The MAK Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Antimony and its inorganic compounds except for stibine

MAK Value Documentation, addendum – Translation of the German version from 2021

A. Hartwig[1,*] MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords

antimony; inorganic; lung; chronic active inflammation; genotoxicity; clastogenicity; carcinogenicity; alveolar/bronchiolar lung carcinomas

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) summarized and re-evaluated the data on the genotoxicity and carcinogenicity of antimony [7440-36-0] and its inorganic compounds except for stibine. The critical effects of antimony and its inorganic compounds are the carcinogenic effects on the lung after inhalation exposure in rats and mice; similar effects may be induced in humans. Overall, the available epidemiological studies indicate that antimony and its inorganic compounds have carcinogenic effects on the human lung. However, because the persons examined were exposed to mixtures of substances and no data for concentrations are available, the classification cannot be made on the basis of these studies alone. A recent 2-year carcinogenicity study in male and female rats and mice showed that exposure to antimony trioxide particles causes lung neoplasms. Mice reacted more sensitively than rats, developing neoplastic lesions beginning at the lowest antimony trioxide concentration of 3 mg/m^3 (2.5 mg Sb/m^3). As a NOAEC for lung tumours and lung effects cannot be derived from the animal or human data and a NOAEC cannot be determined for possible mechanisms of action, no maximum concentration at the workplace (MAK value) can be established, thereby confirming the classification of antimony and its inorganic compounds in Carcinogen Category 2. The clastogenicity of inorganic antimony compounds in vitro is well established. In a recent study, exposure to antimony trioxide by inhalation for 12 months increased the number of micronucleated erythrocytes and DNA strand breaks in lung cells in male and female mice, but not in male or female rats. The lowest effective concentration was 3 mg/m^3 and led to increased DNA strand breaks in lung tissue in male mice. Thus, trivalent antimony was shown to induce genotoxic effects in soma cells after inhalation. This, together with evidence that the substance reaches the testes and ovaries, led to the classification of antimony and its inorganic compounds in Germ Cell Mutagenicity Category 3 A. There are still no reliable positive data for sensitizing effects in humans and no positive results from animal experiments or in vitro investigations. Therefore, antimony and its inorganic compounds continue not to be designated with the “Sh” or “Sa” notation.

Citation Note:
Hartwig A, MAK Commission. Antimony and its inorganic compounds except for stibine. MAK Value Documentation, addendum – Translation of the German version from 2021. MAK Collect Occup Health Saf. 2024 Mar;9(1):Doc007. https://doi.org/10.34865/mb744036e9_1ad

Manuscript completed:
14 Apr 2020

Publication date:
28 Mar 2024

MAK value	–
Peak limitation	–
Absorption through the skin	–
Sensitization	–
Carcinogenicity (2005)	**Category 2**
Prenatal toxicity	–
Germ cell mutagenicity (2020)	**Category 3 A**
BAR value (2019)	**0.2 µg antimony/l urine**[a)]
CAS number	7440-36-0

[a)] including stibine

Documentation for antimony and its inorganic compounds (inhalable fraction) was published in 2006 (Greim 2007).

Antimony trioxide is used as a flame retardant for products such as paper, wall coverings, textiles and furniture and as a catalyst in the production of polyethylene terephthalate (PET) (NTP 2017).

New data for genotoxicity and carcinogenicity have become available since the last documentation was published; these have been used to re-evaluate these 2 end points.

Toxic Effects and Mode of Action

Antimony compounds are absorbed slowly after oral exposure and accumulate in the lungs after inhalation exposure. The findings in humans suggest an increase in lung cancer mortality after exposure to antimony trioxide and antimony ore dust.

A new carcinogenicity study in rats and mice found that antimony trioxide induces carcinogenic effects in the lungs. In mice, carcinomas in the lungs were already detected at the lowest antimony trioxide concentration tested of 3 mg/m^3, which is equivalent to an antimony concentration of 2.5 mg/m^3. At this concentration, chronic active inflammation in the lungs was observed in addition to other effects in the respiratory tract in 96% to 100% of the rats and mice.

The new in vivo genotoxicity studies found that trivalent and pentavalent antimony compounds have a weak clastogenic potency in the lungs and peripheral blood leukocytes of mice, but not in those of rats.

No evidence was found that antimony and its inorganic compounds cause sensitizing effects.

Mechanism of Action

The documentation "Antimony and its inorganic compounds (inhalable fraction)" (Greim 2007) evaluated metallic antimony and antimony compounds in the oxidation states +3 and +5. Of all antimony compounds, those in the oxidation states +3 and +5 represent the most stable species. The toxic potential of the compounds depends on their oxidation state and their solubility in the body. Both the trivalent and the pentavalent compounds are taken up by mammals. In vivo, the pentavalent form is reduced to the trivalent form. All of the available data indicate that trivalent antimony is the stable form of antimony under physiological/cellular conditions; this form has a high affinity for

sulfur. Trivalent antimony reacts with thiol groups, particularly vicinal dithiols, thereby inhibiting enzyme activity. The reaction between trivalent antimony and glutathione leads to reduced glutathione levels in the liver, which may in turn decrease the conjugation of xenobiotics by glutathione (Greim 2007).

The lungs are the target organ after inhalation exposure. Whole-body exposure of Wistar Han [Crl:WI (Han)] rats and B6C3F1/N mice to antimony trioxide for 2 years at concentrations of 3 mg/m^3 (equivalent to an antimony concentration of 2.5 mg/m^3) and above induced alveolar and bronchiolar lung adenomas and carcinomas (NTP 2017).

Inorganic antimony compounds do not induce mutagenic effects in vitro, but clastogenic effects. In the documentation from 2006 (Greim 2007), this lack of mutagenic effects led to the conclusion that antimony does not directly react with the DNA. Impaired DNA replication and repair are discussed as relevant mechanisms (AGS 2018; Grosskopf et al. 2010; Hartwig 2013; Koch et al. 2017). Findings that antimony, like arsenic, inhibits the repair of DNA damage were already included in the documentation from 2006 (Greim 2007). The treatment of A549 human lung carcinoma cells with antimony trichloride at concentrations of 250 µM and above disrupted the nucleotide excision repair of cyclobutane pyrimidine dimers induced by irradiation with UVC. Possible sites of activity are the proteins xeroderma pigmentosum (XP) group A (XPA) and group E (XPE), which are involved in nucleotide excision repair. Antimony was found to interact with the XPA zinc finger domain, which is essential for the protein's function (Grosskopf et al. 2010). In HeLa S3 cells, antimony trichloride interfered with the repair of double-strand breaks induced by γ-irradiation and blocked the activation of checkpoint kinase Chk1 at concentrations of 50 µM and above. The findings suggest interference with both processes involved in the repair of DNA double-strand breaks, homologous recombination and non-homologous recombination (non-homologous end-joining) (Koch et al. 2017).

Another possible mechanism for the induction of carcinogenic effects is the formation of reactive oxygen species (ROS). Antimony trioxide induced ROS and apoptosis in NB4, PLB-985 and HeLa cells at concentrations of 1 µM and above. The data suggest that this is mediated by the SEK1/JNK signalling pathway (Mann et al. 2006). This is further supported by the findings of a genomics analysis in human HepG2 cells, which showed that bis[(+)-tartrato]diantimonate(III) dipotassium trihydrate (200 µM) alters the expression of genes in a manner similar to that of substances known to have a mechanism of action involving the formation of ROS (Kawata et al. 2007). According to the authors, a mechanism involving ROS is plausible because of the decrease in the reductive potential of the cell through the reaction of trivalent (but not pentavalent) antimony with glutathione (AGS 2018). The trivalent forms of antimony and arsenic have an affinity for SH groups, but antimony has a higher affinity than arsenic because of its stronger metallic character (Greim 2007).

It has also been proposed that reduced clearance, resulting in particle deposition in the lungs, may contribute to the mechanism of carcinogenesis. It is assumed that genotoxic effects in the lungs probably occur only at levels of exposure that overload lung clearance. However, no data are available that support this proposed mechanism. Data from in vitro and in vivo studies suggest the existence of an effect threshold and the ability to induce effects at higher concentrations (AGS 2018). The carcinogenicity study did not find that lung clearance was overloaded at the low concentration (NTP 2017). For this reason, the proposed mechanism of hypoxia is not regarded as relevant.

Alveolar or bronchiolar tumours in rats treated with antimony trioxide preferentially harboured *egfr* (epidermal growth factor receptor) mutations, while in mice, these tumours contained roughly equal numbers of *kras* (Kirsten rat sarcoma) and *egfr* mutations. Mutations in *KRAS* and *EGFR* are commonly observed in human non-small-cell lung cancer and occur in a mutually exclusive manner. KRAS and EGFR are major components of the mitogen-activated protein kinase signalling pathway. This association suggests that altered EGFR signalling may play an important role in the carcinogenesis of lung tumours in both rats and mice following exposure to antimony trioxide (NTP 2017).

Overall, the available data suggest the existence of a threshold mechanism for carcinogenic effects (AGS 2018; International Antimony Association 2017).

Toxicokinetics and Metabolism

There are no new studies available. In the documentation from 2006, antimony was found to be absorbed slowly after oral administration and after accumulation in the lungs depending upon particle size. Excretion largely occurs via the kidneys (Greim 2007).

Effects in Humans

Repeated exposure

In an occupational medical study of 60 workers who had been employed for at least 2 years from 1972 to 2017 in 1 of 2 production facilities for antimony trioxide, no substance-related findings were observed in lung function tests (forced vital capacity: FVC, forced expiratory volume in 1 second: FEV1, Tiffeneau index: FEV1/FVC) depending upon the mean antimony concentration in urine or in X-rays of the thorax. After the cohort was divided into a low dose group with antimony concentrations of less than 35 µg/g creatinine and a high dose group with antimony concentrations of at least 35 µg/g creatinine, the Tiffeneau index and the FEV1 were found to be decreased in the high dose group, but without statistical significance. After adjusting for pentavalent antimony, an antimony concentration of 35 µg/g creatinine was calculated to be equivalent to a concentration in air of about 0.5 mg/m^3. The study is not suitable for the derivation of a quantitative limit value because of the small group size, the lack of a control group, the relatively young age of the tested persons and the lack of exposure data, particularly as the parameters investigated are not very sensitive (AGS 2018).

Allergenic effects

The few clinical observations in humans do not provide evidence of noteworthy contact allergenic potential (Greim 2007). No new findings have become available.

Genotoxicity

An indicator test carried out in the DNA from a whole blood sample taken after inhalation exposure to antimony trioxide at the workplace revealed a statistically significant increase in formamidopyrimidine-DNA glycosylase-sensitive oxidative DNA base damage. Organic antimony compounds that release trivalent antimony such as meglumine antimonate (N-methylglucamine antimonate) caused an up to 9-fold increase in the frequency of micronuclei in human peripheral lymphocytes (Greim 2007).

The studies published since the last documentation are described below.

A study was carried out in Egypt in 25 male factory workers (aged 25 to 56) who were exposed to antimony trioxide while working in the polymerization process for polyester for periods ranging from 3 to 30 years. The control group was made up of 25 workers who were in the same age group, but not exposed. The workers had urinary antimony concentrations of 13 ± 4 µg/l (range: 10 to 19 µg/l); the levels in the control persons were below the limit of detection (10 µg/l). DNA damage was assessed by determining apurinic sites in DNA isolated from whole blood. These were increased in exposed workers and correlated with the urinary antimony concentration ($r = 0.873$, $p < 0.001$). By contrast, no correlation was found between the number of apurinic sites and the total oxidative capacity (determined by means of the plasma peroxide concentration). The authors concluded that the DNA damage was not caused by oxidative stress (El Shanawany et al. 2017). However, the number of examined workers was far too small and the scope of the examination too limited to draw conclusions about possible genotoxic effects induced by antimony.

A study population in the United States (n = 2307) consisting of participants who were older than 20 years and had been recruited to participate in the "National Health and Nutrition Examination Survey" (NHANES) between 1999 and 2002 was used to investigate whether there was a relationship between antimony exposure and leukocyte telomere

length. Multivariate linear regression was used to analyse the relationship between the antimony concentration in the urine and telomere length. The antimony concentration in the urine had a geometric mean of 0.12 ng/ml (geometric standard error: 0.003). The participants were divided into quartiles according to the antimony concentration in the urine: less than 0.08, 0.09 to 0.12, 0.13 to 0.18 and higher than 0.18 ng/ml. After adjustment for potential confounders (age, education, ethnicity, alcohol consumption, self-reported smoking status, cotinine in serum, body weight, urinary lead concentration) individuals in the 3rd and 4th quartiles were found to have shorter leukocyte telomeres in comparison with those of the 1st quartile (= referent quartile) (3rd quartile: −4.78%; 95% confidence interval (CI): −8.42 to 0.90, 4th quartile: −6.11%, 95% CI: −11.04 to −1.00). There was evidence of a dose–response relationship (p-value for trend: 0.03). The shorter telomeres associated with exposure to antimony were derived mainly from data from the groups of 40 to 59 and 60 to 85-year-olds. The authors consider a relationship between shorter telomeres and antimony concentrations in the urine to be biologically plausible as antimony is known to lead to oxidative stress and apoptosis, and both effects are associated with reduced telomere lengths (Scinicariello and Buser 2016).

Carcinogenicity

Overall, the available epidemiological studies suggest that antimony induces carcinogenic effects in the lungs in humans. However, because of exposure to a mixture of substances and a lack of concentration data, these studies cannot be included in the evaluation (Greim 2007).

In the workers of a British tin smelter, the weighted cumulative exposure to antimony was found to be correlated with increased lung cancer mortality. However, the workers were concurrently exposed to arsenic and lead. For this reason, the study cannot be used to evaluate the carcinogenic effects of antimony. A United States National Health and Nutrition Examination Survey carried out with 7800 participants over a 6-year period did not establish a relationship between the urinary concentration of antimony and the cancer incidence and mortality (AGS 2018).

Animal Experiments and in vitro Studies

Subacute, subchronic and chronic toxicity

Inhalation

A large number of studies are reviewed in the documentation from 2006. After repeated inhalation exposure (whole-body) of rats to antimony trioxide, damage to the lungs and eyes, an increase in aspartate aminotransferase activity and the urea concentration in serum, and changes in the blood count were observed. After exposure to antimony trioxide, lung damage was induced also in rabbits and miniature pigs. After repeated inhalation exposure (whole-body) to antimony trisulfide, changes in the electrocardiogram, and thus cardiac damage, were observed in rats, rabbits and dogs. In another study in rabbits, the lungs, kidneys, liver and heart were the target organs for toxicity. Antimony ore likewise induced lung and spleen damage in addition to effects on the eyes in rats after repeated inhalation exposure (whole-body) (Greim 2007).

The lungs are considered the only relevant target organ after inhalation exposure to **antimony trioxide** and **antimony ore**. The other target sites may possibly be applicable only for a specific species or sex (AGS 2018; International Antimony Association 2017).

A new inhalation study has been published by the NTP (2017) since the publication of the documentation in 2006. Wistar Han [Crl:WI (Han)] rats and B6C3F1/N mice were exposed to antimony trioxide for either 2 weeks or 2 years. The findings and incidences are shown in Tables 1 and 2. In the 2-year study, both species were exposed to antimony trioxide aerosol at concentration levels of 0, 3, 10, 30 mg/m^3. A high incidence of effects on the lungs was found at the lowest antimony trioxide concentration tested of 3 mg/m^3 (equivalent to an antimony concentration of 2.5 mg/m^3) and above. For example, chronic active inflammation was found in 96% to 100% of the rats and mice. In the 2-year study,

the maximum tolerated dose (MTD) was already reached in female rats at the low antimony trioxide concentration of 3 mg/m³ and in male rats at 30 mg/m³; this was manifest as a marked reduction in body weight by at least 10%. In male mice, this effect was observed at antimony trioxide concentrations of 10 mg/m³ and above and in female mice at 30 mg/m³ (NTP 2017).

Tab. 1 New data for effects after repeated inhalation exposure to antimony trioxide

Species, strain, number per group	Exposure	Findings	References
rat, Wistar Han [Crl:WI (Han)], 5 ♂, 5 ♀	**2 weeks**, 0, 3.75, 7.5, 15, 30, 60 mg Sb_2O_3/m³, 6 hours/day, 5 days/week, whole-body exposure, purity 99.9% [a], aerosol, MMAD = 1.3–1.5 µm; GSD 1.9	**15 mg/m³: NOAEC**; **30 mg/m³ and above**: inflammation in the lungs ↑, absolute and relative lung weights ↑ (♀); **60 mg/m³**: absolute and relative lung weights ↑ (♂); **clearance half-life**: 73–122 days	NTP 2017
rat, Wistar Han [Crl:WI (Han)], 60 ♂, 60 ♀	**2 years**, 0, 3, 10, 30 mg Sb_2O_3/m³, 6 hours/day, 5 days/week, whole-body exposure, purity 99.9% [a], aerosol, MMAD = 0.9–1.5 µm; GSD 1.7 – 2.2	for the incidences of non-neoplastic findings, see Table 2; **no NOAEC**; **3 mg/m³ and above**: **LOAEC**; dose-dependent: abnormal breathing, cyanosis, thinness, absolute and relative lung weights ↑, ♀: 10% decrease in body weights; **10 mg/m³**: ♀: mortality increased as a result of lung proteinosis, 20% decrease in body weights, ♂: mortality trend increased as a result of pneumonia and lung proteinosis; **30 mg/m³**: body weights decreased: ♂ 20% and ♀ 28%	NTP 2017
mouse, B6C3F1/N, 5 ♂, 5 ♀	**2 weeks**, 0, 3.75, 7.5, 15, 30, 60 mg Sb_2O_3/m³, 6 hours/day, 5 days/week, whole-body exposure, purity 99.9% [a], aerosol, MMAD = 1.3–1.5 µm; GSD 1.9	**7.5 mg/m³**: **NOAEC**; **15 mg/m³ and above**: absolute and relative lung weights ↑ (♀); **30 mg/m³ and above**: squamous metaplasia of the epiglottis ↑; **60 mg/m³**: absolute and relative lung weights ↑ (♂); **clearance half-life**: 47–62 days	NTP 2017
mouse, B6C3F1/N, 60 ♂, 60 ♀	**2 years**, 0, 3, 10, 30 mg Sb_2O_3/m³, 6 hours/day, 5 days/week, whole-body exposure, purity 99.9% [a], aerosol, MMAD = 0.9–1.5 µm; GSD 1.7–2.2	for the incidences of non-neoplastic findings, see Tables 2, 4 **no NOAEC**; **3 mg/m³ and above**: **LOAEC**; dose-dependent: abnormal breathing, cyanosis, thinness, absolute and relative lung weights ↑; **10 mg/m³**: body weights decreased: ♂ 10%; **30 mg/m³**: body weights decreased: ♂ 25% and ♀ 11%	NTP 2017

[a] arsenic about 0.019%, lead about 0.016%; GSD: geometric standard deviation; LOAEC: lowest observed adverse effect concentration; MMAD: mass median aerodynamic diameter; NOAEC: no observed adverse effect concentration

Tab. 2 Incidences of non-neoplastic findings in the 2-year inhalation study of antimony trioxide in rats and mice (NTP 2017)

		Exposure concentration (mg Sb_2O_3/m³)			
		0	**3**	**10**	**30**
rat					
surviving animals	♂	30/50 (60%)	30/50 (60%)	28/50 (56%)	18/50 (36%)
	♀	39/50 (78%)	38/50 (76%)	28/50 (56%)	20/50 (40%)
lungs:					
foreign body deposition	♂	1/50 (2%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
	♀	0/50 (0%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
chronic active inflammation	♂	18/50 (36%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
	♀	21/50 (42%)	50/50 (100%)	50/50 (100%)	50/50 (100%)

Tab. 2 *(continued)*

		Exposure concentration (mg Sb_2O_3/m^3)			
		0	**3**	**10**	**30**
suppurative alveolar inflammation	♂	0/50 (0%)	12/50 (24%)	24/50 (48%)	28/50 (56%)
	♀	0/50 (0%)	5/50 (10%)	6/50 (12%)	5/50 (10%)
perivascular cellular infiltration of lymphocytes	♂	3/50 (6%)	25/50 (50%)	19/50 (38%)	9/50 (18%)
	♀	0/50 (0%)	18/50 (36%)	11/50 (22%)	8/50 (16%)
proteinosis	♂	0/50 (0%)	47/50 (94%)	50/50 (100%)	50/50 (100%)
	♀	0/50 (0%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
alveolar epithelial hyperplasia	♂	4/50 (8%)	50/50 (100%)	48/50 (96%)	49/50 (98%)
	♀	5/50 (10%)	50/50 (100%)	49/50 (98%)	50/50 (100%)
bronchial epithelial hyperplasia	♂	3/50 (6%)	34/50 (68%)	36/50 (72%)	33/50 (66%)
	♀	6/50 (12%)	26/50 (52%)	25/50 (50%)	27/50 (54%)
fibrosis	♂	2/50 (4%)	50/50 (100%)	49/50 (98%)	49/50 (98%)
	♀	1/50 (2%)	50/50 (100%)	50/50 (100%)	49/50 (98%)
chronic active arterial inflammation	♂	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
	♀	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
nose:					
foreign body deposition	♂	0/50 (0%)	0/49 (0%)	17/50 (34%)	40/50 (80%)
	♀	0/50 (0%)	5/50 (10%)	26/50 (52%)	45/50 (90%)
respiratory epithelial hyperplasia	♂	6/50 (12%)	15/49 (30%)	13/50 (26%)	25/50 (50%)
	♀	4/50 (8%)	6/50 (12%)	7/50 (14%)	16/50 (32%)
squamous metaplasia of the respiratory epithelium	♂	0/50 (0%)	0/49 (0%)	2/50 (4%)	6/50 (12%)
	♀	0/50 (0%)	2/50 (4%)	3/50 (6%)	5/50 (10%)
larynx:					
foreign body deposition	♂	0/50 (0%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
	♀	0/50 (0%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
chronic active inflammation	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
	♀	0/50 (0%)	8/50 (16%)	0/50 (0%)	3/50 (6%)
trachea:					
foreign body deposition	♂	0/50 (0%)	28/50 (56%)	43/50 (86%)	48/50 (96%)
	♀	0/50 (0%)	39/50 (78%)	47/50 (94%)	49/50 (98%)
bone marrow:					
hyperplasia	♂	0/50 (0%)	3/50 (6%)	4/50 (8%)	8/50 (16%)
	♀	8/50 (16%)	5/50 (10%)	11/50 (22%)	20/50 (40%)
lymph nodes:					
bronchial					
foreign body deposition	♂	0/41 (0%)	35/40 (87%)	45/48 (94%)	42/47 (89%)
	♀	0/35 (0%)	35/36 (97%)	23/28 (82%)	36/41 (88%)
lymphoid hyperplasia	♂	0/41 (0%)	21/40 (52%)	29/48 (60%)	26/47 (55%)
	♀	0/35 (0%)	21/36 (58%)	9/28 (32%)	11/41 (27%)
pigmentation	♂	1/41 (2%)	4/40 (10%)	5/48 (10%)	10/47 (21%)
	♀	14/35 (40%)	12/36 (33%)	8/28 (29%)	18/41 (44%)
mediastinal					
foreign body deposition	♂	0/42 (0%)	41/45 (91%)	41/49 (84%)	43/49 (88%)
	♀	0/46 (0%)	27/46 (59%)	32/46 (69%)	33/46 (72%)
lymphoid hyperplasia	♂	1/42 (2%)	24/45 (53%)	30/49 (61%)	26/49 (53%)
	♀	0/46 (0%)	14/46 (30%)	10/46 (22%)	15/46 (33%)

Tab. 2 *(continued)*

		Exposure concentration (mg Sb_2O_3/m^3)			
		0	**3**	**10**	**30**
pancreas:					
chronic active arterial inflammation	♂	1/50 (2%)	0/50 (0%)	2/50 (4%)	8/50 (10%)
	♀	0/50 (0%)	0/50 (0%)	3/50 (6%)	8/50 (16%)
arterial necrosis	♂	n.i.	n.i.	1/50 (2%)	4/50 (8%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
mesentery:					
chronic active arterial inflammation	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	6/50 (12%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	6/50 (12%)
kidneys:					
accumulation of hyaline droplets in the tubules	♂	0/50 (0%)	1/50 (2%)	3/50 (6%)	14/50 (28%)
	♀	0/50 (0%)	0/50 (0%)	5/50 (10%)	11/50 (22%)
chronic active arterial inflammation	♂	0/50 (0%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
nephropathy	♂	41/50 (82%)	41/50 (82%)	40/50 (80%)	38/50 (76%)
	♀	16/50 (32%)	15/50 (30%)	20/50 (40%)	24/50 (48%)
arteries:					
chronic inflammation in all tissues combined	♂	1/50 (2%)	1/50 (2%)	5/50 (10%)	16/50 (32%)
	♀	0/50 (0%)	0/50 (0%)	5/50 (10%)	15/50 (30%)
eyes:					
acute inflammation of the ciliary body	♂	0/49 (0%)	0/49 (0%)	1/50 (2%)	6/49 (12%)
	♀	0/49 (0%)	0/50 (0%)	1/49 (2%)	6/49 (12%)
retinal atrophy	♂	8/49 (16%)	11/49 (22%)	9/49 (18%)	6/50 (12%)
	♀	6/49 (12%)	21/50 (42%)	18/49 (37%)	19/49 (39%)
mouse					
surviving animals	♂	38/50 (76%)	30/50 (60%)	27/50 (54%)	17/50 (34%)
	♀	36/50 (72%)	31/50 (62%)	26/50 (52%)	15/50 (30%)
lungs:					
cellular infiltration by lymphocytes	♂	13/50 (26%)	47/50 (94%)	48/50 (96%)	45/50 (90%)
	♀	7/50 (14%)	37/50 (74%)	37/50 (74%)	26/50 (52%)
foreign body	♂	0/50 (0%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
	♀	0/50 (0%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
chronic active inflammation	♂	0/50 (0%)	48/50 (96%)	50/50 (100%)	50/50 (100%)
	♀	1/50 (2%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
alveolar fibrosis	♂	0/50 (0%)	12/50 (24%)	30/50 (60%)	37/50 (74%)
	♀	0/50 (0%)	13/50 (26%)	30/50 (60%)	38/50 (76%)
pleural inflammation	♂	1/50 (2%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
	♀	1/50 (2%)	40/50 (80%)	47/50 (94%)	48/50 (96%)
nose:					
foreign body	♂	0/50 (0%)	48/49 (98%)	48/49 (98%)	49/50 (98%)
	♀	1/50 (2%)	44/49 (88%)	45/50 (90%)	48/50 (96%)
chronic epithelial inflammation	♂	3/50 (6%)	9/49 (18%)	9/49 (18%)	6/50 (12%)
squamous metaplasia of the respiratory epithelium	♀	0/50 (0%)	3/49 (6%)	2/50 (4%)	4/50 (8%)
thymus:					
cellular depletion	♂	15/41 (37%)	14/38 (37%)	32/43 (74%)	32/39 (82%)
	♀	9/47 (19%)	18/49 (37%)	23/49 (47%)	29/49 (59%)

Tab. 2 *(continued)*

		Exposure concentration (mg Sb_2O_3/m^3)			
		0	**3**	**10**	**30**
bone marrow:					
hyperplasia	♂	10/49 (20%)	19/50 (38%)	27/48 (56%)	33/50 (66%)
	♀	3/50 (6%)	5/50 (10%)	15/50 (30%)	28/50 (56%)
spleen:					
haematopoietic cell proliferation	♂	13/49 (27%)	10/50 (20%)	10/50 (20%)	13/50 (26%)
	♀	17/50 (34%)	19/50 (38%)	20/50 (40%)	35/50 (70%)
larynx:					
foreign body deposition	♂	0/50 (0%)	15/50 (30%)	29/50 (58%)	44/50 (88%)
	♀	0/50 (0%)	25/50 (50%)	39/50 (78%)	48/50 (96%)
respiratory epithelial hyperplasia	♂	1/50 (2%)	3/50 (6%)	15/50 (30%)	30/50 (60%)
	♀	2/50 (4%)	0/50 (0%)	14/50 (28%)	18/50 (36%)
squamous metaplasia of the respiratory epithelium	♂	0/50 (0%)	0/50 (0%)	8/50 (16%)	18/50 (36%)
	♀	1/50 (2%)	0/50 (0%)	5/50 (10%)	24/50 (48%)
squamous epithelial hyperplasia	♂	2/50 (4%)	0/50 (0%)	4/50 (8%)	13/50 (26%)
	♀	4/50 (8%)	1/50 (2%)	1/50 (2%)	12/50 (24%)
trachea:					
foreign body deposition	♂	0/49 (0%)	3/50 (6%)	1/50 (2%)	20/50 (40%)
	♀	0/50 (0%)	7/50 (14%)	14/50 (28%)	20/50 (40%)
epithelial hyperplasia	♂	0/49 (0%)	0/50 (0%)	2/50 (4%)	5/50 (10%)
lymph nodes:					
bronchial					
lymphoid hyperplasia	♂	2/30 (7%)	21/43 (49%)	26/47 (55%)	13/41 (32%)
	♀	2/41 (5%)	15/47 (32%)	17/48 (35%)	11/49 (22%)
foreign body deposition	♂	0/30 (0%)	34/43 (79%)	47/47 (100%)	38/41 (93%)
	♀	0/41 (0%)	34/47 (72%)	46/48 (96%)	43/49 (88%)
cellular histiocytic infiltration	♂	0/30 (0%)	2/43 (5%)	4/47 (9%)	6/41 (15%)
	♀	0/41 (0%)	2/47 (4%)	7/48 (15%)	7/49 (14%)
mediastinal					
lymphoid hyperplasia	♂	2/37 (5%)	8/45 (18%)	17/48 (35%)	34/49 (69%)
	♀	0/46 (0%)	3/48 (6%)	16/49 (33%)	18/50 (36%)
foreign body deposition	♂	0/37 (0%)	32/45 (71%)	42/48 (87%)	48/49 (98%)
	♀	0/46 (0%)	28/48 (58%)	45/49 (92%)	44/50 (88%)
cellular histiocytic infiltration	♂	0/37 (0%)	4/45 (9%)	13/48 (27%)	34/49 (69%)
	♀	0/46 (0%)	6/48 (12%)	11/49 (22%)	16/50 (32%)
heart:					
chronic active inflammation of the epicardium	♂	0/50 (0%)	2/50 (4%)	7/50 (14%)	16/50 (32%)
	♀	0/50 (0%)	2/50 (4%)	7/50 (14%)	7/50 (14%)
forestomach:					
chronic active inflammation	♂	2/50 (4%)	4/50 (8%)	4/49 (8%)	7/50 (14%)
	♀	n. i.	1/50 (2%)	n. i.	3/50 (6%)

n. i.: not investigated

Allergenic effects

Animals studies that investigated sensitization did not find evidence of a noteworthy contact allergenic potential; however, these studies were of limited validity (Greim 2007).

In a maximization test in guinea pigs carried out according to OECD Test Guideline 406 in 2005, antimony trioxide was not found to induce sensitizing effects. The intradermal and the topical induction treatment were carried out with a 10% and a 50% suspension of antimony trioxide in water, respectively. Prior to the topical induction treatment, the animals were treated for 24 hours with 10% sodium lauryl sulfate in petrolatum. A 50% suspension was used for the 24-hour challenge treatment. A reaction was not obtained in any of the 20 female Dunkin Hartley guinea pigs at the readings after 48 and 72 hours (ECHA 2020).

Genotoxicity

In vitro

Direct mutagenic effects were not induced in most of the tests carried out with bacteria strains incubated with trivalent or pentavalent antimony compounds. Trivalent antimony compounds likewise did not induce gene mutations (mouse lymphoma test) in mammalian cells. However, trivalent antimony compounds were found to induce clastogenic effects in human lymphocytes (DNA strand breaks, chromosomal aberrations, micronuclei), human leukocytes (chromosomal aberrations), human bronchial epithelial cells and fibroblasts (micronuclei), in V79 cells (DNA strand breaks, micronuclei) and CHO-K1 cells (micronuclei) (Beyersmann and Hartwig 2008; Greim 2007).

In vitro genotoxicity studies that were published since the last documentation are shown in Table 3.

In the Salmonella typhimurium strain TA1537, metallic antimony led to an around 2-fold increase in the number of revertants at just below cytotoxic concentrations and in the absence of a metabolic activation system. In the Salmonella typhimurium strains TA98, TA100 and TA1535 as well as in Escherichia coli WP2uvrA/pKM101, metallic antimony did not cause mutagenic effects either in the presence or in the absence of a metabolic activation system (Asakura et al. 2009).

In CHO-9 cells, trimethylantimony(V) dichloride did not lead to an increase in sister chromatid exchange up to a concentration of 1000 µM. Uptake in the cells was 0.05% (Dopp et al. 2006).

Treatment of the human cell lines HepG2 and LS-174T with antimony trichloride increased the phosphorylation of the histone H2AX (γH2AX) at concentrations of 100 µM and above (HepG2) and 250 µM and above (LS-174T), respectively (Kopp et al. 2018). H2AX is phosphorylated at sites of DNA repair. The phosphorylation of H2AX is typically induced by DNA double-strand breaks, but may be initiated also if the DNA is damaged during proliferation or apoptosis. In order to establish with certainty that DNA double-strand breaks are involved, evidence of colocalization with a second protein (for example 53BP1) is required (Rothkamm et al. 2015). As this was not established in the present case, γH2AX quantification yielded evidence of increased DNA damage, but without further specification of the type of damage.

In a test for DNA strand breaks (comet assay) in human lymphocytes, *N*-methylglucamine antimonate, which contains pentavalent antimony, was not found to be clastogenic up to an antimony concentration of 4250 µg/ml (Lima et al. 2010).

Metallic antimony induced chromosomal aberrations, primarily chromatid breaks and exchange, and increased the percentage of endoreduplication in CHL cells at concentrations of 25 µg/ml and above (Asakura et al. 2009). Trimethylantimony(V) dichloride, however, did not induce these types of effects in CHO-9 cells up to a concentration of 1000 µg/ml. The uptake in the cells was 0.05% (Dopp et al. 2006). This pentavalent antimony compound did not induce an increase in micronuclei in CHO-9 cells up to a concentration of 1000 µM with a maximum uptake of 0.05% (Dopp et al. 2006).

Tab. 3 In vitro studies of the genotoxicity induced by antimony compounds carried out after 2006

End point	Test system	Substance	Concentration	Effective concentration	Cytotoxicity	Results		Comments	References
						−m.a.	+m.a.		
gene mutation (preincubation)	Salmonella typhimurium TA1537	metallic antimony, 10 µm	−m.a.: 0, 500, 750, 1250, 1500, 1750, 2000, 2250 µg/plate, +m.a.: 0, 156, 313, 625, 1250, 2500, 5000 µg/plate, solvent: DMSO, purity: 99%	750 µg/plate	−m.a.: ≥ 1500 µg/plate, +m.a.: ≥ 2500 µg/plate	+	–	number of revertants: 2-fold	Asakura et al. 2009
	Salmonella typhimurium TA98, TA100, TA1535, Escherichia coli WP2uvrA/pKM101	metallic antimony, 10 µm	0, 39.1–5000 or 313–1250 µg/plate, solvent: DMSO, purity: 99%	–	−m.a.: TA98: ≥ 1250 µg/plate, TA100: ≥ 2500 µg/plate, TA1535: ≥ 525 µg/plate, WP2uvrA/pKM101: –, +m.a.: TA98: 5000 µg/plate, TA100: ≥ 1250 µg/plate, TA1535: ≥ 1250 µg/plate, WP2uvrA/pKM101: –	–	–		
SCE	CHO-9 cells	trimethylantimony(V) dichloride	0, 10, 100, 1000 µM, solvent: DMSO, purity: analytical grade	–	surviving cells at 500 µM: about 80% after 24 hours	–	not performed	uptake in cells: 0.05%	Dopp et al. 2006
phosphorylation of the histone H2AX (γH2AX)	human cell lines HepG2 and LS-174T (representing the target organs liver and colon)	$SbCl_3$	0, 10, 50, 100, 250, 500 µM, solvent: DMSO, purity: > 95%	HepG2: ≥ 100 µM, LS-174T: 250 µM	+ ≥ 250 µM	+	not performed	induction of γH2AX: HepG2 at 100 µM: 1.3-fold, LS-174T at 250 µM: 1.6-fold	Kopp et al. 2018
DNA strand breaks (comet assay)	human lymphocytes	*N*-methylglucamine antimonate	0, 1060, 2120, 4250 µg Sb(V)/ml, solvent: water	–	–	–	not performed		Lima et al. 2010
CA	CHL cells	metallic antimony, 10 µm	−m.a.: 0, 12.5, 25, 50, 100, 200 µg/ml, +m.a.: 0, 6.25, 12.5, 25, 50, 100, 200 µg/ml, solvent: DMSO, purity: 99%	≥ 25 µg/ml	−m.a.: 200 µg/ml, +m.a.: ≥ 100 µg/ml	+	+	mainly chromatid breaks and exchange in addition to increased percentage of endoreduplication, gaps analysed individually	Asakura et al. 2009

Tab. 3 *(continued)*

End point	Test system	Substance	Concentration	Effective concentration	Cytotoxicity	Results		Comments	References
						−m. a.	+m. a.		
CA	CHO-9 cells	trimethylantimony(V) dichloride	0, 10, 100, 1000 µM, solvent: DMSO, purity: analytical grade	–	surviving cells at 500 µM: about 80% after 24 hours	–	not performed	uptake in cells: 0.05%	Dopp et al. 2006
MN	CHO-9 cells	trimethylantimony(V) dichloride	0, 250, 500, 750, 1000 µM, solvent: DMSO, purity: analytical grade	–	surviving cells at 500 µM: about 80% after 24 hours	–	not performed	uptake in cells: 0.05%; electroporation (method used to temporarily increase the permeability of the cell membrane) in hypo-osmotic buffer: 500 µM: induction of micronuclei and 2-fold intracellular antimony concentration; surviving cells > 90%	Dopp et al. 2006

CA: chromosomal aberrations; DMSO: dimethyl sulfoxide; m. a.: metabolic activation system; MN: micronuclei; SCE: sister chromatid exchange

In vivo

The valid in vivo genotoxicity studies of antimony trichloride and antimony trioxide that were evaluated in 2006 (chromosomal aberrations, micronuclei) yielded only negative results. Antimony trichloride induced an increased number of DNA strand breaks in the spleen of Swiss mice only at highly toxic doses far above the LD_{50}. The findings of chromosomal aberrations in the bone marrow cells induced by antimony trichloride and antimony trioxide are questionable because of methodological shortcomings and the presence of impurities. Like inorganic antimony compounds, the organic antimony compounds and their salts release trivalent antimony and are therefore included in the evaluation of genotoxicity. Oral exposure to the organic compound antimony tris(isooctyl thioglycolate) did not lead to the formation of micronuclei in the bone marrow cells of mice or chromosomal aberrations in the bone marrow cells of rats. However, antimony potassium tartrate and antimony piperazine tartrate given by intraperitoneal injection induced chromosomal aberrations in the bone marrow of rats. Overall, the data suggest that the substances have a clastogenic potential in vivo (Greim 2007).

The in vivo studies of genotoxicity that have become available since the documentation was published in 2006 are listed in Table 4. The study of Kirkland et al. (2007) is the same study as that referred to as IAOIA 2005 by Greim (2007).

In an extensive study carried out by the NTP with inhalation exposure of rats and mice to antimony trioxide for 1 year, DNA strand breaks were determined in the peripheral blood leukocytes and in the lungs by comet assay. The study found that the fraction of tail DNA in the lungs was 30% to 50% higher in male mice at concentrations of 3 mg/m^3 and above and in female mice at 30 mg/m^3. However, this effect was not seen in the leukocytes of mice and in both organs/tissues of rats up to the high concentration (NTP 2017). The lungs are the target organ for inhalation exposure; however, after a certain period of time antimony is detected also in the erythrocytes (Greim et al. 2009).

N-Methylglucamine antimonate, which contains pentavalent antimony, did not induce clastogenic effects in human lymphocytes in vitro (see above; Lima et al. 2010). In Swiss mice, the compound induced DNA strand breaks in the peripheral blood leukocytes and peritoneal exudate macrophages in addition to micronuclei in the bone marrow cells after a single intraperitoneal injection of pentavalent antimony doses of 213 mg/kg body weight and above. In a pairwise comparison of the groups, the findings for these 2 parameters did not reach statistical significance; the authors therefore concluded that the effects were not dose-dependent. The authors suggested that the genotoxic effects are caused by the reduction of pentavalent to trivalent antimony (Lima et al. 2010).

The NTP study investigated the occurrence of micronuclei in the peripheral blood leukocytes after inhalation exposure of rats and mice for 1 year. The study found that the fraction of peripheral blood leukocytes with micronuclei was increased 1.3-fold in male and female mice at a concentration of 30 mg/m^3. By contrast, this effect was not noticeable in rats up to the high concentration and the percentage of reticulocytes in the peripheral blood remained unchanged by treatment (NTP 2017). In mice, erythropoiesis was stimulated and findings of hyperplasia in the bone marrow were obtained by histological examination. This is a sign of rapid cell division, which may lead to an increase in the incidence of errors and suggests indirect micronuclei formation. According to a group of experts, if the in vitro results are negative, then the induction of micronuclei after increased erythropoiesis is to be interpreted as a false positive result (Tweats et al. 2007). However, antimony compounds have been found to be clastogenic in vitro and therefore the induction of micronuclei in mice reported by the NTP study is to be regarded as a substance-induced effect.

Tab. 4 In vivo studies of the genotoxicity of inorganic antimony compounds

Test system, organ	Species, strain, number, sex	Substance	Dose/concentration, administration	Exposure, sampling	Results	Comments	References
DNA strand breaks (comet assay), peripheral blood leukocytes, lung	rat, Wistar Han [Crl:WI (Han)], 10 ♂, 10 ♀,	Sb_2O_3	0, 3, 10, 30 mg/m³, aerosol, whole-body exposure, purity: > 99.9%	12 months, 6 hours/day, 5 days/week	–		NTP 2017
DNA strand breaks (comet assay), peripheral blood leukocytes, lung	mouse, B6C3F1/N, 10 ♂, 10 ♀	Sb_2O_3	0, 3, 10, 30 mg/m³, aerosol, whole-body exposure, purity: > 99.9%	12 months, 6 hours/day, 5 days/week	lungs: + leukocytes: –	lungs: percent tail DNA ↑: ♂: ≥ 3 mg/m³ (3 mg/m³: 1.3-fold, 10 mg/m³: 1.3-fold, 30 mg/m³: 1.5-fold), ♀: 30 mg/m³: 1.4-fold	NTP 2017
DNA strand breaks (comet assay), peripheral blood leukocytes, peritoneal exudate macrophages	mouse, Swiss, 5 ♂, 5 ♀	*N*-methylglucamine antimonate	0, 213, 425, 850 mg Sb(V)/kg body weight, intraperitoneal, vehicle: water	single dose, sampling: 3 hours (macrophages) and 24 hours (leukocytes) after administration	+	≥ 213 mg/kg body weight, leukocytes: DNA damage score: 2.8-fold at 213 mg/kg body weight; 2.9-fold at 425 mg/kg body weight; 3.1-fold at 850 mg/kg body weight; no effects with statistical significance found by a pairwise comparison of the groups; based on this, the authors concluded that there was no dose dependency	Lima et al. 2010
CA, bone marrow	rat, SD, 6 ♂, 6 ♀	$SbCl_3$	0, 250, 500, 1000 mg/kg body weight and day, gavage, vehicle: HPMC/polysorbate, purity: 99.93%	21 days, sampling: 24 hours after last dose	–	body weights decreased by at least 10%	IAOIA 2005 in Greim 2007; Kirkland et al. 2007
MN, peripheral blood leukocytes	rat, Wistar Han [Crl:WI (Han)], 10 ♂, 10 ♀	Sb_2O_3	0, 3, 10, 30 mg/m³, aerosol, whole-body exposure, purity: > 99.9%	12 months, 6 hours/day, 5 days/week	–	percentage of reticulocytes in the peripheral blood unchanged	NTP 2017
MN, peripheral blood leukocytes	mouse, B6C3F1/N, 10 ♂, 10 ♀	Sb_2O_3	0, 3, 10, 30 mg/m³, aerosol, whole-body exposure, purity: > 99.9%	12 months, 6 hours/day, 5 days/week	+	♂ and ♀: 30 mg/m³ (ratio of NCE with MN/NCE: 1.3 times as high)	NTP 2017
MN, bone marrow	rat, SD, 6 ♂, 6 ♀	$SbCl_3$	0, 250, 500, 1000 mg/kg body weight and day, gavage, vehicle: HPMC/polysorbate, purity: 99.93%	21 days, sampling: 24 hours after last dose	–	body weight gains decreased by at least 10%	IAOIA 2005 in Greim 2007; Kirkland et al. 2007

Tab. 4 *(continued)*

Test system, organ	Species, strain, number, sex	Substance	Dose/concentration, administration	Exposure, sampling	Results	Comments	References
MN, bone marrow	mouse, Swiss, 5 ♂, 5 ♀	*N*-methylglucamine antimonate	0, 213, 425, 850 mg Sb(V)/kg body weight, intraperitoneal, vehicle: water	single dose, sampling: 24 hours after administration	+	≥213 mg/kg body weight, fraction of polychromatic erythrocytes with micronuclei: 1.8-fold at 213 mg/kg body weight; 2.2-fold at 425 mg/kg body weight; 2.6-fold at 850 mg/kg body weight; no effects with statistical significance found by a pairwise comparison of the groups; based on this, the authors concluded that there was no dose dependency, PCE/NCE ratio unchanged	Lima et al. 2010

CA: chromosomal aberrations; HPMC: hydroxypropyl methylcellulose; MN: micronuclei; NCE: normochromatic erythrocytes; PCE: polychromatic erythrocytes; SD: Sprague Dawley

Summary

There is strong evidence that antimony compounds induce clastogenic effects. The new in vivo studies demonstrate that antimony compounds have a weak clastogenic potency in mice, but not in rats (Lima et al. 2010; NTP 2017). In the study carried out by the NTP with inhalation exposure of mice for 12 months, the fraction of tail DNA in the lungs was increased by 30% to 50% in male mice at concentrations of 3 mg/m^3 and above and in female mice at 30 mg/m^3; an increase was not observed in the leukocytes. In addition, the fraction of peripheral blood leukocytes with micronuclei was increased 1.3-fold in male and female mice at a concentration of 30 mg/m^3 (NTP 2017).

Carcinogenicity

The documentation published in 2006 includes a detailed review of several studies. These studies found that exposure to antimony trioxide and antimony ore dust led to the development of lung tumours in female rats. In 2 carcinogenicity studies, the incidence of lung tumours in rats was increased with statistical significance after exposure to antimony trioxide for 1 year at concentrations of 4.2 mg/m^3 and 45 to 46 mg/m^3, respectively. In addition, antimony ore dust (46% antimony, predominantly in the form of antimony trisulfide) increased the incidence of lung cancer in rats with statistical significance. Another study did not determine an increased tumour incidence in female rats after exposure to antimony trioxide at a concentration of 4.5 mg/m^3 for 1 year and an observation period of 1 year. This was explained by the fact that the period of exposure and also the survival time of the animals was shorter and the lung burden of antimony was not as extensive. Overall, the findings suggest that long-term exposure to antimony trioxide by inhalation at concentrations ranging from 5 to 50 mg/m^3 may induce lung tumours in female rats (Greim 2007).

A new carcinogenicity study has become available since the documentation was published in 2006. In this inhalation study with exposure of Wistar Han [Crl:WI (Han)] rats and B6C3F1/N mice to antimony trioxide (see Table 5), the combined incidence of alveolar/bronchiolar adenomas and carcinomas in mice was increased in a concentration-dependent manner at the lowest antimony trioxide concentration investigated of 3 mg/m^3 (equivalent to 2.5 mg Sb/m^3) and above.

A positive trend was observed in male rats; however, this was not statistically significant. In female rats, only lung adenomas were observed. In comparison with rats, mice were found to have a greater sensitivity for carcinogenic effects in the lungs. In mice, the incidence of alveolar/bronchiolar carcinomas was increased at the low concentration and above. In rats, the incidences of hyperplasia of the adrenal medulla and of both benign and malignant phaeochromocytomas in the adrenal glands were increased. In male mice, histiocytosis or fibrosarcomas were observed in the skin and the combined incidence of malignant lymphomas in all organs was increased (NTP 2017). The findings in the adrenal glands of rats are not considered relevant for humans (AGS 2018; Greim et al. 2009; Laube et al. 2019).

Tab. 5 Studies of the carcinogenicity induced by antimony trioxide

Author:	NTP 2017
Substance:	antimony trioxide, purity: 99.9%
Species:	**rat**, Wistar Han [Crl:WI (Han)], 50 ♂, 50 ♀
Administration route:	inhalation
Concentration:	0, 3, 10, 30 mg antimony trioxide/m³ (0, 2.5, 8.4, 25 mg Sb/m³)
Duration:	2 years, 5 days/week, 6 hours/day
Toxicity:	3 mg/m³ and above (see Table 1)

		Exposure concentration (mg/m³)			
		0	**3**	**10**	**30**
surviving animals	♂	30/50 (60%)	30/50 (60%)	28/50 (56%)	18/50 (36%)
	♀	39/50 (78%)	38/50 (76%)	28/50 (56%)	20/50 (40%)
tumours and preneoplasms					
lungs:					
alveolar squamous cell metaplasia	♂	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
	♀	0/50 (0%)	5/50 (10%)	3/50 (6%)	1/50 (2%)
alveolar/bronchiolar adenomas	♂	3/50 (6%) [a)]	4/50 (8%)	6/50 (12%)	8/50 (16%)
	♀	0/50 (0%)	2/50 (4%)	6/50 (12%)*	5/50 (10%)*
alveolar/bronchiolar carcinomas	♂	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
alveolar/bronchiolar adenomas or carcinomas	♂	3/50 (6%)	4/50 (8%)	8/50 (16%)	8/50 (16%)
cystic keratinizing epitheliomas or squamous cell carcinomas	♀	0/50 (0%) [b)]	0/50 (0%)	0/50 (0%)	3/50 (6%)
adrenal medulla:					
hyperplasia	♂	1/49 (2%)	2/50 (4%)	4/49 (8%)	8/50 (16%)*
	♀	0/49 (0%)	0/49 (0%)	3/49 (6%)	5/50 (10%)*
benign phaeochromocytomas	♂	1/49 (2%)	0/50 (0%)	2/49 (4%)	7/50 (14%)*
	♀	0/49 (0%)	2/49 (4%)	2/49 (4%)	6/50 (12%)**
benign or malignant phaeochromocytomas	♀	0/49 (0%)	2/49 (4%)	2/49 (4%)	7/50 (14%)**

*p ≤ 0.05, **p ≤ 0.01
[a)] historical controls: inhalation studies 4/150, 2.7% ± 3.1%, 0%–6%; all routes of administration 4/299, 1.3% ± 2.4%, 0%–6%
[b)] historical controls: inhalation studies 0/150; all routes of administration 0/300

Author:	NTP 2017
Substance:	antimony trioxide, purity: 99.9%
Species:	**mouse**, B6C3F1/N, 50 ♂, 50 ♀
Administration route:	inhalation
Concentration:	0, 3, 10, 30 mg antimony trioxide/m³ (0, 2.5, 8.4, 25 mg Sb/m³)
Duration:	2 years, 5 days/week, 6 hours/day
Toxicity:	3 mg/m³ and above (see Table 1)

Tab. 5 *(continued)*

		Exposure concentration (mg/m³)			
		0	**3**	**10**	**30**
surviving animals	♂	38/50 (76%)	30/50 (60%)	27/50 (54%)	17/50 (34%)
	♀	36/50 (72%)	31/50 (62%)	26/50 (52%)	15/50 (30%)
tumours and preneoplasms					
lungs:					
alveolar epithelial hyperplasia	♂	6/50 (12%)	39/50 (78%)**	45/50 (90%)**	49/50 (98%)**
	♀	1/50 (2%)	36/50 (72%)**	49/50 (98%)**	48/50 (96%)**
bronchiolar epithelial hyperplasia	♂	0/50 (0%)	32/50 (64%)**	44/50 (88%)**	44/50 (88%)**
	♀	1/50 (2%)	34/50 (68%)**	48/50 (96%)**	45/50 (90%)**
alveolar/bronchiolar adenomas	♂	10/50 (20%) [a)]	14/50 (28%)	9/50 (18%)	14/50 (28%)
	♀	1/50 (2%) [d)]	10/50 (20%)**	19/50 (38%)**	8/50 (16%)**
alveolar/bronchiolar carcinomas	♂	4/50 (8%) [b)]	18/50 (36%)**	20/50 (40%)**	27/50 (54%)**
	♀	2/50 (4%) [e)]	14/50 (28%)**	11/50 (22%)**	11/50 (22%)**
alveolar/bronchiolar adenomas or carcinomas	♂	13/50 (26%) [c)]	29/50 (58%)**	28/50 (56%)**	34/50 (68%)**
	♀	3/50 (6%) [f)]	22/50 (44%)**	27/50 (54%)**	18/50 (36%)**
skin:					
fibrous histiocytosis	♂	0/50 (0%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
fibrous histiocytosis or fibrosarcomas	♂	0/50 (0%)	1/50 (2%)	3/50 (6%)	4/50 (8%)
squamous cell carcinomas	♀	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
all organs: malignant lymphomas	♀	7/50 (14%)	17/50 (34%)	20/50 (40%)	27/50 (54%)

*$p \leq 0.05$, **$p \leq 0.01$

[a)] historical controls: inhalation studies 35/250, 14% ± 3.7%, 10%–20%; all routes of administration 83/550, 15.1% ± 5.9%, 8%–26%

[b)] historical controls: inhalation studies 42/250, 16.8% ± 5.4%, 8%–22%; all routes of administration 75/550, 27.6% ± 2.6%, 26%–32%

[c)] historical controls: inhalation studies 69/250, 27.6% ± 2.6%, 26%–32%; all routes of administration 147/550, 26.7% ± 6.5%, 16%–38%

[d)] historical controls: inhalation studies 12/249, 4.8% ± 2.7%, 2%–8%; all routes of administration 27/549, 4.9% ± 3.5%, 0%–10%

[e)] historical controls: inhalation studies 17/249, 6.8% ± 3.7%, 2%–10%; all routes of administration 24/549, 4.4% ± 3.5%, 0%–10%

[f)] historical controls: inhalation studies 28/249, 11.3% ± 5.5%, 6%–18%; all routes of administration 50/549, 9.1% ± 5.2%, 2%–18%

Manifesto (MAK value/classification)

The critical effects of antimony and its inorganic compounds are the effects on the lungs after inhalation exposure, which lead to the development of lung tumours in rats and mice. Similar effects may be induced in humans.

Carcinogenicity. The findings suggest that lung cancer mortality in humans may be increased after exposure to antimony trioxide and antimony ore dust. However, because of exposure to a mixture of substances and a lack of data for the concentrations, the epidemiological studies cannot be used for classification (Greim 2007). A new carcinogenicity study in rats and mice demonstrated carcinogenic effects in the lungs after inhalation of antimony trioxide, primarily in mice, to a lesser degree in male rats and hardly in female rats. Mice exhibited a much higher sensitivity than rats (NTP 2017). The animal studies that were previously described had found mainly lung tumours in female rats (Greim 2007). As a NOAEC (no observed adverse effect concentration) cannot be derived for lung tumours in mice and for effects on the lungs of humans or in animal studies and a NOAEC can also not be determined for possible mechanisms of action, antimony and its inorganic compounds remain classified in Carcinogen Category 2.

Germ cell mutagenicity. Substantial evidence is available that antimony compounds cause clastogenic effects in vitro. The new in vivo studies found that antimony compounds have a weak clastogenic potency in mice, but not in rats (Lima et al. 2010; NTP 2017). In the NTP inhalation study, exposure for a period of 12 months led to a 30% to 50% increase in the fraction of tail DNA in the lungs, but not in the leukocytes, at concentrations of 3 mg/m³ and above in male mice and at 30 mg/m³ in female mice. In addition, the fraction of peripheral blood leukocytes with micronuclei

was 1.3 times as high in male and female mice at a concentration of 30 mg/m³ (NTP 2017). Even though antimony compounds do not demonstrate a marked clastogenic potency in vivo, inhalation as the main route of absorption at the workplace is especially important. Trivalent antimony has been found to induce genotoxic effects in soma cells after exposure by inhalation. In addition, the evidence shows that antimony reaches the testes and the ovaries (Greim 2007). On the basis of these findings, antimony and its inorganic compounds have been reclassified in Category 3 A for germ cell mutagens. This classification is supported by the likewise classification of arsenic and its inorganic compounds in Category 3 A for germ cell mutagens. Both metals have a similar effect profile, but that of antimony is less pronounced. This is because trivalent antimony compounds have a more highly metallic character and thus a higher affinity for SH groups than trivalent arsenic compounds.

MAK value, peak limitation and developmental toxicity. In the 2-year inhalation study in rats and mice, chronic inflammation and other findings such as foreign body deposition, lymphocyte infiltration, hyperplasia and fibrosis in the lungs were observed in 96% to 100% of the animals of both species at the lowest Sb_2O_3 concentration tested of 3 mg/m³, which is equivalent to an antimony concentration of 2.5 mg/m³ (NTP 2017). As antimony remains classified in Carcinogen Category 2, the derivation of a MAK value, the peak limitation and the assignment to a pregnancy risk group are omitted.

Sensitization. There are no reliable positive findings for sensitizing effects in humans and no positive results from animal studies or in vitro studies. Therefore, antimony and its inorganic compounds do not require either the "Sh" or "Sa" designation (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

References

AGS (Ausschuss für Gefahrstoffe) (2018) Begründung zu Antimontrioxid und Antimontrisulfid (A-Staub) in TRGS 900, Antimontrioxid und Antimontrisulfid (CAS-Nr.: 1309-64-4 / 1345-04-6). Dortmund: AGS. https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/900/900-antimontrioxid-antimontrisulfid.pdf?__blob=publicationFile&v=2, accessed 30 Jan 2018

Asakura K, Satoh H, Chiba M, Okamoto M, Serizawa K, Nakano M, Omae K (2009) Genotoxicity studies of heavy metals: lead, bismuth, indium, silver and antimony. J Occup Health 51(6): 498–512. https://doi.org/10.1539/joh.l9080

Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82(8): 493–512. https://doi.org/10.1007/s00204-008-0313-y

Dopp E, Hartmann LM, Florea A-M, von Recklinghausen U, Rabieh S, Shokouhi B, Hirner AV, Rettenmeier AW (2006) Trimethylantimony dichloride causes genotoxic effects in Chinese hamster ovary cells after forced uptake. Toxicol In Vitro 20(6): 1060–1065. https://doi.org/10.1016/j.tiv.2006.01.018

ECHA (European Chemicals Agency) (2020) Diantimony trioxide (CAS Number 1309-64-4). Registration dossier. Joint submission, first publication 17 Mar 2011, last modification 29 Apr 2020. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15500/1, accessed 04 May 2020

El Shanawany S, Foda N, Hashad DI, Salama N, Sobh Z (2017) The potential DNA toxic changes among workers exposed to antimony trioxide. Environ Sci Pollut Res Int 24(13): 12455–12461. https://doi.org/10.1007/s11356-017-8805-z

Greim H, editor (2007) Antimony and its inorganic compounds (inhalable fraction). MAK Value Documentation, 2006. In: The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations. Volume 23. Weinheim: Wiley-VCH. p. 1–73. Also available from https://doi.org/10.1002/3527600418.mb744036e0023

Greim H, Hartwig A, Reuter U, Richter-Reichhelm H-B, Thielmann H-W (2009) Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. Crit Rev Toxicol 39(8): 695–718. https://doi.org/10.1080/10408440903190861

Grosskopf C, Schwerdtle T, Mullenders LHF, Hartwig A (2010) Antimony impairs nucleotide excision repair: XPA and XPE as potential molecular targets. Chem Res Toxicol 23(7): 1175–1183. https://doi.org/10.1021/tx100106x

Hartwig A (2013) Metal interaction with redox regulation: an integrating concept in metal carcinogenesis? Free Radic Biol Med 55: 63–72. https://doi.org/10.1016/j.freeradbiomed.2012.11.009

International Antimony Association (2017) i2a's assessment of NTP's long-term carcinogenicity studies on antimony trioxide (ATO). Brussels: International Antimony Association. https://www.antimony.com/download/21/publications/245/i2a-ntp-study-assessment-final-170130, accessed 14 Aug 2018

Kawata K, Yokoo H, Shimazaki R, Okabe S (2007) Classification of heavy-metal toxicity by human DNA microarray analysis. Environ Sci Technol 41(10): 3769–3774. https://doi.org/10.1021/es062717d

Kirkland D, Whitwell J, Deyo J, Serex T (2007) Failure of antimony trioxide to induce micronuclei or chromosomal aberrations in rat bone-marrow after sub-chronic oral dosing. Mutat Res 627(2): 119–128. https://doi.org/10.1016/j.mrgentox.2006.10.012

Koch B, Maser E, Hartwig A (2017) Low concentrations of antimony impair DNA damage signaling and the repair of radiation-induced DSB in HeLa S3 cells. Arch Toxicol 91(12): 3823–3833. https://doi.org/10.1007/s00204-017-2004-z

Kopp B, Zalko D, Audebert M (2018) Genotoxicity of 11 heavy metals detected as food contaminants in two human cell lines. Environ Mol Mutagen 59(3): 202–210. https://doi.org/10.1002/em.22157

Laube B, Michaelsen S, Meischner V, Hartwig A, Epe B, Schwarz M (2019) Classification or non-classification of substances with positive tumor findings in animal studies: guidance by the German MAK Commission. Regul Toxicol Pharmacol 108: 104444. https://doi.org/10.1016/j.yrtph.2019.104444

Lima MIS, Arruda VO, Alves EVC, de Azevedo APS, Monteiro SG, Pereira SRF (2010) Genotoxic effects of the antileishmanial drug Glucantime. Arch Toxicol 84(3): 227–232. https://doi.org/10.1007/s00204-009-0485-0

Mann KK, Davison K, Colombo M, Colosimo AL, Diaz Z, Padovani AMS, Guo Q, Scrivens PJ, Gao W, Mader S, Miller WH Jr (2006) Antimony trioxide-induced apoptosis is dependent on SEK1/JNK signaling. Toxicol Lett 160(2): 158–170. https://doi.org/10.1016/j.toxlet.2005.06.017

NTP (National Toxicology Program) (2017) NTP technical report on the toxicology and carcinogenesis studies of antimony trioxide (CASRN 1309-64-4) in Wistar Han [Crl:WI (Han)] rats and B6C3F1/N mice (inhalation studies). NTP TR 590. Research Triangle Park, NC: NTP. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr590_508.pdf, accessed 14 Aug 2018

Rothkamm K, Barnard S, Moquet J, Ellender M, Rana Z, Burdak-Rothkamm S (2015) DNA damage foci: meaning and significance. Environ Mol Mutagen 56(6): 491–504. https://doi.org/10.1002/em.21944

Scinicariello F, Buser MC (2016) Urinary antimony and leukocyte telomere length: an analysis of NHANES 1999–2002. Environ Res 150: 513–518. https://doi.org/10.1016/j.envres.2016.06.044

Tweats DJ, Blakey D, Heflich RH, Jacobs A, Jacobsen SD, Morita T, Nohmi T, O'Donovan MR, Sasaki YF, Sofuni T, Tice R (2007) Report of the IWGT working group on strategies and interpretation of regulatory in vivo tests I. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards. Mutat Res 627(1): 78–91. https://doi.org/10.1016/j.mrgentox.2006.10.005