based on the middle of the concentration range that workers are exposed to, so it would not be expected to be at the maximally tolerated level. The concentrations fluctuated dramatically, up to 191.1 mg/m^3 for daily TWAs, while a mean daily TWA was 45, 46 mg/m^3 (two chambers) and the target concentration was 50 mg/m^3. It is not clear whether the chamber air was humidified. Survival and body weights were similar to controls, but total neoplasms in the lung were significantly increased over untreated controls.
Exposure duration	++	Exposure duration was 53 weeks.
Dose-response	+	Only one exposure level was used and it was based on the middle of the concentration range that workers are exposed to (i.e., well below animals' maximal tolerated dose). The actual exposure concentration fluctuated greatly until about 5 months into the study when the target concentration was reached.
Outcome		
Pathology	+++	Most organs were histologically examined.
Consistency between groups	++	No indication of differential treatments.
Study duration	++	The study duration was 71 to 73 weeks long, roughly 1.4 years, with only 5 months of observation after the end of 53 week-long exposure. These lengths were likely limited because the rats were 8 months old at the beginning of the study.
Confounding		
Confounding	+	The chemical was only 80% antimony, with various other metal contaminants, such as tin, lead, cesium, aluminum, arsenic, and bromine. Lead and arsenic are carcinogenic.
Reporting and analysis		
Reporting data and statistics	NR	Statistical analysis was reported for body weights, tissue levels of antimony, and neoplasms. Statistical significance, but not the method was reported for neoplasm incidences in females.
Combining lesions	+++	Neoplasms were combined by site. While no numbers of each pathological type were provided, the tumor combining is fine

Overall utility: ++. The chemical was well characterized, but was found to only be 80% pure, with lead and arsenic as contaminants. The low purity makes it difficult to distinguish effects caused by antimony from possible effects caused by the contaminants. The sensitivity of the study to detect neoplasms was low as only one dose level was used and it was based on the level of exposure to workers and not the maximally tolerated dose. Further, the exposure concentration varied widely until 5 months into the study when the target concentration was reached. The exposure duration was more than a year and full necropsies with histological examinations were performed. Neoplasms were reported with statistical analysis as total neoplasms combined per organ site.

Table D-7. Groth *et al.* (1986) study of female rats exposed to antimony trioxide by inhalation for 53 weeks followed by post-exposure observation for 71 to 73 weeks

Utility question	Rating	Rationale
Study design		
Randomization	NR	Not reported.
Controls	+++	Concurrent untreated chamber controls (filtered air) were used.
Historical data	No	
Animal model	+++	Male and female inbred rats were used.
Statistical power	+++	A large number of rats (90/sex/group) were used.
Exposure		
Chemical characterization	+	The low purity (80%) and contamination by carcinogens (arsenic and lead) and others (tin, cesium aluminum, and bromine) was reported. The aerosol concentrations didn't reach target levels of 50 mg/m^3 until after 5 months of adjustment and modifications on the exposure equipment. MMAD of aerosol of 2.80 um was fine, but the GSD was not reported. Aerosol size appeared only measured once at 6 month of exposure.
Dosing regimen	++	The exposure level was based on the middle of the concentration range that workers are exposed to, so it would not be expected to be at the maximally tolerated level. The concentrations fluctuated dramatically, up to 191.1 mg/m^3 for daily TWAs, while a mean daily TWA was 45, 46 mg/m^3 (two chambers) and the target concentration was 50 mg/m^3. It is not clear whether the chamber air was humidified. Survival and body weights were similar to controls, but total neoplasms in the lung were significantly increased over untreated controls.
Exposure duration	++	Exposure duration was 53 weeks.
Dose-response	+	Only one exposure level was used and it was based on the middle of the concentration range that workers are exposed to (i.e., well below animals' maximal tolerated dose). The actual exposure concentration fluctuated greatly until about 5 months into the study when the target concentration was reached.
Outcome		
Pathology	+++	Most organs were histologically examined.
Consistency between groups	++	No indication of differential treatments.
Study duration	++	The study duration was 71 to 73 weeks long, roughly 1.4 years, with only 5 months of observation after the end of 53 week-long exposure. These lengths were likely limited because the rats were 8 months old at the beginning of the study.
Confounding		
Confounding	+	The chemical was only 80% antimony, with various other metal contaminants, such as tin, lead, cesium, aluminum, arsenic, and bromine. Lead and arsenic are carcinogenic
Reporting and analysis		
Reporting data and statistics	NR	Statistical analysis was reported for body weights, tissue levels of antimony, and neoplasms. Statistical significance, but not the method was reported for neoplasm incidences in females.
Combining lesions	+++	Neoplasms were combined by site. While no numbers of each pathological type were provided, the tumor combining is fine.

Overall utility: ++. The chemical was well characterized, but was found to only be 80% pure, with lead and arsenic as contaminants. The low purity makes it difficult to distinguish effects caused by antimony from possible effects caused by the contaminants. The sensitivity of the study to detect neoplasms was low as only one dose level was used and it was based on the level of exposure to workers and not the maximally tolerated dose. Further, the exposure concentration varied widely until 5 months into the study when the target concentration was reached. The exposure duration was more than a year and full necropsies with histological examinations were performed. Neoplasms were reported with statistical analysis as total neoplasms combined per organ site.

Table D-8. Newton *et al.* (1994) study of male rats exposed to antimony trioxide by inhalation for 12 months followed by post-exposure observation for 24 months

Utility question	Rating	Rationale
Study design		
Randomization	+++	Used a computer program to randomly sort animals so that mean group weights were comparable.
Controls	+++	Use concurrent control at the same number of animals as exposure groups.
Historical data	No	
Animal model	+++	Normal Fischer rats, which are often used in carcinogenicity studies.
Statistical power	+++	A large number of rats (65/sex/group) were used.
Exposure		
Chemical characterization	+++	A blend of lots from 9 producers of antimony trioxide. Highly pure material. Particle size was characterized as having a mass median aerodynamic diameter of 3.76 +/- 0.84 μm and a geometric standard deviation of 1.79 +/- 0.32. Exposure concentration was analyzed four times a day and particle sizes were analyzed before the study and every three months. Homogeneity of the exposure chamber was verified by measuring 10 different locations.
Dosing regimen	++	There were not differences in body weight, survival, or neoplasm incidence, suggesting the dose was not at the maximally tolerated dose.
Exposure duration	+++	12 month exposure.
Dose-response	+++	Three exposed levels were used, which covered a 100-fold range.
Outcome		
Pathology	++	Only heart, airway, and peribronchial lymph nodes were histologically examined.
Consistency between groups	+++	Consistent treatment and evaluation of groups.
Study duration	+++	The study duration was 2 years, with 12 months of exposure and 12 months of observation.
Confounding		
Confounding	+++	Material of high purity. Animal husbandry reported in detail. No significant body weight loss in the treated groups, compared to the control compared to the control.
Reporting and analysis		
Reporting data and statistics	+++	Since neoplasm incidences were not reported, as they were negative, statistical analysis wasn't reported.
Combining lesions	+++	No tumor combining, as only three cases [2 males (including one from control), 1 female] were seen.

Overall utility: ++. There was little concern for confounding as the chemical was pure, exposure conditions were well characterized, and groups were treated consistently with animals randomly assigned to exposure groups. The sensitivity of detecting neoplasms was good as high numbers of both sexes were tested. Exposure were at three concentrations for about half a life-span duration (1 year), though observations (1 year) continued to a near life-span duration. However, the highest exposure level did not reach the maximally tolerated level. Most organs were histologically examined, so most neoplasms had the ability of being detected. Although aerosol size was not ideal (slightly over the current upper limit of test guidelines), this paper did show Sb2O3 accumulation and increased clearance half-life in the lung (by 80% in the 4.5 mg/m^3 group). For inert particle, such overload would/could cause lung tumor. The overload was observed at relatively low exposure concentrations (compared to inert particles, such as TiO2) and Sb2O3 toxicity was suspected. It appears conditions that could potentially lead to cancer did persist (Table 9, post-exposure, chronic inflammation in most animals, although hyperplasia was in only very few animals).

Table D-9. Newton *et al.* (1994) study of female rats exposed to antimony trioxide by inhalation for 12 months followed by post-exposure observation for 24 months

Utility question	Rating	Rationale
Study design		
Randomization	+++	Used a computer program to randomly sort animals so that mean group weights were comparable.
Controls	+++	Use concurrent control at the same number of animals as exposure groups.
Historical data	No	
Animal model	+++	Normal Fischer rats, which are often used in carcinogenicity studies.
Statistical power	+++	A large number of rats (65/sex/group) were used.
Exposure		
Chemical characterization	+++	A blend of lots from 9 producers of antimony trioxide. Highly pure material. Particle size was characterized as having a mass median aerodynamic diameter of 3.76 +/- 0.84 μm and a geometric standard deviation of 1.79 +/- 0.32. Exposure concentration was analyzed four times a day and particle sizes were analyzed before the study and every three months. Homogeneity of the exposure chamber was verified by measuring 10 different locations.
Dosing regimen	++	There were not differences in body weight, survival, or neoplasm incidence, suggesting the dose was not at the maximally tolerated dose.
Exposure duration	+++	12-month exposure.
Dose-response	+++	Three exposed levels were used, which covered a 100-fold range.
Outcome		
Pathology	++	Only heart, airway, and peribronchial lymph nodes were histologically examined.
Consistency between groups	+++	Consistent treatment and evaluation of groups.
Study duration	+++	The study duration was 2 years, with 12 months of exposure and 12 months of observation.
Confounding		
Confounding	+++	Material of high purity. Animal husbandry reported in detail. No significant body weight loss in the treated groups, compared to the control.
Reporting and analysis		
Reporting data and statistics	+++	Since neoplasm incidences were not reported, as they were negative, statistical analysis wasn't reported.
Combining lesions	+++	No tumor combining, as only three cases [2 males (including one from control), 1 female] were seen.

Overall utility: ++. There was little concern for confounding as the chemical was pure, exposure conditions were well characterized, and groups were treated consistently with animals randomly assigned to exposure groups. The sensitivity of detecting neoplasms was good as high numbers of both sexes were tested. Exposure were at three concentrations for about half a life-span duration (1 year), though observations (1 year) continued to a near life-span duration. However, the highest exposure level did not reach the maximally tolerated level. Most organs were histologically examined, so most neoplasms had the ability of being detected. Although aerosol size was not ideal (slightly over the current upper limit of test guidelines), this paper did show Sb2O3 accumulation and increased clearance half-life in the lung (by 80% in the 4.5 mg/m^3 group). For inert particle, such overload would/could cause lung tumor. The overload was observed at relatively low exposure concentrations (compared to inert particles, such as TiO2) and Sb2O3 toxicity was suspected. It appears conditions that could potentially lead to cancer did persist (Table 9, post-exposure, chronic inflammation in most animals, although hyperplasia was in only very few animals).

Table D-10. Watt (1983) study of female rats exposed to antimony trioxide by inhalation for 1 year followed by post-exposure observation for 2 years

Utility question	Rating	Rationale
Study design		
Randomization	NR	Not reported.
Controls	+++	Concurrent controls were used, although animals were housed in different rooms (housing chambers separated to control, low concentration, and high concentration). Otherwise, treatments were the same.
Historical data	No	
Animal model	++	Only female rats were used
Statistical power	+	Small number of animals were used. 13-18 animals per group sacrificed at the end of exposure. Less than 10 per group sacrificed between 2 to 12 months post exposure. Less than 20 per group sacrificed 12-months post exposure.
Exposure		
Chemical characterization	+++	Detailed chemical analysis verified that Sb2O3 was of high purity. Small amounts of arsenic (0.02%) and lead (0.2%) were found as contaminates. Dust size (measured by SEM) was reported as Feret diameter. Presumably this is average from the same particle with rotation. Aerosol concentration in the exposure chamber. The equipment generated aerosols of MMAD less than 15 um, but aerosol sizes were not measured. Based on conversion done in Newton et al 1994 paper Table 2, the MMAD is 5.06 um, which is above the ideal range of rat inhalation study (no more than 4 um).
Dosing regimen	+++	Another potential concern is the use of pine shaving in the exposure chamber. The rats were not in direct contact with shaving, but metabolism change from pine cannot be excluded. This does not affect the interpretation of this study as all groups were treated the same, but has been suggested by Newton et al 1994 as a factor affecting outcome even though it is based on concerns of increased particulates (rather than rat metabolism). Survival was not reported, but body weight gain was greater than controls, indicating the dose is not close to maximal tolerant dose. Significant increases in neoplasia occurred, indicating the dose level was high enough to cause carcinogenesis.
Exposure duration	+++	Exposure occurred for up to 1 year, with intermediate sacrifices at 3, 6, 9 months.
Dose-response	++	Only two dose levels, ranging over 2.5 folds, were used, limiting the examination of a dose response curve.
Outcome		
Pathology	++	Major organs were examined microscopically.
Consistency between groups	+++	Consistent treatment among groups, except housed in different rooms.
Study duration	+++	The study duration was 2 years, with 1 year of exposure and 1 year of observation.
Confounding		
Confounding	++	Animals in high dose group were heavier than low dose group at the beginning, suggesting slightly different development level. Not all organs appear to have been examined during necropsy.
Reporting and analysis		
Reporting data and statistics	++	While statistic methods were not specified, the data were reported with raw numbers and therefore enables statistical analysis

Utility question	Rating	Rationale
Combining lesions	+++	Tumor types were not combined. Scirrhous carcinomas, a pathologically distinctive lung cancer, alone, was significantly increased compared to negative controls.

Overall utility: ++. The chemical purity was high and exposure was characterized, though the particle size (converted by Newton et al (year) to be around MMAD 5 um) was over the recommended (1-4 um). Only female rats were used, which eliminates the ability to detect sex differences. The sensitivity to detect neoplasms was low as a small number of rats were used at only two dose levels, though the exposure was near life-span duration. The ability to detect neoplasms, if they exist, was moderate as the organs examined during necropsy were not fully reported. The statistical methods used were not reported. The use of large exposure chamber with pigs inside and pine shaving also increased the chance of exposure to non-Sb2O3 particles (and possible metabolism alternation due to pine shaving and therefore affecting susceptibility).

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Appendix E: Mechanistic and Other Relevant Information

This appendix first lists the 10 characteristic of carcinogens proposed by Smith *et al.* (2016) and used to organize the information in Section 6 (see Table E-1). The remainder of the appendix contains animal carcinogenic studies of antimony potassium tartrate (Appendix E.1), genotoxicities of antimony compounds (Appendix E.2), effects of antioxidant and inhibitors of enzymes on antimony effects (Appendix E.3), immune effects of compounds containing pentavalent antimony (Appendix E.4), the top ten canonical pathways affected by 6-hour exposure to 20 μM antimony(III) potassium tartrate trihydrate (Appendix E.5), and the top 10 upstream regulators of antimony (Appendix E.6).

Table E-1. Ten characteristics of carcinogens

Number	Characteristic, i.e. the ability of an agent to have an effect to…
1	Act as an electrophile either directly or after metabolic activation
2	Be genotoxic
3	Alter DNA repair or cause genomic instability
4	Induce epigenetic alterations
5	Induce oxidative stress
6	Induce chronic inflammation
7	Be immunosuppressive
8	Modulate receptor-mediated effects
9	Cause immortalization
10	Alter cell proliferation, cell death, or nutrient supply

Source: Smith *et al.* 2016.

E.1: Studies of antimony(III) potassium tartrate carcinogenicity in experimental animals

This appendix includes neoplasms induced in experimental animals exposed to antimony potassium tartrate (Table E.1-1), details of these animal studies (Table E.1-2) and risk of bias rating of Schroeder et al. (1970) study (male rats in Table E.1-3, female rats in Table E.1-4) and Kanisawa and Schroeder (1969) study (Table E.1-5)

Table E.1-1. Neoplasms induced in experimental animal carcinogenicity studies by drinking water studies of antimony potassium tartrate

Studies are presented in the order of descending overall utility.

Species strain/stock*	Site	Classification	Neoplasms (Sex of animal)	Reference
Rat, Long-Evans	None	None	None – (M and F)	Schroeder et al. 1970
Mouse, Swiss CD-1	None	None	None – (M and F)	Schroeder et al. 1968, Kanisawa and Schroeder 1969

F = female, M = male.

Table E.1-2. Cancer studies in experimental animals exposed to antimony(III) potassium tartrate

Reference and study design	Exposure	Tumor site – Tumor type		Comments
		Dose levels	Tumor incidence (n/N) (%)	
Schroeder et al. 1970 **Animal**: Rat — Long-Evans (random bred) M, F **Animal age at the beginning of exposure**: NR (possibly at weaning) **Study duration**: ~4 years	**Agent and purity**: Antimony potassium tartrate NR **Exposure route**: Drinking water **Exposure concentrations, frequency, and duration**: 0 5 ppm not clearly reported (possibly ad libitum x life-span)	**Whole body – Tumor NOS (M)**		**Survival**: The survival of females at 50% death ($P < 0.025$ by chi-square analysis) and males and females for longevity (mean age of the last surviving 10%) ($P < 0.001$ by Student's t test) was significantly reduced compared to untreated controls. **Body weight**: Both males and females were similar to controls. **Overall utility: [+]** The study has low utility because of many limitations, including only reporting grossly visible tumors without organ site or tumor type.
		0	10/50 (20%)	
		5	6/50 (12%)	
		Whole body – Tumor NOS (F)		
		0	14/39 (35.9%)	
		5	18/47 (38.3%)	
Kanisawa and Schroeder 1969	**Agent and purity**: Antimony potassium tartrate	**Whole body – Tumor NOS**		**Survival**: Survival was similar to controls. **Body weight**: Males were sporadically lower than controls at 90, 150, and 540 days, while females were more consistently
		0	24/71 (33.8%)	
		5	18/76 (23.7%)	

Reference and study design	Exposure	Tumor site – Tumor type		Comments
		Dose levels	Tumor incidence (n/N) (%)	
Animal: Mouse — White Swiss CD-1 (Random bred) M+F (combined) **Animal age at the beginning of exposure**: Weanling **Study duration**: Life span	NR **Exposure route**: Drinking water **Exposure concentrations, frequency, and duration**: 0 5 µg/mL in double deionized water ad libitum x life span	**Whole body – Malignant tumor NOS**		lower at 150, 360, and 540 days. **Other comments**: The incidences were reported for both sexes combined, but it was stated that none of the neoplasms were significantly increased. **Overall utility: [+]** This study is of low utility due to many limitations, including only one tested concentration (below maximally tolerated dose for males, and close to or at maximally tolerated dose for females), unknown test substance purity, tumor incidences only reported in combined sexes with no histologic information, and lack of site specific information (except incidences of three sites in sexes combined). Data lack sufficient details to allow us determine whether any specific type of tumor had increased in a sex.
		0	8/71 (11.3%)	
		5	6/76 (7.9%)	
		Whole body – Benign tumor NOS		
		0	16/71 (22.5%)	
		5	12/76 (15.8%)	
		Mammary gland – Tumor NOS		
		0	1/71 (1.4%)	
		5	3/76 (3.9%)	
		Lung – Tumor NOS		
		0	15/71 (21.1%)	
		5	10/76 (13.2%)	
		Liver – Tumor NOS		
		0	4/71 (5.6%)	
		5	1/76 (1.3%)	

F = female; M = male; n/N = number of animals with neoplasms divided by the total number of animals tested in that dose group; NR = not reported; NOS = not otherwise specified

Table E.1-3. Schroeder *et al.* (1970) study of male rats exposed to antimony(III) potassium tartrate in drinking water for the life span of the animals

Utility question	Rating	Rationale
Study design		
Randomization	NR	Randomization and initial body weights were not reported.
Controls	+++	Concurrent control group, exposed to untreated drinking water, had the same number of animals as exposure group did.
Historical data		No
Animal model	+++	Both sexes of a random bred strain, which increases external validity.
Statistical power	+++	Large numbers of animals (51 males, 59 females) per concentration group were used.
Exposure		
Chemical characterization	NR	Not reported, not even purity.
Dosing regimen	++	The maximally tolerated dose level was not reached, because the treated group did not show decrased body weight compared to the control group, although the median life spans and longivity (mean age of the last surviving 10%) for both sexes were decrased by the treatment. The dose might not have been high enough to detect neoplastic effects.
Exposure duration	+++	Though exposure duration was never clearly stated, this study appears to use a life time exposure.
Dose-response	+	Only one dose level was tested and no basis for that level was reported. All other elements were administered at the same level, except for lead.
Outcome		
Pathology	+	Only grossly visible tumors were reported. The methods stated that gross tumors were fixed, but did not state that they were stained or microscopically examined. Consequently, small tumors might have been missed.
Consistency between groups	++	A pneumonia epidemic killed many rats and the death rates varied among the groups.
Study duration	+++	Life time study, because the animals were observed until their nature death (as compared to scheduled euthanization after a predetermined exposure period).
Confounding		
Confounding	++	Pneumonia killed various numbers of animals per group, before penicillin treatment controlled the disease. It is unclear that all rats, or only visibly sick rats, received penicillin. Furthermore, the disease might in effect select stronger/healthier animals (than the general population) to complete the study. Additionally, test substance purity was unknown.
Reporting and analysis		
Reporting data and statistics	++	The statistical methods and results of survival measures were reported, but statistical analysis of tumor incidences were not reported.
Combining lesions	+	Tumors were counted based on gross observation, not histological analysis occurred.

Overall utility: +. The study has low utility because of many limitations, including only reporting grossly visible tumors without organ site or tumor type.

Table E.1-4. Schroeder *et al.* (1970) study of female rats exposed to antimony(III) potassium tartrate in drinking water for the life span of the animals

Utility question	Rating	Rationale
Study design		
Randomization	NR	Randomization and initial body weights were not reported.
Controls	+++	Concurrent control group, exposed to untreated drinking water, had the same number of animals as exposure group did.
Historical data		No
Animal model	+++	Both sexes of a random bred strain, which increases external validity.
Statistical power	+++	Large numbers of animals (51 males, 59 females) per concentration group were used.
Exposure		
Chemical characterization	NR	Not reported, not even purity.
Dosing regimen	++	The maximally tolerated dose level was not reached, because the treated group did not show decreased body weight compared to the control group, although the median life spans and longevity (mean age of the last surviving 10%) for both sexes were decreased by the treatment. The dose might not have been high enough to detect neoplastic effects.
Exposure duration	+++	Though exposure duration was never clearly stated, this study appears to use a life time exposure.
Dose-response	+	Only one dose level was tested and no basis for that level was reported.
Outcome		
Pathology	+	Only grossly visible tumors were reported. The methods stated that gross tumors were fixed, but did not state that they were stained or microscopically examined. Consequently, small tumors might have been missed.
Consistency between groups	++	A pneumonia epidemic killed many rats and the death rates varied among the groups.
Study duration	+++	Life time study, because the animals were observed until their nature death (as compared to scheduled euthanization after a predetermined exposure period).
Confounding		
Confounding	++	Pneumonia killed various numbers of animals per group, before penicillin treatment controlled the disease. It is unclear that all rats, or only visibly sick rats, received penicillin. Furthermore, the disease might in effect select stronger/healthier animals (than the general population) to complete the study. Additionally, test substance purity was unknown.
Reporting and analysis		
Reporting data and statistics	++	The statistical methods and results of survival measures were reported, but statistical analysis of tumor incidences were not reported.
Combining lesions	+	Tumors were counted based on gross observation, not histological analysis occurred.

Overall utility: +. The study has low utility because of many limitations, including only reporting grossly visible tumors without organ site or tumor type.
In the row of species, R = rats, M = mice. In the row of sex, M = males, F = females. In rows of each signaling question, NR = Not reported, +++ = High utility, ++ = Moderate utility, + = Low utility.

Table E.1-5. Kanisawa and Schroeder (1969) study of male and female (combined) mice exposed to antimony potassium tartrate in drinking water for the lifespan of the animals

Utility question	Rating	Rationale
Study design		
Randomization	NR	Not reported.
Controls	+++	Concurrent control group, exposed to doubly deionized water with added essential trace elements, had the same number of animals as the exposure group did.
Historical data		No
Animal model	+++	Both sexes of random bred mice were used, giving a high level of external validity.
Statistical power	+++	A large number (54) of mice per sex per group were used.
Exposure		
Chemical characterization	NR	No chemical characterization was reported, not even purity.
Dosing regimen	+	The maximally tolerated dose level was not reached, because the treated group did not show decreased body weight compared to the control group. The dose might not have been high enough to detect neoplastic effects.
Exposure duration	+++	Mice were exposed for their lifetimes.
Dose-response	+	Only one concentration was tested and no rational for the dose selection was reported. Dose response relationships cannot be evaluated due to only one dose level.
Outcome		
Pathology	++	Only gross lesions were microscopically evaluated.
Consistency between groups	++	The treated and control groups were treated the same while mice were alive. The examination of organs/tissues varied, because only gross lesions (not all major organs) were examined microscopically.
Study duration	+++	The study duration was lifetime, up to the animals' natural death.
Confounding		
Confounding	+++	Testing substance purity and supplier were unknown. Exposure to antimony via other sources (feed, housing) was negligible because the feed was antimony free and metal exposure via housing was minimized.
Reporting and analysis		
Reporting data and statistics	++	Although tumor incidents were not statistically analyzed in the study, the data were reported and enabled us to conduct statistical analysis. Statistical methods were described as "numerical data were treated by Chi-squire analysis and by Student's t test", but the reported probability in tables did not specify the result was from which method.
Combining lesions	++	Tumor incidence was reported for two sexes combined only, instead of male and female separately. Site specific (lung, liver, mammary gland, and other) information was limited to tumor incidence, with no subtype. Tumors were also grouped by origin (epithelial, non-epithelial) along with being benign or malignant. Overall information did not allow detecting of specific tumor type increase in either sex.

Overall utility: +. Due to many limitations, including only one tested concentration (below maximally tolerated dose), unknown test substance purity, tumor incidences only reported in combined sexes with no histologic information, and lack of site specific information (except incidences of three sites in sexes combined), this study is of low utility. Data lack sufficient details to allow us determine whether any specific type of tumor had increased.

E.2: Genetox tables

The genotoxic tables are organized by endpoints: mutations (Table E.2-1), mutations in the lung of mice and rats (Table E.2-2), DNA damage (Table E.2-3), chromosomal aberrations (Table E.2-4).

Table E.2-1. Genotoxicity of antimony compounds: Mutations[a,b c]

Mutation studies are listed hierarchically according to the following criteria:

1 By genotoxicity endpoints;
2 By domain of target species (eukaryote and then prokaryote);
3 By testing system (e.g., *E. coli* strains and then *Salmonella* strains); and
4 By compound in the order of antimony(III) trioxide (bold) and then antimony(III) trichloride.

Genotoxicity endpoint	Antimony form	Testing system/exposure duration	Assay endpoint	Comments	Reference
Mammalian cells					
Point mutations and chromosome deletions	**Antimony trioxide**	L5178Y mouse lymphoma cell line (+/-S9, 2 experiments) 4-hour exposure duration	Negative (concentrations tested: 6–50 µg/mL)	Precipitate formed at top dose level; authors report no significant toxicity at these doses	Elliott *et al*. 1998
Bacteria					
A/T base pair substitutions	**Antimony trioxide**	*E. coli* B/r WP2 *try* and WP2 *hcr try* (-S9, spot test method)	Negative (concentrations tested: 0.05–0.5 M)	Microbial toxicity not reported	Kanematsu *et al*. 1980
A/T base pair substitution	**Antimony trioxide**	*E. coli* WP2P (+/-S9; plate incorporation and pre-incubation protocols) 3-day exposure duration	Negative (concentrations tested: 100–5000 µg/plate)	Microbial toxicity not reported	Elliott *et al*. 1998
A/T base pair substitution	**Antimony trioxide**	*E. coli* WP2P*uvr*A (+/-S9; plate incorporation and pre-incubation protocols) 3-day exposure duration	Negative (concentrations tested: 100–5000 µg/plate)		Elliott *et al*. 1998
A/T base pair substitutions	**Antimony trichloride**	*E. coli* B/r WP2 *try* and WP2 *hcr try* (-S9, spot test method)	Negative (concentrations tested: 0.05–0.5 M)	Microbial toxicity not reported	Kanematsu *et al*. 1980

Genotoxicity endpoint	Antimony form	Testing system/exposure duration	Assay endpoint	Comments	Reference
G/C base pair substitutions	**Antimony trioxide**	*S. typhimurium* TA 1535, TA 1537, TA100, TA98 (+/-S9 ; plate incorporation and pre-incubation protocols) 3-day exposure duration	Negative (concentrations tested: 100–5000 µg/plate)	Microbial toxicity not reported	Elliott *et al*. 1998
Frameshift mutations	**Antimony trioxide**	*S. typhimurium* TA 1537 and 98 (+/-S9; plate incorporation and 60 min pre-incubation protocols) 3-day exposure duration	Negative (concentrations tested: 100–5000 µg/plate)	Microbial toxicity not reported	Elliott *et al*. 1998
Base pair substitution and frameshift mutations	**Antimony trioxide**	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1568 (-S9, spot test method)	Negative (concentrations tested: 0.05–0.5 M)	Duration of chemical exposure for spot test assay not reported; microbial toxicity not reported	Kanematsu *et al*. 1980
Base pair substitution and frameshift mutations	**Antimony trioxide**	*S. typhimurium* TA100, TA98 (+/-S9; 20 min pre-incubation modification)	Negative in 3 experiments (concentrations tested: 0.43–1.71 µg/plate)	Survival after pre-incubation step reported	Kuroda *et al*. 1991
Base pair substitution and frameshift mutations	**Antimony trichloride**	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1568 (-S9, spot test method)	Negative (concentrations tested: 0.05–0.5 M)	Duration of chemical exposure for spot test assay not reported; microbial toxicity not reported	Kanematsu *et al*.1980
Base pair substitution and frameshift mutations	**Antimony trichloride**	*S. typhimurium* TA100, TA98 (+/-S9; 20 min pre-incubation modification)	Negative in 3 experiments (concentrations tested: 625–5000 µg/plate)	Survival after pre-incubation step reported	Kuroda *et al*. 1991

[a]All data in prokaryotes were derived bacterial reverse mutation assays. The single eukaryotic study data was derived from the mouse lymphoma TK gene mutation assay.
[b]Levels of significance are designated as follows: $*P < 0.05$; $**P < 0.01$.

Table E.2-2. Mutations in the lung of mice and rats after two-year inhalation exposure to antimony trioxide (NTP 2016).

Genotoxicity endpoint	Antimony form	Testing system	Assay endpoint		Comments	Reference
Egfr mutations	**Antimony trioxide**	Lung tumors from exposed B6C3F1/N mice. Both non-tumor lung and spontaneous tumors from control mice.	**Mutation Frequency**			NTP 2016
			Concentration (mg/m^3)	**# with mutation/# tissues assayed**		
			0 (nontumor lung)	0/10		
			0 (tumor lung)	0/9		
			3 (tumor lung)	11/28*		
			10 (tumor lung)	11/26*		
			30 (tumor lung)	15/26**		
Egfr mutations	**Antimony trioxide**	Lung tumors from exposed Wistar Han rats. Both non-tumor lung and spontaneous tumors from control mice.	**Mutation Frequency**		Increase was not statistically significant.	NTP 2016
			Concentration (mg/m^3)	**# with mutation/# tissues assayed**		
			0 (nontumor lung)	0/11		
			0 (tumor lung)	0/4		
			3 (tumor lung)	3/5		
			10 (tumor lung)	6/11		
			30 (tumor lung)	4/10		
Kras mutations	**Antimony trioxide**	Lung tumors from exposed Wistar Han rats. Both non-tumor lung and spontaneous tumors from control mice.	**Mutation Frequency**		Increase was not statistically significant.	NTP 2016
			Concentration (mg/m^3)	**# with mutation/# tissues assayed**		
			0 (nontumor lung)	0/11		
			0 (tumor lung)	0/4		
			3 (tumor lung)	0/5		
			10 (tumor lung)	1/11		
			30 (tumor lung)	0/10		
Kras mutations	**Antimony trioxide**	Lung tumors from exposed B6C3F1/N mice.	**Mutation Frequency**		Increase was not statistically	NTP 2016
			Concentration	**# with mutation/#**		

Genotoxicity endpoint	Antimony form	Testing system	Assay endpoint		Comments	Reference
		Both non-tumor lung and spontaneous tumors from control mice.	(mg/m^3)	tissues assayed	significant.	
			0 (nontumor lung)	0/10		
			0 (tumor lung)	3/9		
			3 (tumor lung)	9/28		
			10 (tumor lung)	15/26		
			30 (tumor lung)	10/26		

Table E.2-3. Genotoxic DNA damaging effects of antimony compounds

Listing order of the studies are as follows:

I Assay, in the order of metaphase analysis, micronucleus assay, and sister chromatid exchange assay;

II Target system, in the order of studies in human cells, animal studies, *in vitro* studies, and biochemical studies;

III Compound, in the order of antimony(III) trioxide (bold), antimony(III) trichloride, and other antimony(III) compounds.

Genotoxicity endpoint	Antimony form	Assay name	Testing system	Assay endpoint[a]			Comments	References
DNA Damage (epidemiological studies)[b]								
DNA strand breaks, alkali-labile sites, oxidized purines	Occupational **antimony trioxide**	Alkaline FPG-modified comet assay	Blood lymphocytes from occupationally exposed workers (-S9)	**Frequency of subjects with oxidative DNA damage**			Sb_2O_3 levels for direct and indirect exposure groups lower than OSHA/NIOSH PEL and REL for workplace. Moderate oxidative DNA damage observed in direct exposure group (0.12 ± 0.11 µg/m3); potential concomitant exposures not addressed.	Cavallo et al. 2002
				Conc. (µg/m^3)		**# with oxidative damage/total**		
				0		3/23		
				0.12 ± 0.11		11/17		
				0.052 ± 0.038		1/6		
				Relative risk of DNA damage				
				Conc. (µg/m^3)	**Adjusted relative risk**	**95% CI**		
				0	1	n/a		
				0.12 ±	14.2**	2.7–73.4		

Genotoxicity endpoint	Antimony form	Assay name	Testing system	Assay endpoint[a]			Comments	References
				0.11				
				0.052 ± 0.038	1.7	0.1–22.5		
				Tail moment values for FPG-treated Cells				
				Conc. (µg/m^3)		**Mean ± SD**		
				0		24.4 ± 9.51		
				0.12 ± 0.11		32.4 ± 16.3		
				0.052 ± 0.038		28.8 ± 5.61		
				Tail moment values for untreated cells				
				Conc. (µg/m^3)		**Mean ± SD**		
				0		16.3 ± 6.59		
				0.12 ± 0.11		14.6 ± 8.29		
				0.052 ± 0.038		18.3 ± 8.78		
DNA strand breaks, alkali-labile sites, oxidized purines	Occupational **antimony trioxide**	AP sites quantified using ELISA technique	Blood lymphocytes from occupationally exposed workers (-S9)	The quantity of DNA damage (determined by the number of AP sites/1 × 10^5 bp) among the studied workers was significantly (p-0.004) higher compared to that recorded for the control group and a significant positive correlation was found between the quantity of DNA damage (in the form of increased AP sites) and urinary antimony level among workers (r = 0.873, p < 0.001); Total oxidative capacity (also measured by ELISA) was not different between workers and controls.			The number of measured abasic sites ranged from 17.22 (control group) to 26.88 (exposed workers)/1×10^5bp. This range is higher than expected.	El Shanawany *et al.* 2017
DNA damage (*in vitro* studies in human cells)								
DNA strand breaks, alkali-labile sites, DNA-protein	Antimony trichloride (concentra-tions tested:	Alkaline comet assay +/- proteinase K	Human whole blood or human lymphocytes exposed ex vivo	**Mean tail moment in human whole blood in comet assay without proteinase K**			Significance tested by Kruskal-Wallis one-way ANOVA on ranks.	Schaumloffel and Gebel 1998
				Conc. (µM)	**Time. (hrs)**	**Mean ± SD**		

Genotoxicity endpoint	Antimony form	Assay name	Testing system	Assay endpoint[a]			Comments	References
crosslinks	1–50 μM)		(-S9)	0	2.5	1.28 ± 0.10		
				1	2.5	1.26 ± 0.01		
				5	2.5	1.32 ± 0.08		
				10	2.5	1.32 ± 0.04		
				25	2.5	1.47 ± 0.07		
				50	2.5	1.75 ± 0.08*		
				Mean tail moment in human lymphocytes in comet assay without proteinase K				
				Conc. (μM)	**Time (hrs)**	**Mean ± SD**		
				0	2.5	1.00 ± 0.02		
				1	2.5	1.23 ± 0.28		
				5	2.5	1.39 ± 0.19*		
				10	2.5	1.56 ± 0.04*		
				25	2.5	1.64 ± 0.03***		
				50	2.5	2.14 ± 0.01***		
				Mean tail moment in human lymphocytes in comet assay with proteinase K				
				Conc. (μM)	**Time (hrs)**	**Mean ± SD**		
				0	2.5	1.08 ± 0.11		
				1	2.5	1.13 ± 0.09		
				5	2.5	1.30 ± 0.20		
				10	2.5	1.47 ± 0.13*		
				25	2.5	1.53 ± 0.08*		
				50	2.5	1.94 ± 0.30***		
DNA damage (animal studies)								
DNA strand breaks and	**Antimony trioxide**	*In vivo* exposure		**Percent tail DNA**			Trend tests show significant increase for	NTP 2016
				Dose	**Time**	**Mean ± SE**		

Genotoxicity endpoint	Antimony form	Assay name	Testing system	Assay endpoint[a]			Comments	References
alkali labile sites	NC: air	(inhalation) Alkaline comet assay		(mg/m^3)	(mo.)		both lung tissue of males and females exposed to trioxide; No increase in percent tail DNA observed in leukocytes of males or females exposed to trioxide. Normally distributed data analyzed by independent sample's t-test and linear regression; data that were not normally distributed were analyzed by the Mann-Whitney test followed by the Kendall rank correlation test	
				0	12	25.6 ± 0.78		
				3	12	33.7 ± 2.62*		
				10	12	33.5 ± 2.02**		
				30	12	37.5 ± 2.28***		
			Lung of female mice exposed via inhalation for 12 months	Percent tail DNA				
				Dose (mg/m^3)	Time (mo.)	Mean ± SE		
				0	12	32.8 ± 1.11		
				3	12	35.8 ± 2.09		
				10	12	36.4 ± 2.65		
				30	12	45.5 ± 2.32***		
DNA strand breaks and alkali labile sites	**Antimony trioxide** NC: air	*In vivo* exposure (inhalation) Alkaline comet assay	Lung and blood leukocytes of male and female rats exposed via inhalation for 12 months	No statistically significant increases were observed in percent tail DNA in blood leukocytes or lung tissue in exposed rats of either sex			Normally distributed data analyzed by independent sample's t-test; data that were not normally distributed were analyzed by the Mann-Whitney test followed by the Kendall rank correlation test	NTP 2016
DNA damage (*in vitro* studies in non-human mammalian cells)								
DNA strand breaks and alkali labile sites	Antimony trichloride	Alkaline comet assay	V79 Chinese hamster cells exposed *in vitro* (-S9)	Tail moment was significantly* elevated at a minimum dose of 1 µM Sb(III); no difference could be found comparing the results obtained in presence and absence of proteinase K.			DNA damage observed below cytotoxic levels; antimony uptake measured	Gebel et al. 1998
DNA damage (bacterial systems)								

Genotoxicity endpoint	Antimony form	Assay name	Testing system	Assay endpoint[a]		Comments	References
Growth in recombination-repair deficient bacterial strain	**Antimony trioxide** NC: Kanamycin (5, 10 20 µg/plate) PC: Mitomycin C (0.05, 0.1, and 0.2 µg/plate)	B. subtilis rec assay	B. subtilis M45(rec-) and H17(rec+)	**HI17 (Rec+) and M45 (Rec-) inhibition length**		Used spore plate method	Kuroda et al. 1991
				Conc. (µg/plate)	**Difference in Inhibition length (mm)**		
				NC (5)	0		
				NC (10)	0		
				NC (20)	0.5		
				PC (0.05)	8.0		
				PC (0.1)	8.0		
				PC (0.2)	7.0		
				0.3	2.5		
				0.6	4.0		
				1.1	4.5		
Growth in recombination-repair deficient bacterial strain	**Antimony trioxide** PC and NC: other metals tested	*B. subtilis* rec assay	*B. subtilis* M45(rec-) and H17(rec+) (-S9)	**HI17 (Rec+) and M45 (Rec-) inhibition length**		Examined 127 metals; Used streak plate method; Included cold incubation step to increase contact of metal with bacteria	Kanematsu *et al*. 1980
				Solution conc. (M)	**Difference in inhibition length (mm)**		
				0.05	5		
Growth in recombination-repair deficient bacterial strain	Antimony trichloride NC: Kanamycln (5, 10 20 µg/plate) PC: Mitomycin C (0.05, 0.1,	B. subtilis rec assay	B. subtilis M45(rec-) and H17(rec+) (-S9)	**HI17 (Rec+) and M45 (Rec-) inhibition length**		Used spore plate method	Kuroda et al., 1991
				Conc. (µg /plate)	**Difference in inhibition length (mm)**		
				NC (5)	0		
				NC (10)	0		
				NC (20)	0.5		
				PC (0.05)	8.0		
				PC (0.1)	8.0		
				PC (0.2)	7.0		

Genotoxicity endpoint	Antimony form	Assay name	Testing system	Assay endpoint[a]		Comments	References
	and 0.2 μg/plate)			6.3	1.5		
				12.5	4.5		
				23	4.5		
Growth in recombination-repair deficient bacterial strain	Antimony trichloride	*B. subtilis* rec assay	*B. subtilis* M45(rec-) and H17(rec+) (-S9)	Antimony trichloride result was negative in rec assay (tested at 0.05M)		Antimony pentachloride also negative	Nishioka *et al*. 1975
Growth in recombination-repair deficient bacterial strain	Antimony trichloride PC and NC: other metals tested	*B. subtilis* rec assay	*B. subtilis* M45(rec-) and H17(rec+) (-S9)	**HI17 (Rec+) and M45 (Rec-) inhibition length**		Examined 127 metals; Used streak plate method; Included cold incubation step to increase contact of metal with bacteria	Kanematsu et al. 1980
				Solution Conc. (M)	**Difference in inhibition length (mm)**		
				0.01	7		
Induction of recombination-repair genes	Antimony trichloride	SOS chromotest for genotoxicity	*E. coli* PQ37 derived from strain GC4436 (-S9)	SOS chromotest was negative for antimony trichloride (concentration tested: 11–707 μM)		Cytotoxicity observed at 354 μM	Lantzsch H and Gebel T, 1997
Induction of recombination-repair genes	Antimony trichloride	Umu test for genotoxicity	*S. typhimurium* TA1535/pSK1002 (-S9)	Umu test was negative for antimony trichloride (concentrations tested: 1.6–820 μM)		Data not reported	Yamamoto et al., 2002
DNA Damage (biochemical assay)							
plasmid DNA nicking	Trimethyl-stibine potassium antimony tartrate PC: Trimethyl-arsine	Plasmid DNA nicking assay	Plasmid pBR322 exposed *in vitro* (gaseous phase) to test reactions for 30 min.	**Estimated Quantity of Open Circular form of Plasmid[d]**		Chemical reactions to produce trimethylstibine were conducted in situ; Plus and minus designations were estimated from images only (no quantitation of nicked and supercoiled forms). Negative results were reported for potassium	Andrewes et al. 2004
				Dose (μM)		**Result**	
				Trimethyl-stibine	NC	+/-	
					5	+/-	
					20	+/-	
					50	+	
					200	++	
					500	+++	

Genotoxicity endpoint	Antimony form	Assay name	Testing system	Assay endpoint[a]			Comments	References
					5000	+++	antimony tartrate.	

ALL = Acute lymphoblastic leukemia; avg = average; CI = confidence interval; conc. = concentration; ELISA = enzyme-linked immunosorbent assay; FPG = formamidopyrimidine-DNA glycosylase; hr =hour(s); mo = month(s); NC = negative control; NR=not reported; PC = positive control; SD = standard deviation; SE = standard error; VC = vehicle control.i

[a]Levels of significance are designated as follows: *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[b]DNA damage estimated as quantity of open circular (vs supercoiled) forms from images of plasmids electrophoretically separated in ethidium bromide-stained agarose gels.

Table E.2-4. Genotoxicity of antimony compounds – chromosomal aberrations, micronucleus, and sister chromatic exchange[a, b, c]

Studies are listed hierarchically according to the following criteria:

1 Assay, in the order of assays for chromosomal aberrations, micronucleus, and sister chromatid exchange.

2 Target system, in the order of studies in human cells, animal studies, *in vitro* studies, biochemical studies.

3 Compound, in the order of antimony trioxide (bold), antimony trichloride, other antimony(III) compounds.

Substance	Exposure and assay name	Testing system and exposure duration	Assay endpoint			Comments	References
Chromosomal aberrations							
Antimony trioxide NC: dimethyl sulfoxide (10 µL/mL) PC: mitomycin C (0.2 µg/mL for-S9) or cyclo-phosphamide (50 µg/mL for +S9)	*In vitro* exposure Metaphase analysis	Human peripheral lymphocytes with 2 hr exposure to colcemid (-S9) Exposure time: 20 hr and 44 hr Dose: 10, 50, 100 µg/mL	**Mean % aberrant cells excluding gaps**			Precipitate formed at top dose level	Elliot *et al.* 1998
			Group	**HIC/LEC (µg/mL, unless specified)**	**Mean (%)**		
			NC	–	0.5–1.5		
			PC	–	22.0 -32.0**		
			Donor 1, 20 hr	100	2.0		
			Donor 2, 20 hr	100	12.5**		
			Donor 2. 44 hr	100	4.5*		
		Human peripheral lymphocytes with 2 hr exposure to colcemid (+S9) Dose: Same as above	NC	–	1.0–1.5		
			PC	–	26–34.0**		
			Donor 1, 20 hr	50	4.5*		
			Donor 2, 20 hr	100	9.5**		

Substance	Exposure and assay name	Testing system and exposure duration	Assay endpoint			Comments	References
			Donor 2. 44 hr	100	2.0		
Antimony sodium tartrate	*In vitro* exposure Metaphase analysis	Human leucoytes Exposure time: 48 hr Concentration: 2.3 nM	12% of cells with chromatid breaks ($P < 0.05$)			Purity of test compound not reported; toxicity (marked reduction in mitotic index) reported at 10 nM	Paton and Allison 1972
Antimony trioxide VC: HPMC/poly-sorbate PC: Cyclo-phosphamide	*In vivo* exposure *Ex vivo* metaphase analysis	Sprague-Dawley rat bone marrow cells (-S9) Exposure time: Once daily for 21 consecutive days by oral gavage (except PC administered on only on day 21) Dose: 250, 500, 1000 mg/kg	**Frequency of cells with chromosomal aberration excluding gaps in male rats**			Body-weight gain was reduced (<10%) in the top dose group of treated rats of both sexes over the 3-week dosing period.	Kirkland *et al*. 2007
			Group	**HIC/LEC (mg/kg)**	**Mean% ± SD**		
			VC	**20**	0 ± 0		
			PC	20	13 ± 6.63***		
			Male rat	1000	0 ± 0		
			Female rat	1000	0 ± 0		
Antimony trioxide NC: distilled water	*In vivo* exposure *Ex vivo* metaphase analysis	Male Swiss albino mice bone marrow cells (-S9) Exposure by daily oral gavage on days 7, 14 and 21. Dose: 400, 666.7, 1000 mg/kg	**Frequency of aberrations excluding gap**			Purity of test compound not reported; Test for trend significant for 7 and 14 days for analysis including and excluding gaps (not shown in this table). No increases in chromosomal aberrations was observed after single acute exposure at same doses and measured 6, 12, 18 and 24 hours); Highest dose was lethal.	Gurnani *et al*. 1992b
			LEC (mg/kg)	**Time (days)**	**Mean % ± SD**		
			NC	7	1.4 ± 1.140		
			400	7	2.2 ± 0.447*		
			NC	14	1.6 ± 0.547		
			400	14	3.2 ± 0.447*		
			NC	21	1.6 ± 0.547		
			400	21	4.6 ± 0.547*		
Antimony	*In vivo*	Female Swiss albino	**Frequency of aberrations including gap**			Source and purity of test	Gurnani *et al*.

Substance	Exposure and assay name	Testing system and exposure duration	Assay endpoint			Comments	References
trichloride NC: distilled water	exposure *Ex vivo* metaphase analysis	mice bone marrow cells (-S9) Dose: 70, 140, 233.3 mg/kg Single exposure by oral gavage analyzed at 6, 12, 18 and 24 hrs	**LEC (mg/kg)**	**Time (hrs)**	**Mean% ± SD**	compound not reported Test for trend significant for 6, 12, 18, and 24 hr analysis including and excluding gaps (not shown in this table).	1992a
			NC	6	1.6 ± 0.547		
			70	6	2.6 ± 0.547		
			NC	12	1.0 ± 1.0		
			70	12	3.0 ± 0.0		
			NC	18	1.6 ± 0.547		
			70	18	3.2 ± 0.836		
			NC	24	1.0 ± 0.0		
			70	24	4.2 ± 1.095		
Potassium antimony tartrate Control: untreated animals	*In vivo* exposure *Ex vivo* metaphase analysis	Male rats bone marrow (-S9) Exposure via single intraperitoneal injection at each dose; Also, tested repeated exposure (daily for 5 days) at each dose. Dose: 2.0, 8.4, 14.8 mg/kg	**Metaphases with aberrations excluding gap**			Similar findings for aberrations including gaps but statistical analysis not performed	El Nahas *et al*. 1982
			LEC (mg/kg, unless specified)	**Time after treatment (hr, unless specified)**	**%**		
			NC	n/a	0.7		
			2.0	6	2.0*		
			2.0	24	2.4*		
			8.4	48	5.2*		
			2.0 mg/kg/day x 5 days	-	7.6*		
Micronuclei							
Occupational **antimony trioxide**	Epidemiology study Sister chromatid exchange assay	Blood lymphocytes from textile workers exposed to low levels of antimony trioxide 23 exposed workers: 17 high exposure (0.12 ± 11 μ/m^3) and 6 lower exposure (0.052 ± 0.038 μ/m^3)	Mean micronuclei/1000 binucleated cells did not differ between controls and two exposure groups			High exposure well below OHSA permissible exposure levels and NIOSH recommended exposure levels Exposure groups had similar ages, and smoking habits	Cavallo *et al*. 2002

Substance	Exposure and assay name	Testing system and exposure duration	Assay endpoint			Comments	References
		23 controls					
Antimony trichloride NC: DMSO PC: mitomycin C (data not shown)	*In vitro* exposure Micronucleus test	Human peripheral lumphocytes (-S9) Doses: 0, 0.5, 2, 5, 25 µM	**Induction of micronuclei by Sb(III)**			Co-incubation with SOD or CAT had no effect on micronucleus frequency; Statistical significant in MN observed in second experiment at 5, 10 and 25µM	Schaumloffel N and Gebel T, 1998
			LEC (µM)	**Time (hrs)**	**MN/1000 BN, mean ± SD**		
			0	20	10 ± 1.4		
			5	20	30.5 ± 2.1		
Antimony trioxide NC: air	*In vivo* exposure *Ex vivo* micronucleus test	Male mice peripheral blood erythrocytes exposed via inhalation for 12 months. Dose: 0, 3, 10, 30 mg/m^3	No significant increase in micronucleated PCEs/1,000 PCEs in male mice			Twenty thousand CD71+ reticulocytes (PCE) were scored per animal for the presence of micronuclei and 1 ×10^6 erythrocytes (NCE) were counted for micronuclei. William's and Dunn's test were used for pairwise significance, and Jonckheere's test and linear regression used for trend significance. MN frequency in NCEs but not PCEs significant by trend test ($P < 0.001$) in both sexes.	NTP 2017a
			Micronucleated NCEs/1,000 NCEs				
			LEC (mg/m^3)	**Time (mo.)**	**Mean±SE**		
			30	12	1.93 ± 0.10***		
		Female mice peripheral blood erythrocytes exposed via inhalation for 12 months Dose: 0, 3, 10, 30 mg/m^3	No significant increase in micronucleated PCEs/1,000 PCEs in female mice				
			Micronucleated NCEs/1,000 NCEs				
			LEC (mg/m^3)	**Time (mo.)**	**Mean±SE**		
			30	12	1.38 ± 0.09***		
Antimony trioxide NC: air	*In vivo* exposure *Ex vivo* micronucleus test	Male rat peripheral blood erythrocytes exposed via inhalation for 12 months	No significant increase in micronucleated PCEs/1,000 PCEs or micronculeated NCEs/1000 NCEs in male rats.			Twenty thousand CD71+ reticulocytes (PCE) were scored per animal for the presence of micronuclei and 1 ×10^6 erythrocytes (NCE)	NTP 2017a
		Female rat peripheral blood erythrocytes	No significant increase in micronucleated PCEs/1,000 PCEs or micronculeated NCEs/1000				

Substance	Exposure and assay name	Testing system and exposure duration	Assay endpoint	Comments	References
		exposed via inhalation for 12 months	NCEs in female rats.	were counted for micronuclei. William's and Dunn's test were used for pairwise significance, and Jonckheere's test and linear regression used for trend significance. No significant changes were observed in MN frequency in rats of either sex.	
Antimony trichloride	*In vitro* exposure Micronucleus test	Human fibroblast cells (-S9) Human bronchial epithelial cells (BES-6) (-S9) Chinese hamster ovary cells (CHO-K1) (-S9) Exposure time: 4 hr, Dose: 50–400 μM	Positive findings for all cell types at all doses	LD_{50} = 40 μM in fibroblast cells LD_{50} = 80 μM in BES-6 cells LD_{50} = 180 μM in CHO-K1 cells	Huang *et al*. 1998
Antimony trioxide VC: DMSO PC: Cyclo-phosphamide (20 mg/kg)	*In vivo* exposure Micronucleus test	Mouse bone marrow (-S9) male and females Single dose study Exposure time: 24 and 48 hr Dose: 5000 mg/kg by oral gavage Repeated dose study: Exposure time: 8, 15 and 22 days Dose: 400, 667, or 1000 mg/kg by oral gavage	No increases in mean incidence of MPE/1000 PE in the single dose study (males and females) or in the repeated dose study (sex not identified).	Significantly decreased frequency of polychromatic erythrocytes observed in females at 24 hr in the single dose experiment.	Elliot *et al*. 1998
Antimony	*In vivo*	Sprague-Dawley male	No increase in the frequency of micronucleated		Kirkland *et al.*

Substance	Exposure and assay name	Testing system and exposure duration	Assay endpoint	Comments	References
trioxide VC: HPMC/poly-sorbate PC: Cyclo-phosphamide (20 mg/kg)	exposure Micronucleus test	and female rat bone marrow cells (-S9) Exposure time: 21 days (except for PCs) by oral gavage Dose: 250, 500, 1000 mg/kg	PCE in male and female rats		2007
Antimony trioxide VC: DMSO (25 µL) PC: Mitomycin C (0.5 µM)	*In vitro* Micronucleus test with cytokinesis block	Chinese hamster V79 cells Exposure time: 24 hr Dose: 2–50 µM	**Mean number of micronuclei** **Group** / **LEC (µM)** / **Mean** VC / – / 9.5 PC / – / 45.5 Antimony trioxide / 25 / 17.5	Study measured both antimony uptake in cells and cytotoxicity (50% neutral red uptake was found with $SbCl_3$ at 83 µM)	Gebel T *et al.,* 1998
Sister chromatid exchange					
Occupational **antimony trioxide**	Epidemiology study Sister chromatid exchange assay	Peripheral blood lymphocytes from textile workers exposed to low levels of antimony trioxide 23 exposed workers: 17 high exposure (0.12 ± 11 µ/m^3) and 6 lower exposure (0.052 ± 0.038 µ/m^3) 23 controls	Mean SCE did not differ between controls and two exposure groups	High exposure well below OHSA permissible exposure levels and NIOSH recommended exposure levels Exposure groups had similar ages, and smoking habits	Cavallo *et al*. 2002
Antimony trioxide	*In vitro* exposure	Human peripheral blood lymphocytes	**SCE/cell** **LEC (µM)** / **Mean ± SD**	NC was DMSO, and it is unclear whether the 0	Gebel *et al*., 1997

Substance	Exposure and assay name	Testing system and exposure duration	Assay endpoint		Comments	References
(dissolved in distilled water) NC: DMSO	Sister chromatid exchange assay	from healthy non-smokers aged 25-Human 35 years (-S9) Exposure time: 24 hrs	0	8.6 ± 3.4	uM result was from distilled water or DMSO. No PC was stated in the study. Results are from 60 metaphase scored on two slides.	
			0.5	11.5 ± 4.4*		
Antimony trichloride (dissolved in DMSO) NC: DMSO	*In vitro* exposure Sister chromatid exchange assay	Human peripheral blood lymphocytes from healthy non-smokers aged 25–35 years (-S9) Exposure time: 24 hr	**SCE/cell**		No PC was stated in the study. Results are from 60 metaphases scored on two slides.NC was DMSO, and it is unclear whether the 0 uM result was from distilled water or DMSO.	Gebel *et al*., 1997
			LEC (µM)	**Mean ± SD**		
			0	8.8 ± 4.0		
			1	13.8 ± 5.5**		
Antimony trioxide NC: Water (100 µL) PC: Mytomycin C (0.01 µg/mL)	*In vitro* exposure Sister chromatid exchange assay	Chinese hamster V79 cells Exposure time: 28 hr Dose: 0.09–0.34 µg/mL	**Frequency of sister chromatid exchanges/metaphase**		$Sb^{V}_2O_5$ was negative in the SCE assay; Similar results in experiment 1, although LEC was 0.17 µg/mL	Kuroda *et al*., 1991
			LEC (µg/mL)	**Time (hrs)**	**Mean ± SD**	
			NC	28	6.3 ± 2.5	
			PC	28	56.0 ± 9.3**	
			0.09	28	10.6 ± 3.7**	
Antimony trichloride NC: Water (100 µL) PC: Mytomycin C (0.01 µg/mL)	*In vitro* exposure Sister chromatid exchange assay	Chinese hamster V79 cells Exposure time: 28 hr Dose: 1.3–20 µg/mL	**Frequency of sister chromatid exchanges/metaphase**		$Sb^{V}Cl_5$ was negative in the SCE assay. Toxic at 20µg/mL; Similar results in experiment 2, although LEC was 5 µg/mL.	Kuroda *et al*., 1991
			Conc. (ug/mL)	**Time (hrs)**	**Mean ± SD**	
			NC	28	4.5 ± 2.2	
			PC	28	46.8 ± 8.6**	
			2.5	28	7.5 ± 4.3*	

[a]Provided are the form of the test compound, study details including the testing system and exposure duration, assay endpoint results for test compounds and positive and negative controls, comments provided by reviewers, and reference.
[b]Abbreviations used in this table are as follows:
b.w. = body weight
HIC=Highest ineffective concentration
LEC=Lowest effective concentration
NC=Negative control
PC=Positive Control
VC=Vehicle Control
hr(s)=Hour(s)
mo=Months
NR=not reported
CMC-Na= sodium carboxymethylcellulose
FISH= fluorescence in situ hybridization
[c]Levels of significance are designated as follows:
*$p<0.05$
**$p<0.01$
***$p<0.001$

E.3: Effects of antioxidants and inhibitors of oxidative stress related enzymes on cells exposed to compounds containing trivalent antimony

Table E.3-1. Effects of antioxidants and inhibitors of oxidative stress related enzymes on cells exposed to compounds containing trivalent antimony

Cell types	Additional treatment (besides antimony exposure)	Oxidative stress and damage	MMP and cell death	Comparison group (cells)	Reference
Antimony (III) trioxide					
LOUCY, CCRF-CEM, HL-60, K-562	BSO, an inhibitor of γ-glutamylcysteine synthetase	↓ GSH	↓ MMP[a] ↑ cell death	exposed to Sb_2O_3 alone	Lösler *et al.* 2009
HL-60, K-562	MS, an inhibitor of glutathione peroxidase		↑ cell death	exposed to Sb_2O_3 alone	Lösler *et al.* 2009
K-562	AT, an inhibitor of catalase		↑ cell death	exposed to Sb_2O_3 alone	Lösler *et al.* 2009
CCRF-CEM, K-562	NaAsc, an antioxidant, but able to act as an oxidant under oxidative stress		↑ cell death	exposed to Sb_2O_3 alone	Lösler *et al.* 2009
NB4	None	↑ ROS	↑ cell death	negative control	Mann et al. 2006
NB4-M-AsR3[a]	None	↑ GSH	↓ cell death	parental NB4 cells	Mann et al. 2006
NB4	BSO, an inhibitor of γ-glutamylcysteine synthetase	↓ GSH ↑ ROS	↑ cell death	cells not treated with BSO	Mann et al. 2006
NB4-M-AsR3[b]	BSO, an inhibitor of γ-glutamylcysteine synthetase	↓ GSH	↑ cell death	cells not treated with BSO	Mann et al. 2006
Antimony (III) trichloride					
Primary rat hepatocytes	none	↑ ROS ↑ lipid peroxidation	↓ MMP ↑ cell death		Hashemzaei et al. 2015
Primary rat hepatocytes	nBP, a GSH-depleting agent	↓ GSH ↑ ROS ↑ lipid peroxidation	↓ MMP ↑ cell death	exposed to $SbCl_3$ alone	Hashemzaei et al. 2015
Primary rat hepatocytes	Dimethyl sulfoxide, a ROS scavenger	↓ ROS ↓ lipid peroxidation	↑ MMP ↑cell death	exposed to $SbCl_3$ alone	Hashemzaei et al. 2015
Primary rat hepatocytes	Mannitol, a ROS scavenger	↓ ROS ↓ lipid peroxidation	↑ MMP ↓cell death	exposed to $SbCl_3$ alone	Hashemzaei et al. 2015
Primary rat hepatocytes	Trifluoperazine, a mitochondria permeability transition pore sealing agent	↓ ROS ↓ lipid peroxidation	↑ MMP ↑cell death	exposed to $SbCl_3$ alone	Hashemzaei et al. 2015
Primary rat	Carnitine, a	↓ ROS	↑ MMP	exposed to	Hashemzaei

Cell types	Additional treatment (besides antimony exposure)	Oxidative stress and damage	MMP and cell death	Comparison group (cells)	Reference
hepatocytes	mitochondria permeability transition pore sealing agent	↓ lipid peroxidation	↓cell death	$SbCl_3$ alone	et al. 2015
Primary rat hepatocytes	L-Glutamine, an adenosine triphosphate (ATP) generating agent	↓ ROS ↓ lipid peroxidation	↑ MMP ↑cell death	exposed to $SbCl_3$ alone	Hashemzaei et al. 2015
Antimony (III) potassium tartrate					
HL-60	none	↑ ROS	↓ MMP ↑ cell death	negative control	Lecureur et al. 2002
HL-60	BSO		↑ cell death	exposed to antimony alone	Lecureur et al. 2002
HL-60	NAC		↓ cell death	exposed to antimony alone	Lecureur et al. 2002

↑ = Increased.
↓ = Decreased.
[a] Only tested in HL-60 cells.
[b] Arsenic resistant subclone of parental NB4 due to increased GSH levels.
AT = 3-amino-1,2,4-azole.
BSO = DL-buthionine-[*S,R*]-sulfoximine.
CCRF-CEM = Acute Lymphoblastic Leukemia cells.
HL-60 = Acute Promyelocytic Leukemia cells.
K-562 = chronic myelogenous leukemia cells.
LOUCY = T cell Acute Lymphoblastic Leukemia cells.
MMP = mitochondrial membrane potential.
MS = mercaptosuccinic acid.
NaAsc = sodium ascorbate.
NB4 = Acute Promyelocytic Leukemia cells.
NB4-M-AsR3 cells = Arsenic resistant APL cells derived in Miller lab (ref).
nBP = *n*-bromoheptane.
NAC = N-acetylcysteine.

E.4: Immune effects from compounds containing pentavalent antimony

This appendix lists immune function from compounds containing pentavalent antimony (Table E.4-1).

Table E.4-1. Effects of compounds containing pentavalent antimony on immunity

Patients, species or experimental system	Antimony compound	Immune effects	Functional or mechanistic association	Reference
Human studies				
Healthy active duty soldiers treated for leishmaniasis	Sodium stibogluconate	Transient lymphopenia (decreased $CD4^+$ and $CD8^+$ T cells)	Increased susceptibility to Herpes Zoster infections	Wortmann *et al.* 1998
Patients treated for cutaneous leishmaniasis	Glucantime (meglumine antimoniate)	Elevated IL-1β, TNF-α, IL-6 and IL-8	Amplified pro-inflammatory cytokines upon exposure to antimonials	Kocyigit *et al.* 2002
Patients treated for visceral leishmaniasis	Sodium stibogluconate	Elevated IL-1β, TNF-α, IL-6, GM-CSF, and C1q-binding circulating immune complexes (CIC)	Amplified pro-inflammatory cytokines and CIC-induced GM-CSF upon exposure to antimonials	Elshafie *et al.* 2007
Animal studies				
BALB/c mice	Antimony sodium gluconate	Activation of peritoneal macrophages associated with enhanced antigen presentation to T cells	Increased macrophage membrane fluidity and enhanced antigen presentation capacity	Ghosh *et al.* 2013
Normal C57BL/6 mice, IFNγ gene knockout mice, inducible nitric oxide synthase-knockout (iNOS KO) mice, and respiratory burst-deficient $gp91^{phox-/-}$ (X-linked chronic granulomatous disease [X-CGD]) mice	Sodium stibogluconate	In IFNγ gene knockout mice, pentavalent antimony inhibited but did not kill intracellular *Leishmania donovani;* treatment was effective in killing the parasite in normal, iNOS KO, and X-CGD mice.	Results support a role for T cell-derived IFNγ as a critical host factor required for the efficacy of antimony in promoting parasite killing	Murray and Delph-Etienne 2000
BALB/c mice	Antimony sodium gluconate	Sodium stibogluconate synergizes with IL-2 to promote IFNγ-dependent anti-Renca tumor immune response	Supports a role for pentavalent antimony in promoting IFNγ-dependent anti-tumor immune response	Fan *et al.* 2009
***In vitro* studies**				
Murine Baf 3 cell line and TF-1 human myeloid leukemia cells	Sodium stibogluconate	Sodium stibogluconate is a potent inhibitor of protein tyrosine phosphatases including Src homology PTPase1 (SHP-1), SHP-2, and PTP1B	Sodium stibogluconate, which contains a pentavalent antimony atom, (but not antimony(III) potassium tartrate) can alter	Pathak and Yi 2001

Patients, species or experimental system	Antimony compound	Immune effects	Functional or mechanistic association	Reference
			signaling of multiple cytokines (IL-3, IFNα, and GM-CSF) that signal through receptor tyrosine kinases regulated by PTPases	
Various cancer cell lines	Sodium stibogluconate	Sodium stibogluconate enhanced IFNα-induced Stat1 tyrosine phosphorylation, inactivated intracellular SHP-1 and SHP-2, and induced cellular protein tyrosine phosphorylation in cancer cell lines	Sodium stibogluconate treatment was found to synergize with IFNα to overcome cancer cell lines that were refractory to the anti-cancer effects of IFNα *in vitro* and *in vivo*	Yi *et al.* 2002
Human CD4+ and CD8+ T lymphocytes from healthy donors and melanoma patients	Sodium stibogluconate	Sodium stibogluconate synergizes with IL-2 to potentiate induction of IFNγ + T cells	Sodium stibogluconate treatment may potentiate T cell function in the presence of IL-2	Fan *et al.* 2009

E.5 Top ten canonical pathways affected by 6-hours exposure to 20 μM antimony(III) potassium tartrate trihydrate

Table E.5-1. Top ten canonical pathways affected by 6-hour exposure to 20 μM antimony(III) potassium tartrate trihydrate

Order	Ingenuity Canonical Pathways	-log(p-value)	Ratio	Molecules
1	Agranulocyte Adhesion and Diapedesis	4.29	0.358	CLDN7,CCL8,SELL,CLDN8,PODXL2,MYH3,CXCR4,IL1RN,CLDN14,C5AR1,MYH11,CXCL1,MYH7,MMP11,MADCAM1,MYL6B,CDH5,CXCL8,IL18,XCL2,CXCR2,SELPLG,IL1A,IL36RN,ITGA4,ACTA1,CXCL3,CD34,CXCL14,MMP1,MMP25,ITGA3,MMP12,ITGB7,CXCL5,CCL4,MMP28,ICAM2,CLDN17,CCL7,CLDN18,CCL1,MMP17,CCL21,CXCL2,MMP7,CCL22,MMP19,CXCL13,MMP20,MMP10,MYH8,MMP14
2	Granulocyte Adhesion and Diapedesis	3.79	0.35	CLDN7,CCL8,SELL,CLDN8,CXCR4,IL1RN,CLDN14,C5AR1,CXCL1,MMP11,TNFRSF1B,FPR2,CDH5,CXCL8,HSPB1,IL18,XCL2,CXCR2,SELPLG,IL1A,IL36RN,ITGA4,CXCL3,CXCL14,MMP1,HRH2,MMP25,ITGAM,ITGA3,MMP12,FPR1,HRH4,CXCL5,CCL4,MMP28,ICAM2,CLDN17,CCL7,CLDN18,CCL1,MMP17,CCL21,CXCL2,MMP7,CCL22,MMP19,CXCL13,MMP20,MMP10,MMP14
3	Eicosanoid Signaling	3.63	0.449	DPEP3,ALOX12,FPR2,PTGER1,LTB4R2,PLA2G7,PLA2G6,DPEP1,PLA2G3,PLA2G5,PTGER2,PTGIS,PTGFR,PLA2G4C,TBXA2R,PLA2G2E,ALOX12B,PTGES,PTGIR,PTGER3,ALOX15,TBXAS1
4	Role of Cytokines in Mediating Communication between Immune Cells	3.28	0.444	IFNA10,IL3,CSF2,IFNG,IL4,IFNA7,IFNA14,CXCL8,IL26,IL18,IL1RN,IL25,IFNA1/IFNA13,IL24,IL1A,IL36RN,IL17A,IFNA16,IFNB1,IFNA4
5	Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	2.91	0.447	IFNA10,IFNG,CCR1,IFNA7,IFNA14,CXCL8,CCR5,IL18,IL1RN,IFNA1/IFNA13,IL1A,IL36RN,IL17A,CCL4,IFNA16,IFNB1,IFNA4
6	Bladder Cancer Signaling	2.33	0.351	FGF5,MMP25,FGF1,MMP12,E2F1,THBS1,FGF20,SUV39H1,CDKN1A,MMP28,FGF12,MMP11,FGF21,FGF7,FGF3,FGF2,MMP17,CXCL8,MMP7,MMP19,FGF16,MMP20,MMP10,FGF8,MMP1,FGF9,MMP14
7	Crosstalk between Dendritic Cells and Natural Killer Cells	2.24	0.346	CAMK2B,CCR7,IL3,CSF2,IFNG,TREM2,HLA-F,FSCN2,TLR4,CD209,FSCN1,FSCN3,KIR2DL2,TNFRSF1B,PRF1,IL4,LTA,NECTIN2,CD69,IL3RA,KLRD1,IL18,CD40LG,CD28,IFNA1/IFNA13,ACTA1,IFNB1
8	Role of IL-	2.2	0.583	S100A9,CXCL1,IL17A,DEFB4A/DEFB4B,CXCL3,CXCL5,CX

Order	Ingenuity Canonical Pathways	-log(p-value)	Ratio	Molecules
	17A in Psoriasis			CL8
9	Role of Wnt/GSK-3β Signaling in the Pathogenesis of Influenza	2.1	0.362	IFNA10,IFNG,WNT5A,LEF1,FZD2,WNT5B,IFNA7,IFNA14,FZD7,DVL1,FZD9,WNT2B,WNT11,WNT8B,IFNA1/IFNA13,WNT7A,IFNA16,IFNB1,APC2,IFNA4,WNT10B
10	Oxidized GTP and dGTP Detoxification	1.99	1	RUVBL2,NUDT1,DDX6

Pathways 1, 2, 4, 5, 7, 8, and 9 (light green background) are related to immune reactions. Pathway 6 (with peach background) is related to cancer. Pathway 10 (with yellow background) is related to oxidative stress.

E.6. Top 10 upstream regulators of antimony

Table E.6-1. Top 10 upstream regulators for antimony

	Upstream Regulator	Expr Fold Change	Molecule Type	Predicted Activation State	Activation z-score	Flags	p-value of overlap	Target molecules in dataset	Mechanistic Network
1	**Vegf**		group	Activated	9.487	bias	1.88E-09	ANGPT2,ANGPTL4,AQP4,ATF3,AURKA,AURKB,BCL2A1,BIRC5,BNC1,BTN1A1,CA2,CALB1,CALCRL,CCL7,CCNF,CD3EAP,CDC14A,CDC20,CDC25A,CDC25B,CDC25C,CDC45,CDH5,CDK1,CDKN2C,CDKN3,CELSR1,CHI3L1,CHIA,CHRNB2,CHST7,CKS1B,CLCF1,CNN1,CNTFR,CPA3,CRLF1,CRYAB,CSF2,CXCL1,CXCL8,CXCR2,CXCR4,CYR61,DBF4,DPF3,DRD3,DTYMK,DUSP4,DUSP5,EDN1,EGR1,EGR3,EMCN,EMP2,ESM1,FABP4,FAIM2,FANCG,FGF16,FGF2,FLNA,FOSB,FOSL1,GATA1,GEM,GH1,GPR4,GPRC5B,HBE1,HBEGF,HDC,HMOX1,HOXB8,HPSE,HTR7,IL18,IL1A,IL3RA,IL4,ITGB3BP,JAM2,JUN,KIF15,KIF22,KIF2C,KITLG,LEF1,LPAR1,LRAT,LYVE1,MCM2,MCM5,MID1,MKI67,MMP10,MMP14,MT1G,MYCN,NDC80,NEK2,NFATC1,NGB,NR4A2,NR4A3,NRCAM,NRG1,PLK1,PLXNA2,PMAIP1,PRC1,PRKCB,PSMC3IP,PTH,RGS2,RGS20,SOCS2,SOCS3,ST8SIA4,STK10,TAAR5,TACR1,TACSTD2,TBXA2R,THBD,TNC,TNFRSF9,TNFSF15,TPX2,TRAF5,TRAIP,TRPC4,TTK,UBE2C,XCR1	
2	**CSF2**	8.025	cytokine	Activated	8.308	bias	1.85E-08	ADA,ADAM8,ADGRE5,ANXA1,AURKA,BCL2A1,BIRC5,C5AR1,CCL4,CCNF,CCR1,CCR5,CCR7,CD1C,CD209,CD28,CD33,CD40LG,CD69,CD8A,CDC20,CDK1,CDKN1A,CDKN2B,CDKN2C,CENPE,CHAF1A,CHAF1B,CKS1B,CLCF1,COL8A1,CSF1,CSF2,CTLA4,CXCL1,CXCL2,CXCL8,CXCR4,CYBB,EDN1,EGR1,EGR2,EGR3,EPOR,EXO1,FANCA,FCGR2B,FOLR2,FOS,FOSL1,FPR2,GATA1,GCLM,GDF15,HBEGF,HDC,HLA-DQB1,HRH4,HRK,HSPH1,IER3,IFNG,IGF1,IL1A,IL1RN,IL24,IL3RA,IL4,ITGA4,ITGAM,LEP,MCM5,MKI67,MMP1,MMP14,MRC1,NEK2,NFATC1,NFE2,NFKBIA,NR4A2,NUSAP1,OSM,PDE1B,PIM1,PLK1,POLD1,POLE,PPP1R15A,PRC1,PTGER2,RARA,RECQL4,RELB,RRM2,SERPINB9,SLC1A5,SOCS2,SOCS3,SPAG5,SPI1,STMN1,THBS1,TLR2,TLR4,TNFAIP3,TNFRSF1B,TNFRSF9,TNFSF14,TNFSF15,TNFSF8,TPM4,TPX2,UBE2C,ZFP36	352 (5)

	Upstream Regulator	Expr Fold Change	Molecule Type	Predicted Activation State	Activation z-score	Flags	p-value of overlap	Target molecules in dataset	Mechanistic Network
3	**TREM1**	1.62	transmembrane receptor	Activated	4.945	bias	0.000000203	ATF3,CASP5,CCL7,CCR7,CCRL2,CDK1,CDKN2B,CEBPB,CKS2,CSF1,CSF2,CXCL1,CXCL2,CXCL3,CXCL5,CXCL8,CXCR4,DCSTAMP,DEFB4A/DEFB4B,DUSP4,EDN1,EGR1,EGR2,EGR3,FOSL1,GADD45B,GCLM,GEM,GIPR,GLA,HAS1,HBEGF,IFNG,IL17A,IL36RN,IL4,LPL,MAD1L1,MAFF,MMP1,MMP10,MMP19,NFKBIA,NOD2,NR4A2,OSGIN1,RGS1,RRAD,SLC1A3,SNAPC1,TCEAL9,THBD,THBS1,TLR2,TLR4,TNFSF14,TNFSF15,WNT5A	
4	**GATA2**	2.854	transcription regulator		1.922		0.000000237	ADGRE5,ANGPT2,ANGPTL4,ARPP21,C9,CCL21,CCR8,CD177,CD34,CD36,CD69,CD96,CDH5,CDK6,CDKN1A,CEL,CELSR3,CHGA,CHI3L1,CLDN18,CMA1,CPA1,CPA3,CST7,CYBB,CYP2F1,CYP4F11,DDX4,DLK1,E2F2,EDN1,ELANE,EMCN,EPHA3,FABP4,FCN1,GABRP,GATA1,GATA2,GP5,GP9,GPR65,GUCA2A,HBQ1,HDC,HOXA10,HSD17B1,ICAM2,IKZF1,IL3RA,IL4,IL4R,ITGAM,KLF2,KLK3,LYL1,MAFB,MEP1A,MMRN1,MPIG6B,NFE2,PAX3,PDE9A,PLK2,PRG3,RAG1,REG1A,S100A5,S100A9,S100G,SERPINB10,SLC4A1,SLC9A5,SOX18,SPI1,SSTR2,TAC3,TACSTD2,TAL1,THBS1,TUBA8,UBASH3A	
5	**calcitriol**		chemical drug		0.412		0.000000494	ADAM19,ALPI,ANGPT2,ANKRD2,ATP5D,BIRC5,CA2,CALB1,CALCB,CASR,CCNA1,CCR8,CDC20,CDC45,CDK1,CDK5R1,CDKN1A,CEBPB,CELSR3,CHAF1A,CHAF1B,CHGA,CKM,COL4A1,CSF1,CSF2,CXCL2,CXCL3,CXCL8,CYP24A1,CYP2C9,CYP3A4,CYP46A1,CYR61,DCSTAMP,DEFB4A/DEFB4B,DUSP1,DUSP10,EDN1,EGR1,ETFB,EXO1,FABP4,FAM107A,FCER2,FOS,GADD45A,GADD45G,GEM,HBEGF,HSPB7,IER3,IFITM1,IFNG,IGF1,IGFBP5,IL10RA,IL17A,IL18,IL1A,IL1RN,IL4,INCENP,INS,ITGA4,ITGAM,ITGB7,JUN,KIF20A,KIF22,KL,KLK13,KLK5,LEP,LIG1,LPAR1,LPL,LTBP1,MAOA,MCM2,MCM5,MMP1,MRC1,MYH8,NEK2,NFATC1,NKX2-1,NME4,NPHS1,NTHL1,NUPR1,NUSAP1,PDE9A,POLE,POU1F1,PRC1,PRKCB,PRKCD,PTGER2,PTGFR,PTH,RAB38,RAD51AP1,RARRES1,RBPMS,REL,RRM2,RUNX1T1,S100A9,S100G,SERPINB7,SERPINB9,SLC2A4,SLC7A7,SNPH,SOCS3,SPAG5,STMN1,SUV39H1,TACC3,TERT,THBD,THBS1,THRA,TK1,TLR2,TLR4,TNFAIP3,TPX2,TSPO,WNT11	140 (2)
6	**ID2**	1.706	transcript		-		0.0000005	AICDA,ASCL2,BATF,CCR10,CCR7,CCR8,CD40LG,CDC25B,CDK1,CD	

	Upstream Regulator	Expr Fold Change	Molecule Type	Predicted Activation State	Activation z-score	Flags	p-value of overlap	Target molecules in dataset	Mechanistic Network
			ion regulator		1.136		14	KN1A,CDKN2C,CEBPB,CSF1,CXCR4,CXCR5,DUSP1,DUSP10,DUSP4,E2F2,EGR2,EGR4,FLT3LG,FOXO3,FSHB,GADD45B,GADD45G,IFNG,IL10RA,IL4,IL4R,IL9R,IRF8,KLF6,LTA,MAP3K14,MPZ,NFAT5,NFATC1,NR4A3,PDCD1,PTPN13,PTPN14,PTPN22,RAPGEF4,REL,RPS6KA2,SELL,SEMA3F,SH2D1A,SOCS3,SOX4,SOX5,TNFRSF25,TNFSF14,TNFSF8,TRAF1,TRAF5	
7	**phorbol myristate acetate**		chemical drug	Activated	7.684	bias	0.000000604	ADAM28,ADAM8,ADM,ADRB3,AGER,ALOX12,ANGPT2,ANGPTL4,ANXA1,AQP4,ATP2A3,AURKA,AURKB,BCL2A1,BDNF,BIRC5,BLM,BTG2,C5AR1,CA2,CA8,CAV1,CCL1,CCL4,CCNA1,CCR7,CD209,CD28,CD36,CD40LG,CD69,CDK1,CDK5R1,CDK5R2,CDKN1A,CDKN2B,CGA,CHGA,CKM,CLCF1,CRH,CRHR1,CSF1,CSF2,CTLA4,CXCL13,CXCL2,CXCL3,CXCL8,CXCR2,CXCR4,CYBB,CYP24A1,CYP2A6 (includes others),CYR61,DEFB4A/DEFB4B,DSG1,DUSP1,DUSP2,DUSP5,E2F1,E2F3,EGR1,EGR2,EGR3,EGR4,EIF4EBP1,ELANE,EN1,EP300,EPOR,ERBB4,FGF2,FGF7,FOS,FOSB,FOSL1,FSHB,FUT9,GABRP,GAP43,GATA1,GATA2,GDF15,GEM,GML,GNRH1,GRIN2A,H1FX,HAS1,HBEGF,HDC,HMGA1,HPSE,HSD11B1,HSD17B1,HSD3B1,HTR2A,HTR7,IFNG,IGF1,IGFBP2,IGFBP5,IL12RB1,IL17A,IL18,IL1A,IL1RN,IL20RA,IL24,IL4,ITGAM,ITM2A,JUN,JUNB,JUND,KCNJ10,KIF2C,KLF2,KLF6,KLK3,KRT35,LAMB3,LOR,LPL,LTA,LYVE1,MAD1L1,MMP1,MMP11,MMP12,MMP14,MMP19,MMP7,MPZ,MRC1,MSR1,MST1R,MT2A,MUC4,MYH7,MYOZ2,NCR1,NFAT5,NFATC1,NFKBIA,NFKBIE,NKX2-1,NOCT,NR4A2,NTS,OLR1,OSM,OSR2,PAK2,PDCD1,PDE1C,PDPN,PIM1,PLIN3,PODXL2,PON1,POU1F1,PPP1R15A,PRKCB,PRKCD,PRKD1,PTGER2,PTGES,PTGFR,PTPRE,PTPRN,PTPRO,RAE1,RARA,RARB,RASGRP1,RECQL4,REL,RELB,RGS1,RGS2,RUVBL2,S100A9,SELL,SELPLG,SERPINB10,SERPINB7,SERPINB9,SLC22A1,SLC6A2,SLC6A7,SLC7A11,SNAI1,SNAP25,SOCS3,SP4,SPHK1,SRC,SRD5A2,SSTR2,STATH,TACR1,TBXAS1,TEAD4,TERT,TH,THBS1,TIE1,TK1,TLR2,TLR4,TLR6,TMOD2,TNFAIP3,TNFRSF1B,TNFSF14,TRAF1,TRPC6,ULBP2,USF2,VIP,WT1,XCR1,ZFP36	276 (3)
8	**HDAC1**	0.743	transcription		-0.945		0.000000942	ADIPOQ,AMPD3,ANGPT2,ASCL2,ATF3,BDNF,CCNA1,CCNB2,CCR8,CD27,CD34,CDC25A,CDC25C,CDK1,CDKN1A,COL1A2,COL9A1,CXC	414 (12)

	Upstream Regulator	Expr Fold Change	Molecule Type	Predicted Activation State	Activation z-score	Flags	p-value of overlap	Target molecules in dataset	Mechanistic Network
			regulator					L8,E2F2,EGR1,EHMT2,FABP4,FAM107A,FOS,H2AFX,HBE1,HBG2,IFNB1,IL17A,IL24,IL4,INA,ITGB4,KLK3,LIG1,MAD1L1,MCM5,MPZ,MT1G,MUC4,MYH7,NEFH,NFATC1,NFKBIA,NKX2-5,PAX3,PLK1,PMAIP1,POLL,PPP2R2B,PRIM2,PTH,RAD54L,RAG1,RECQL4,RELB,RGS10,RRM2,RUNX2,S100A9,SATB1,SNAI1,SOX10,TAGLN,TAL1,TBX1,TBX2,TERT,TUBB3,TYMS	
9	**PTGER2**	2.853	g-protein coupled receptor	Activated	5.127	bias	0.00000162	AURKA,CCNB2,CCR7,CDKN3,CENPE,CFP,CKS2,CXCL8,CXCR2,CXCR4,EGR1,FPR1,H2AFX,HAMP,HDC,HIST1H2AB,IFNG,IL17A,IL1A,KIF15,KIF20A,KIF22,KIF2C,KLRD1,MKI67,NEK2,NUSAP1,PIM1,PLK1,PRC1,PTGER3,PTGES,SPAG5,THBS1,TPX2,TROAP,TTK	
10	**TNF**	1.621	cytokine	Activated	8.752	bias	0.00000184	ACTA1,ADAM8,ADAMTS5,ADIPOQ,ADM,ADRB1,ADRB3,AEBP1,AGER,AICDA,AMPD3,ANGPT2,ANGPTL4,ANXA1,ARHGDIB,ATF3,AURKC,BCL2A1,BDKRB1,BDKRB2,BDNF,BIK,BIRC5,BTG2,BTG3,C5AR1,CA2,CABP1,CAV1,CCK,CCL1,CCL22,CCL4,CCL7,CCR1,CCR5,CCR7,CCR8,CD1C,CD209,CD247,CD28,CD36,CD3E,CD40LG,CD5,CD69,CD82,CDC25C,CDH13,CDH5,CDK5R1,CDKN1A,CDKN2C,CDX1,CEBPB,CEBPG,CHI3L1,CHRNA4,CHRNB2,CHRNE,CHRNG,CHST4,CHST7,CIB2,CKM,CLCF1,CLDN7,CNN1,COL15A1,COL16A1,COL1A2,COLQ,COTL1,CPA3,CRH,CRHR1,CRLF1,CRYAB,CSF1,CSF2,CSN2,CST7,CTLA4,CTSF,CX3CR1,CXCL1,CXCL13,CXCL2,CXCL3,CXCL5,CXCL8,CXCR2,CXCR4,CXCR5,CYBB,CYP26B1,CYP2C8,CYR61,CYTH3,DCSTAMP,DEFB4A/DEFB4B,DPF3,DUSP1,DUSP10,DUSP2,DUSP4,DUSP5,DVL1,E2F1,EDN1,EGR1,EGR2,EGR3,ELF3,EMCN,EMP2,ENG,ENPP3,EREG,ESM1,FABP4,FAT2,FCAR,FCER2,FCGR2B,FGF2,FGF5,FOS,FOSB,FOSL1,FOXF1,FOXF2,FPR1,FPR2,FSCN1,G0S2,GABRA1,GADD45A,GADD45B,GADD45G,GATA2,GCLM,GDF15,GEM,GNA15,GNL1,GPR176,GPRC5B,GRIA1,HAS1,HBEGF,HDC,HIVEP1,HLA-F,HMOX1,HOXB8,HRK,HSD11B1,HSPA1A/HSPA1B,HSPG2,ICAM2,IER2,IER3,IFI27,IFITM1,IFNA1/IFNA13,IFNB1,IFNG,IGF1,IGFBP2,IGFBP5,IL10RA,IL17A,IL18,IL18R1,IL1A,IL1RN,IL24,IL3,IL36RN,IL3RA,IL4,IL4R,INS,IRF8,ITGA4,ITGAM,ITGB7,JUN,JUNB,JUND,KIF20A,KITLG,KL,KLF10,KLF2,KLF6,KLK3,LAMA4,LAMB3,LBP,LEP,LPL,LTB4R2,LYVE1,MADCAM1,MAFF,MAP3K14,MC1R,MCF2,MECOM,MFHAS1,M	611 (12)

Upstream Regulator	Expr Fold Change	Molecule Type	Predicted Activation State	Activation z-score	Flags	p-value of overlap	Target molecules in dataset	Mechanistic Network
							GMT,MMP1,MMP10,MMP12,MMP14,MMP28,MMP7,MSR1,MST1R,MSTN,MT2A,MUC1,MUC4,MYH7,NCAN,NCF2,NEFH,NFATC1,NFKBIA,NFKBIE,NKX2-1,NKX6-1,NOCT,NOD2,NPHS1,NPPB,NR4A2,NR4A3,NR6A1,OAS2,OLR1,OSM,OTUD7B,P2RY6,PAK2,PAX6,PDCD1,PDE2A,PDGFRA,PDPN,PIM1,PLA2G3,PLA2G4C,PLA2G5,PLIN1,PLK2,PLP1,PMAIP1,PPP1R15A,PRKCD,PRSS23,PTGES,PTGFR,PTPRN,PYCARD,RARA,RBPMS,RCAN2,REL,RELB,RFX2,RGS1,RGS2,RGS20,RGS3,RGS5,RND1,RRAD,RRM1,RRM2,RUNX2,S100A9,SCNN1B,SCO2,SCUBE2,SELL,SELPLG,SERPINB10,SERPINB9,SLC12A1,SLC16A2,SLC1A3,SLC2A4,SLC7A8,SNAI1,SNN,SOCS2,SOCS3,SOX4,SPHK1,ST8SIA4,STMN1,SYNGR3,TAGLN,TBXAS1,TERT,TH,THBD,THBS1,THBS2,TIE1,TK1,TLR2,TLR4,TNC,TNFAIP3,TNFRSF1B,TNFRSF9,TNFSF14,TNFSF15,TNFSF8,TNFSF9,TNNC1,TRAF1,TRAF2,TRAF5,TREM2,TRIM15,TRPC3,TRPC6,TWIST1,TXNRD1,VIP,WNT10B,WNT5A,WNT7A,YY1,ZFP36	