Health Council of the Netherlands

Antimony and antimony compounds

Evaluation of the carcinogenicity and genotoxicity

Gezondheidsraad
Health Council of the Netherlands

Aan de staatssecretaris van Sociale Zaken en Werkgelegenheid

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Geachte staatssecretaris,

Graag bied ik u hierbij het advies aan over de gevolgen van beroepsmatige blootstelling aan antimoon en antimoonverbindingen.

Dit advies maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Daarbij heeft de subcommissie op verzoek van uw ministerie de formulering van de categorie waarin sommige antimoonverbindingen vallen, aangepast; niet een numerieke aanduiding maar een standaardzin vormt de hoofdformulering. Het advies is getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb het advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. L.J. Gunning-Schepers,
voorzitter

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Subcommittee on the Classification of Carcinogenic Substances of
the Dutch Expert Committee on Occupational Safety,
a Committee of the Health Council of the Netherlands

to:

the State Secretary of Social Affairs and Employment

No. 2011/33, The Hague, December 2, 2011

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, Agriculture & Innovation, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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INAHTA

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. De evaluatie en beoordeling worden verricht door de Subcommissie Classificatie van carcinogene stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de raad, hierna kortweg aangeduid als de commissie. In het voorliggende advies neemt de commissie antimoon en antimoonverbindingen onder de loep. Antimoontrioxide is commercieel het meest belangrijk en ook de meest onderzochte stof.

Antimoontrioxide wordt door de commissie ervan verdacht kankerverwekkend te zijn voor de mens. Daarom beveelt de commissie aan deze stof in categorie 2 te classificeren. Van antimoon en de overige antimoonverbindingen zijn de gegevens niet voldoende om de kankerverwekkende eigenschappen ervan te evalueren (categorie 3).*

* Volgens het nieuwe classificatiesysteem van de Gezondheidsraad (zie bijlage H).

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the Subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Standards of the Health Council, hereafter the Committee. In this report the Committee evaluated antimony and antimony compounds. Antimony trioxide is commercially the most important and also the most investigated compound.

The Committee suspects that antimony trioxide is carcinogenic to humans. Therefore, it recommends classifying the compound in category 2. The Committee is furthermore of the opinion that the available data are insufficient to evaluate the carcinogenic properties of antimony and other antimony compounds (category 3).*

* According to the new classification of the Health Council (see Annex H).

Chapter 1

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification (see Annex A). In addition to classifying substances, the Health Council also assesse the genotoxic properties of the substances in question. The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex H). The criteria used for classification are partly based on an EU-directive (see Annex I). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question.

This report contains the evaluation of the carcinogenicity of antimony and antimony compounds. Since stibine has been evaluated recently by the Health Council, the compound was not included in this report.[1]

1.2 Committee and procedures

The evaluation is performed by the Subcommittee on Classifying Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee. The members of the Committee are listed in Annex B.

In 2011 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation and recommendation of the Committee is standardly based on scientific data, which are publicly available. The starting points of the Committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of antimony and antimony compounds, such an IARC-monograph is available, of which the summary and conclusion of IARC is inserted in Annex D.

More recently published data were retrieved from the DIMDI database Medline and XToxline, and Chemical Abstracts (Scifinder CAPlus). The last updated online search was in November 2011. The new relevant data were included in this report.

Chapter 2

General information

2.1 Identity and physicochemical properties

The element antimony (stibium; Sb) is a member of Group V of the Periodic Table of Elements. It can exhibit a valency of -3, 0, +3 or +5. Antimony is a metalloid (it displays both metallic and nonmetallic characteristics) that occurs in over 100 mineral ores, also in combination with other metals such as lead, copper, arsenic.[2,3] The organic and inorganic antimony compounds for which genotoxicity and carcinogenicity data were available, are evaluated in this report.

Among the antimony compounds antimony trioxide is the commercially most significant compound worldwide. It is produced from stibnite ores (antimony trisulphide) or as a by-product of lead smelting and production. In EU antimony trioxide is no longer produced from ores but by oxidation of the antimony metal.

In aqueous solution, both trivalent and pentavalent antimony are stable and both forms may interconvert with the trivalent form being dominant under (reducing) physiological conditions.[4]

Most severe occupational exposure occurs during handling of antimony ores, and manufacturing of antimony compounds (inhalation). As most antimony compounds are solids the particle size is an important parameter for the presence of inhalable/respirable particles.[2]

The identity, some physicochemical properties and use of antimony and its inorganic and organometal compounds are given in Tables 1 and 2 on the next pages.[2,3,5-7]

2.2 IARC conclusion

In 1989, IARC concluded that there is inadequate evidence for the carcinogenicity of antimony trioxide and antimony trisulphide in humans.[5] Sufficient evidence was present for the carcinogenicity of antimony trioxide in experimental animals and limited evidence for antimony trisulphide in experimental animals. Therefore, according to the IARC guidelines, antimony trioxide was considered to be possibly carcinogenic to humans (Group 2B) and antimony trisulphide is not classifiable as to its carcinogenicity to humans (Group 3).

The other antimony compounds were not evaluated by the IARC.

See Annex D for summary of IARCs conclusions.

Table 1 Chemical and physical properties of the evaluated antimony and antimony compounds.

	Antimony	Antimony trioxide	Antimony trisulphide	Antimony trichloride
Physical form	silvery or gray, lustrous metalloid	white, crystalline solid	natural stibnite: pale to dark greyish, crystalline solid; pure $Sb_2(III)S_3$: yellow-red amorphous powder	colourless, hygroscopic crystals (fuming in air)
Molecular weight	121.75	291.50	339.68	228.10
Melting/boiling point	630/1635 °C	656 °C/sublimes	563/1150 °C	73.4/220 °C
Density (g/cm^3)	6.684 at 25 °C	5.2 (senarmontite) 5.67 (valentinite)	4.12 (amorphous solid) 4.64 (stibnite)	3.14 at 25 °C
Vapour pressure				1 mm at 49.2 °C (subl)
Solubility	insoluble in water; soluble in hot concentrated H_2SO_4	very slightly in water; soluble in KOH, HCl and acetic acid; insoluble in organic solvents	1.75 mg/L at 18 °C in water; soluble in HCl and ethanol; insoluble in acetic acid	soluble in ethanol, carbon disulphide, diethyl ether, carbon tetra-chloride, methylene chloride, tartaric acid and water (6.0 g/L at 0°C); insoluble in quinoline and other organic bases
Conversion factor	no data	no data	no data	no data
Use	present in alloys, *e.g.* with lead in lead-batteries	flame-retardant in rubber, plastics, adhesives, textiles, and paper; other uses: production of	production of explosives, pigments, antimony salts and ruby glass	mordant for patent leather and for dyeing, for bronzing iron, as chemical reagent/

			production of explosives, pigments, antimony salts and ruby glass	catalyst, colouring zinc black, in manufacture of other antimony salts and as veterinary escharotic and dehorning agent
EU classification	not classified	H351: Suspected of causing cancer	not classified	H314: causes severe burns and eye damage H411: toxic to aquatic life with long lasting health effects
	Antimony pentoxide	Antimony pentachloride	Antimony potassium tartrate	Potassium antimonate
Physical form	yellowish-white powder or deep yellow crystals	colourless or red-yellow oil or liquid; offensive odour	colourless crystals to white powder	no data
Molecular weight	323.5	299.01	667.80	208.86
Melting/boiling point	decomp at 380 °C	4/140 °C	loses H_2O at 100 °C	no data
Density (g/cm^3)	3.78	no data	2.607	no data
Vapour pressure	no data	1 mm at 22.7 °C	no data	no data
Solubility	insoluble in water; slightly soluble in warm KOH solution	decomposes in water; soluble in HCl, HBr, carbon disulphide, carbon tetrachloride and chloroform	no data	no data
Conversion factor	no data	no data	no data	no data
Use	no data	chemical reagent	anti-parasitic (e.g. anti-schistosomal) drug until 1990s	chemical reagent
EU classification	not classified	H314: causes severe burns and eye damage H411: toxic to aquatic life with long lasting health effects	H302 : Harmful if swallowed H322: Harmful if inhaled H411: toxic to aquatic life with long lasting health effects	no data
	Potassium hexahydroxo-antimonate	Trimethylanti-mony dichloride	Trimethylstibine	
Physical form	no data	no data	liquid	
Molecular weight	262.90	237.77	166.9	
Melting/boiling point	no data	no data	no data/80.6 °C	
Density (g/cm^3)	no data	no data	1.523 at 15 °C	
Vapour pressure	no data	no data	no data	
Solubility	no data	no data	slightly soluble in water	
Conversion factor	no data	no data	no data	
Use	chemical reagent		present in biological fermentation gases from landfills and sewage treatment plants	
EU classification	no data	no data	no data	

Table 2 Identification of the evaluated antimony and antimony compounds.

Compound	Molecular formula	Synonyms	CAS#	EC/EINECS#
Antimony	Sb	antimony black; antimony powder; antimony regulus; CI 77050; stibium	7440-36-0	231-146-5
Antimony trioxide	Sb_2O_3	diantimony trioxide; antimonious oxide; antimony (III) oxide; antimony sesquioxide; antimony white; AP 50; flowers of antimony; CI 77052; CI Pigment White 11; senarmontite (CAS 12412-52-1); valentinite (CAS 1317-98-2)	1309-64-4	215-175-0
Antimony trisulphide	Sb_2S_3	diantimony trisulfide; antimonous sulfide; antimony sesquisulfide; antimony needles; antimony orange or vermilion or black; CI 77060; CI Pigment Red 107; stibnite (CAS 1317-86-8)	1345-04-6	215-713-4
Antimony trichloride	$SbCl_3$	antimonous chloride; antimony(III) chloride; CI 77056; trichlorostibine	10025-91-9	233-047-2
Antimony pentoxide	Sb_2O_5	antimonic 'acid'; antimonic oxide; diantimony pentoxide; stibic anhydride	1314-60-9	215-237-7
Antimony pentachloride	$SbCl_5$	antimony(V) chloride; antimonic chloride; antimony perchloride; pentachloroantimony	7647-18-9	231-601-8
Antimony potassium tartrate	$C_8H_4O_{12}Sb_2K_2 \bullet 3H_2O$	antimonyl potassium tartrate; ENT 50,434; potassium antimonyl-d-tartrate; tartar emetic; tartarized antimony; tartrated antimony	28300-74-5	none
Potassium antimonate	$KSbO_3$	no data	14459-60-0	238-451-2
Potassium hexahydroxoantimonate	$KSb(OH)_6$	potassium antimonate, hydrated	12208-13-8	235-387-7
Trimethylantimony dichloride	$(CH_3)_3SbCl_2$	dichlorotrimethylantimony	13059-67-1	235-952-8
Trimethylstibine	$Sb(CH_3)_3$	antimony trimethyl	594-10-5	209-824-7

Chapter 3

Carcinogenicity

3.1 Observations in humans

Human data are summarized in Annex E.

Several studies cited by Integrated Risk Information System (US EPA) and IARC, report that smelter workers exposed to antimony compounds may develop pneumoconiosis. Antimony accumulates in the lung and is retained for long periods of time dependent on solubility and particle size. No carcinogenic effects were reported in these studies.

In a cohort study among male workers of an antimony smelter in England mortality was followed from 1961-1992 involving 357 men.[8] They were divided into four occupational groups: antimony workers, maintenance workers, zirconium workers and others (office workers and management staff). Subsequently, the workers were subdivided according to their cause of death and subdivided into groups employed before 1961 and after 1960. Expected death rates were based on local rates. A significant increase in deaths from lung cancer was found (32 versus 14.7 expected (regional mortality rate), $p<0.001$) in antimony workers before 1961. Since antimony workers had been exposed to several agents, including antimony and its oxides, arsenic and arsenic oxide, sulphur dioxide, and polycyclic aromatic hydrocarbons, the excess of lung cancer could not be linked to a certain substance. No estimate of antimony exposure was reported.

In another study among male workers of an antimony smelter in Texas employed between 1937 and 1971, a standard mortality ratio of 1.39 (90% confidence interval 1.01-1.88) was found for lung cancer when white males and Spanish-surnamed males were combined and irrespective of duration of employment.[9] A positive trend for mortality was seen with increased duration of employment. Only arsenic is considered here as possible confounding factor. Industrial hygiene estimates of antimony and arsenic levels (1975 and 1976) range from 50-6,200 μg/m^3 for antimony, and 1-47 μg/m^3 for arsenic.

Another study investigated the correlation between occupational cancer and the metals used in art glass industry in Sweden.[10] Cases (n=888) were men that had died between 1950 and 1982 from eleven parishes. Cases were evaluated against local men that had died from causes other than cancer or cardiovascular diseases. Exposure estimates were based on questionnaires on metal consumption by seven glassworks to yield none, low or high exposure for 10 metals, mainly as oxides. Co-exposure to these metals occurred. For colon cancer an increased risk was found for high exposure to antimony (OR 5.0 (95% confidence interval 2.6-9.6), together with one for lead (OR 2.5 (95% confidence interval 1.4-4.4). For lung cancer no correlation with any metal could be found.

No conclusive studies without co-exposure to other possibly carcinogenic agents were available.

3.2 Carcinogenicity studies in animals

Animal data are summarized in Annex F.

Antimony trioxide

Fischer 344 rats (n=65/sex/dose) were exposed by inhalation to 0, 0.06, 0.5 or 4.5 mg/m^3 of antimony trioxide for 6 hr/day, 5 days/week for 52 weeks.[11] Five animals per sex per group were terminated after six and twelve months of exposure and 6 months post-exposure. All survivors were terminated at twelve months post-exposure. The Mass Median Aerodynamic Diameter (MMAD) was 3.76 μm with a Geometric Standard Deviation (GSD) of 1.79 at all concentrations indicating the dust consisted mostly of respirable particles. Survival was comparable between exposed and control groups. Body and lung weights were unaffected by the exposure. Clinical pathology showed no treatment related effects. Foreign particulate material was noted in the lungs and peribronchial lymph nodes during the exposure, as well as the observation period (near-steady state lung burden in all groups already at six months exposure). Histopathologi-

cal effects observed were chronic interstitial inflammation, interstitial fibrosis, granulomatous inflammation, and bronchiolar/alveolar hyperplasia in the lungs. Pulmonary carcinomas were only seen in one male at 0 and at 4.5 mg/m^3 and in one female at 0.5 mg/m^3. These carcinomas are not considered to be related to antimony trioxide exposure. Concentrations in lungs at the highest dose level amounted up to 1420-1500 µg antimony trioxide per g at the end of exposure and resulted in a reduced clearance at this dose level during the post-exposure period.

Watt (in an otherwise unpublished PhD thesis, 1983) exposed female, 19 weeks old Fischer rats (n=50/dose) to 0, 1.6 and 4.2 mg/m^3 of antimony trioxide for 6 hours/day, 5 days/week for up to 13 months.[5,12] Particle size (Ferret's diameter) was 0.44 and 0.40 µm with standard deviations of 2.23 and 2.13 at low and high concentration, respectively. Non-neoplastic pulmonary effects including focal fibrosis, adenomatous hyperplasia (high dose only), multinucleated giant cells, cholesterol clefts, pneumonocyte hyperplasia and pigmented macrophages were observed in all exposed animals; severity of these effects increased with concentration and duration of exposure. The incidence of lung tumours (scirrhous carcinomas, squamous cell carcinomas, and bronchoalveolar adenomas) was increased to 14/18 at 4.2 mg/m^3 (p <0.001) compared to 0/13 in control animals.

Groth *et al.* (1986) exposed eight months old Wistar rats to 0 or 45 mg/m^3 antimony trioxide for 7 hours/day, 5 days/week for up to 52 weeks.[5,13] The MMAD was 2.80 µm (GSD not given). Histologically, interstitial fibrosis, alveolar-wall cell hypertrophy and hyperplasia, and cuboidal and columnar cell metaplasia of the lungs was observed with an increased size of area affected after twelve months of exposure and an increased extent of fibrosis after four-five months of recovery. The incidence of lung tumours (squamous cell carcinomas, scirrhous carcinomas, bronchioloalveolar adenomas/carcinomas) was increased in females (19/70 animals; p <0.001; none in control animals). The concentration of elemental antimony in all organs of exposed animals was significantly higher than in the control group (p < 0.01; lungs: 38,300 µg Sb/g for males and 25,600 µg Sb/g for females; control: 9.2 for males and 10.5 for females). Arsenic values were not significantly different from controls; male lungs contained a significantly higher value than female lungs (213 and 150 µg As/g, respectively; $p \leq 0.02$).

The Committee is aware that the US National Toxicology Programme is at the moment (November 2011) performing a two-year inhalation study in rats and mice (n=50/sex/dose) administering 0, 3, 10 or 30 mg/m^3 of antimony trioxide.

Regarding the three animal studies described above, the Committee noted that all these studies showed deficiencies in exposure design, in that for instance only exposure for only 1 year was accomplished and not for longer periods. Another deficiency in design was seen in the study by Groth et al. They exposed the animals not before they were 8 month old. Furthermore, in the Groth-study only one level was used that was ten times higher than that used in the Newton- and Watt-studies. Also in the Groth-study, lung burden was consequently much higher compared to the other two studies. The Watt-study only exposed females (49-51 in total per dose level), and animals were necropsied in seven groups of varying size at different time intervals making the number of animals alive towards the end of the experiment small (13, 17 and 18 at 0, 1.6 and 4.2 mg/m^3, respectively). This study also had widely varying levels of exposure over time. Similar particle sizes were calculated by Newton calculated as MMAD from the different parameters determined, for the three studies.[11], However, the pathologist who had also seen the slides from the Watt- and Groth-studies stated that, although the exposure level of Watt was similar to the high exposure level Newton used, the exposed rats from the Watt-study showed more damage and appeared to have considerably more test material in the lungs.[11]

In the study by Newton *et al.* it was shown that antimony trioxide reduced the pulmonary clearance rate in a dose dependent manner, interpreted by the authors as a toxic effect of diantimony trioxide rather than a general effect due to pulmonary overload.[11,14] However, it is wellknown that reduced lung clearance rate at chronic exposure of rats to poorly soluble particles can result in pulmonary overload, subsequently followed by an inflammatory response, epithelial cell hypertrophy and/or hyperplasia and squamous metaplasia. The persistence of these recurrent tissue damage and repair over chronic time periods can lead to secondary development of lung tumours. Thus, it could be speculated that the neoplastic effects seen in the Watt and Groth studies is a result of pulmonary overload and an inflammatory response to particulate diantimony trioxide.[14] The tumour development as a consequence of pulmonary overload is an inflammatory-driven process which usually takes over a year (15-18 months) of poorly soluble particles exposure via inhalation. In the present studies on antimony trioxide, development of lung tumours occurred earlier.

Antimony and antimony compounds

Groth *et al.* (1996) have also exposed rats to antimony ore (antimony trisulphide) under the same conditions as for antimony trioxide, and at a comparable high

concentration.[13] The incidence of lung tumours (squamous cell carcinomas, scirrhous carcinomas, bronchioloalveolar adenomas/carcinomas) was increased in females (17/68 animals; $p < 0.001$) compared to none in the control group. The antimony lung burden at nine months of exposure in the treated group was significant ($p \leq 0.05$; 7,140 µg Sb/g for males and 4,520 µg Sb/g for females; control: 9.2 for males and 10.5 for females). Arsenic lung burden in the treated groups was comparable to the control lung burden.

Limitedly described studies in mouse and rat exposed to antimony potassium tartrate by drinking water for life to a dose below the maximum tolerated dose, were available with comparable tumour incidence compared to controls.[15,16]

Chapter 4

Genotoxicity

In vitro and *in vivo* genotoxicity data are summarized below, and are presented in Annex G. No data on antimony trisulphide (antimony ore) were available.

4.1 *In vitro* assays

Antimony trioxide, trichloride, pentachloride, pentoxide and antimony potassium tartrate are not mutagenic in bacterial reverse mutation assays using *Salmonella typhimurium* or *Escherichia coli* with and without metabolic activation.[17-22] Antimony trichloride did not induce DNA damage in an SOS chromosome test without metabolic activation and was negative in the umu test.[23,24] Antimony trioxide, trichloride and pentachloride showed differential killing in DNA repair-proficient compared to repair-deficient strains of Bacillus subtilis, while antimony pentoxide did not.[20,21]

The trivalent antimony compounds antimony trioxide and antimony trichloride were positive in *in vitro* tests including chromosomal aberration[19], sister chromatid exchange[21,25], micronucleus test[26-28] and Comet assay[26,28]. Exogenous metabolic activation was only included in a chromosomal aberration test. A mouse lymphoma test was negative for antimony trioxide with or without metabolic activation.[19]

In general, the pentavalent antimony compounds antimony pentoxide, antimony pentachloride, trimethylantimony dichloride and potassium hexahydroxoantimonate were negative. Antimony pentachloride, antimony pentoxide and

trimethylantimony dichloride were negative in the sister chromatid exchange.[21,29] Trimethylantimony chloride was negative in the chromosomal aberration and micronucleus assay possibly due to inefficient uptake of the compound.[29] Potassium antimonate was positive in a micronucleus test and FISH analysis showed that both centromeric and non-centromeric micronuclei were increased relative to controls indicating clastogenic as well as aneugenic mechanisms may be active.[30] Potassium hexahydroxoantimonate and trimethylantimony dichloride, and the trivalent antimony potassium tartrate did not induce DNA damage in a plasmid DNA-nicking assay, while the organoantimony compounds stibine and trimethylstibine did induce DNA damage.[31]

4.2 *In vivo* assays

In vivo tests are only available for trivalent antimony compounds. Gurnani reported induction of chromosomal aberrations after repeated treatment (21 times) of mice with 400-1000 mg/kg bw antimony trioxide or single treatment with 70 mg/kg bw antimony trichloride.[32,33] Antimony trioxide did not induce chromosomal aberrations after a single dose in mice or repeated dose in rats, nor micronuclei after a single or repeated dose in the bone marrow of mice or rats.[34] However, the purity of the antimony oxide and/or the health status of the animals in the Gurnani-study is questionable as the severe toxicity observed on repeated exposure in the Gurnani-study at the same period and same level of exposure of 1000 mg/kg bw/day as in the studies performed by Elliott *et al.* and Kirkland *et al.* were not seen in the latter two studies.[19,34]The fact that a different strain or species was used may also have played a role: Gurnani used Swiss albino mice, Elliott used CD-1 mice, and Kirkland used Sprague-Dawley rats.

Antimony trioxide did not induce unscheduled DNA repair in rat hepatocytes *in vivo* or affect the sperm morphology in mice after repeated exposure.[19,33] Cavallo studied genotoxicity in human peripheral lymphocytes from a group of 23 textile workers exposed to antimony trioxide as judged from their work tasks. By monitoring via personal air sampling two levels of exposure were distinguished: 0.052 and 0.12 µg/m^3. No increased incidence of sister chromatid exchanges or micronuclei was noted. However, the enzyme (Fpg)-modified Comet assay showed a probable relationship (RR= 14.2) between moderate levels of oxidative DNA damage and exposure to antimony, with a significantly higher proportion of workers of the high exposure group having oxidative DNA damage compared to the control group.[35]

4.3 Carcinogenic mechanism of action

Occupational exposure to antimony is predominantly via inhalation of dust or aerosol. Antimony compounds accumulate in the lung, and can be retained for long periods of time dependent on solubility and particle size. This may cause inflammation resulting in pneumoconiosis. Absorption of antimony is known to occur as it was excreted in urine of humans.[2,5,36]

From the above mentioned genotoxicity studies it is clear that the antimony compounds antimony trioxide, antimony trichloride and potassium antimonate show signs of clastogenicity *in vitro*. *In vivo* however, negative results on chromosome aberrations and micronuclei were obtained in mouse and rat for all trivalent and pentavalent antimony salts mentioned in this report. It is not possible to conclude whether the negative results are due to a lack of genotoxic potential or due to inability of the test to detect an effect at low concentrations achieved in the bone marrow (EU-RAR). The only positive *in vivo* studies in mice (Gurnani *et al.* 1992[32], Gurnani *et al.* 1992[33]) showed experimental deficiencies.

Despite this lack of conclusive data on local genotoxicity in the lung, the overall expert judgement by EU Technical Committee of New and Existing Substances (EU TC NES) is that the most likely mechanism for carcinogenicity for antimony trioxide appears to be impaired lung clearance and particle overload followed by an inflammatory response, fibrosis and tumours.[14] However, the issue whether genotoxicity or particle overload may be the reason for antimony-trioxide induced lung tumours is still not entirely clear and several mechanistic options are still being explored.

An increased incidence of delayed DNA-fragmentation as a marker of apoptosis has been observed in human fibroblasts, CHO cells and human bronchial epithelial cells (BES-6) after a four-hour treatment with antimony trichloride (50-200 µM); apoptosis was confirmed by microscopy.[27] Potassium antimony tartrate induces apoptosis in human myeloid leukaemic HL60 cells and in lymphoic tumoral cells.[37] The apoptosis was associated with loss of mitochondrial potential, enhanced cellular production of reactive oxygen species and depended on caspase activity. Depleting or increasing GSH levels resulted respectively, in increase or decrease of the apoptotic activity of potassium antimony tartrate suggesting the redox-status of the cell is an important factor in antimony toxicity.

Trivalent antimony is known to react with sulfhydryl groups of proteins.[2] Andrewes *et al.* showed that a certain amount of the thiol-reducing agents glu-

tathione and L-cysteine is sufficient to convert the pentavalent trimethylantimony dichloride to trimethylstibine and the resulting trivalent antimony may induce DNA damage to DNA plasmid if sufficient thiols are present (pentavalent does not).[31] Tirmenstein et al. showed that potassium antimony tartrate caused oxidative stress in primary cardiomyocytes.[3] Since it coincided with cytotoxicity it was not clear whether this finding could explain the mechanism of antimony's genotoxicity. Schaumlöffel and Gebel showed that the number of micronuclei formed upon incubation with antimony trichloride could not be suppressed by co-incubation with superoxide dismutase or catalase, suggesting that oxidative stress induction is no crucial step in the production of DNA damage.[28]

Kawata *et al.* (2007) compared the gene expression pattern of heavy metals including antimony potassium tartrate, to those of two chemicals known to generate reactive oxygen species and one chemical known to be a carcinogen and mutagen.[38]

Microanalysis of the DNA in treated human hepatoma derived cell line (HepG2) showed that all metals, including antimony, showed a gene expression pattern similar to DMNQ, a substance which is known to generate reactive oxygen species. Gebel and coworkers showed that while arsenic(III) did generate DNA strand lesions and DNA-protein crosslinks, antimony(III)trichloride did induce DNA strand lesions, but no DNA-protein crosslinks, indicating that there are differences between antimony and arsenic although they are often mentioned in one breath[26,28] They also determined that not more than 11% of antimony(III) had been oxidized to the pentavalent species.

Takahashi *et al.* (2002) showed that antimony trichloride and antimony potassium tartrate may affect the repair of DNA-double strand breaks induced by gamma-irradiation in CHO cells.[39] The inhibition was dose-dependent and for antimony trichloride at the level of the mean cytotoxic dose, while for potassium antimony tartrate inhibition was only seen above the mean cytotoxic dose. Arsenic compounds also inhibit DNA repair through inhibition of the incision step of nucleotide excision repair. The authors do not comment on a possible relationship between cytotoxicity and induction of DNA-double strand breaks.

Chapter 5

Classification

5.1 Evaluation of data on carcinogenicity and genotoxicity

Data on epidemiological studies are insufficient, in that all exposures concerned mixed exposure with other compounds with possible or unknown carcinogenic properties. Three animal inhalation studies have been reported, all using rats and antimony trioxide. One study showed no exposure-related tumour development, whereas in the other two studies an increased incidence of lung tumours in female rats was found (in one of these studies also for antimony trisulphide).

Despite some deficiencies in study design of the animal studies, at least two of them showed positive results. The Committee emphasizes that in these studies the development of carcinomas already occurred within a year. Therefore, the Committee is of the opinion that the animal data show limited evidence for carcinogenicity of antimony trioxide. Animal data on antimony and other antimony compounds are insufficient to evaluate the carcinogenic potential.

Limited data are available on the genotoxicity of antimony compounds. Some positive *in vitro* results were available for antimony trioxide and antimony trichloride, but not for antimony pentoxide. The only positive *in vivo* study was questionable. Additional *in vitro* studies indicate that mechanisms may include induction of oxidative stress and inhibition of DNA repair.

A possible non genotoxic mechanism underlying tumour induction by antimony trioxide may be impaired lung clearance and particle overload followed by an inflammatory response, fibrosis and tumours.[14] In this case antimony trioxide can be regarded as a threshold carcinogen.[14]

5.2 Recommendation for classification

The Committee suspects that antimony trioxide is carcinogenic to humans. Therefore, it recommends classifying the compound in category 2. The Committee is furthermore of the opinion that the available data are insufficient to evaluate the carcinogenic properties of antimony and other antimony compounds (category 3).* The Committee is aware of the NTP 2-year inhalatory study on antimony trioxide. Since the studies on which the current proposal is based showed deficiencies the Committee will reevaluate the classification as soon as the NTP data become available.

* According to the new classification of the Health Council (see Annex H).

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as

part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10-4 and 10-6 per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the committee is given in Annex B.

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Quality of Life, Zeist; Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- J. van Benthem
  Genetic Toxicologist, National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, SHELL International BV, The Hague
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- Ms M.J.M. Nivard
  Molecular Biologist and Genetic Toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Dow Chemicals NV, Terneuzen
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- G.B. van der Voet, *scientific secretary*
  Toxicologist, Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2011 for public review. The following organisations and persons have commented on the draft document:

- National Institute for Occupational Safety and Health, Cincinnati, USA
- International Antimony Asssociation, Brussels, Belgium

Annex **D**

IARC Monograph

VOL: 47

Antimony trioxide

CAS No.: 1309-64-4 (1317-98-2 – Valentinite and 12412-52-1 – Senarmontite)

Antimony trisulfide

CAS No.: 1345-04-6 (1317-86-8 – Stibnite)

Summary of Data Reported and Evaluation

Exposure data

Antimony trioxide is produced from stibnite ores (antimony trisulfide) or as a by-product of lead smelting and production. It is used mainly in fire-retardant formulations for plastics, rubbers, textiles, paper and paints. It is also used as an additive in glass and ceramic products and as a catalyst in the chemical industry. Occupational exposure may occur during mining, processing and smelting of antimony ores, in glass and ceramics production, and during manufacture and use of products containing antimony trioxide.

Antimony trisulfide is used in the production of explosives, pigments, antimony salts and ruby glass. Occupational exposure may occur during these processses and also during the mining, processing and smelting of ores containing antimony trisulfide.

Experimental carcinogenicity data

Antimony trioxide was tested for carcinogenicity by inhalation exposure in male and female rats of one strain and in female rats of another strain, producing a significant increase in the incidence of lung tumours (scirrhous and squamous-cell carcinomas and bronchioloalveolar tumours) in females in both studies. No lung tumour was seen in male rats.

Antimony ore concentrate (mainly antimony trisulfide) was tested for carcinogenicity by inhalation exposure in male and female rats of one strain, producing a significant increase in the incidence of lung tumours (scirrhous and squamous-cell carcinomas and bronchioloalveolar tumours) in females. No lung tumour was seen in males.

Human carcinogenicity data

The available data were inconclusive.

Other relevant data

Antimony trioxide causes pneumoconiosis in humans. One study of women exposed to dusts containing metallic antimony, antimony trioxide and antimony pentasulfide suggested that they may have had an excess incidence of premature births and spontaneous abortions and that their children's growth may have been retarded.

Evaluation

There is *inadequate evidence* for the carcinogenicity of antimony trioxide and antimony trisulfide in humans.
There is *sufficient evidence* for the carcinogenicity of antimony trioxide in experimental animals.
There is *limited evidence* for the carcinogenicity of antimony trisulfide in experimental animals.

Overall evaluations

Antimony trioxide is possibly carcinogenic to humans (Group 2B).

Antimonytrisulfide is not classifiable as to its carcinogenicity to humans (Group 3).

Annex **E**

Human data

See page 39.

Study type	Population	Total dose, Exposure duration, Follow-up	Effect	Relative Ratio (95% Conf. Interval)
prospective cohort (control: regional mortality rates)[8]	antimony workers (n=1420)	no data on dose, ≥3 months before 1993, up to >35 years	lung cancer mortality (employees joined before 1961: 32 cases (14.7 control, $p<0.001$)	-
retrospective cohort (control: Texas white male/Spanish-surnamed population)[9]	white male (n=91)/ Spanish-surnamed male (n=923) antimony smelter workers (total n = 1014)	no data on dose, ≥3 months in 1937-1971, from 1989	lung cancer mortality: 28 cases (control combined white males/Spanish surnamed; positive trend with increased duration of employment)	SMR: 1.39 (90% CI 1.01-1.88)
			liver/gall bladder cancer mortality: 6 cases (control Spanish surnamed)	SMR: 1.58 (0.57-3.44)
			stomach cancer mortality: 7 cases (control Spanish surnamed)	SMR: 1.24 (0.5-2.55)
retrospective cohort (control local male population deceased 1950-1982; n=3523)[10]	art glass industry male workers in Sweden (n<888)	glassworks consuming no, small or large amounts of the metal; inhalation and/or oral exposure (glass blowers) no data on duration, no data on follow- up	colon cancer mortality	OR: 1.4 (0.6-3.3), 1.8 (0.8-13.8) and
			for lung cancer mortality: no relation with any metal	5.0 (2.6-9.6) for no, low or high exposure

Annex **F**

Animal data

Species, sex (no./ group)	Dose, freq	X_{po}	X_{pe}	no. survivors, no. animals with tumours	comments/specified tumours	Reference
Antimony ore concentrate (antimony trisulphide)						
rat (Wistar), M/F (90/sex)	0, 36-40 mg/m^3 (inhalation)[a], 7 h/d; 5d/week	up to 52 weeks	up to 72 weeks	survivors not specified, tumours M: 0, 0 F: 0, 17/68	36-40: 9 squamous-cells carcinomas, 4 scirrhous carcinomas, 6 bronchioloalveolar adenomas or carcinomas (not further specified) Sb lung burden: 0: 9.2 (M) and 10.5 (F); 36-40: 7,140 (M) and 4,520 (F) µg Sb/g at 9 months of exposure ($p \leq 0.05$) As lung burden: 0: 6.5 (M) and 18.5 (F); 10.1 (M) and 13.9 (F) µg As/g	[13]

Antimony trioxide

rat (Fischer 344), M/F (65/sex)	0, 0.06, 0.5, 4.5 mg/m^3 (inhalation)[b], 6 h/d, 5d/week	up to 52 weeks	up to 24 months	survivors M: 56, 56, 58, 56% F: 48, 40, 66, 60% tumours M: 1/65, 0, 0, 1/65 F: 0, 0, 1/65, 0	pulmonary carcinomas: not antimony trioxide related Sb_2O_3 lung burden: 1420 (M) and 1500 (F) µg Sb_2O_3/g, at end of exposure (control: no Sb_2O_3)	11
rat (Wistar), M/F (90/sex)	0, 45 mg/m^3 (inhalation)[c] , 7 h/d; 5d/week	up to 52 weeks	up to 72 weeks	survivors not specified tumours M: 0, 0 F: 0, 19/70	45: 9 squamous-cells carcinomas, 5 scirrhous carcinomas, 11 bronchioloalveolar adenomas or carcinomas (not further specified) Sb lung burden: 0: 9.2 (M) and 10.5 (F); 45: 38,300 (M) and 25,600 (F) µg /g at 9 months of exposure ($p < 0.01$) As lung burden: 0: 6.5 (M) and 18.5 (F); 45: 213 (M) and 150 (F) µg /g ($p \leq 0.05$)	13
rat (Fischer, CDF from Charles River), F (49-51)	0, 1.6 ± 1.5, 4.2 ± 3.2 mg/m^3 (inhalation) [d], 6 h/d; 5d/week	up to 13 months	up to 25 months	survivors not specified tumours 0: 0/13 1.6: 1/17 4.2: 14/18 at 12 months after exposure	1.6: 1 bronchioloalveolar adenoma 4.2: 3 bronchioloalveolar adenomas, 9 scirrhous carcinomas ($p<0.01$), 2 squamous-cell carcinomas Another 6/18 scirrhous carcinomas between end of exposure and final termination at 4.2 (control 1/6 bronchioloalveolar adenoma)	5,12
Sinclair S-1 miniature swine, F(2, 3, 3 at 0, 1.6 and 4.2 mg/m^3)	0, 1.6 ± 1.5, 4.2 ± 3.2 mg/m^3 (inhalation)[e], 6 h/d; 5d/week	1 year	1 year	survivors not specified tumours none	'no evidence of exposure related alterations'	12

Antimony potassium tartrate

rat, M/F (≥50/sex)	0, 5 µg/ml (drinking water), not specified, probably daily	lifetime	lifetime	survivors not applicable (decreased longevity) tumours 0: for M 10/50 and F 14/39 5: for M 6/50 and F 18/47	not specified	[16]
mouse (Swiss CD-1), M/F (54/sex)	0, 5 µg/ml (drinking water), not specified, probably daily	lifetime	lifetime	survivors not applicable tumours 24/71 and 18/76 at 0 and 5 µg/ml (M/F not specified)	only specified as (non)epithelial or categorised according to location	[15]

[a] 46% antimony, principally as antimony trisulphide; <4% titanium, 0.5% aluminium, 0.2% tin, 0.3% lead, 0.3% iron, 0.08% arsenic

[b] 99.68% pure

[c] ≥95% pure; 0.004% arsenic; <3% titanium

[d] 99.4% pure (measured as antimony; 0.02% arsenic, particle size 0.4 µm ± 2.13 (for the high concentration) and 0.44 µm ± 2.23 (for the lower concentration)

[e] 99.4% pure (measured as antimony; 0.02% arsenic, particle size 0.4 µm ± 2.13 (for the high concentration) and 0.44 µm ± 2.23 (for the lower concentration)

Abbrevations used:
no. = number; freq= frequency; X_{po} = duration of exposure; X_{pe} = duration of the experiment; M = male; F = female.

Annex **G**

Genotoxicity data

In vitro genotoxicity data.

Test system	Type of assay	Dose[a] (LED or HID)	Result [b]		Reference
			exogenous metabolic activation		
			without	with	
Antimony trioxide					
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, reverse mutation	5 mg/plate	–	–	19
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538, reverse mutation, spot test	not specified	–	NT	20
	Salmonella typhimurium TA98, TA100, reverse mutation	1.71 µg/plate	–	–	21
	Escherichia coli WP2P and WP2P *uvr*A, reverse mutation	5 mg/plate	–	–	19
	Escherichia coli B/r WP2, reverse mutation	not specified	–	NT	20
	DNA-repair-proficient/deficient strains of *Bacillus subtilis*	not specified	+	NT	20
	Bacillus subtilis M45 (rec-) and H17(rec+), spore plate method	0.6 µg/disk	+	NT	21
	Bacillus subtilis M45 (rec-) and H17(rec+), spore plate method	0.05M	+	NT	20
	Gene mutation, mouse lymphoma L5178Y cells, tk locus	50 µg/ml	–	–	19

	Chromosome aberrations, human peripheral blood lymphocytes	50 µg/ml	+	+	[19]
	Sister chromatid exchange, V79 cells	0.09 µg/ml	+	NT	[21]
	Sister chromatid exchange, human peripheral lymphocytes	0.5 µM	+	NT	[25]
Antimony trichloride					
	Salmonella typhimurium TA1535/ pSK1002, umu test	8.2×10^{-4} M	–	–	[24]
	Salmonella typhimurium TA98, TA100, reverse mutation	5 mg/plate	–	–	[21]
	Salmonella typhimurium TA98, TA100, reverse mutation	1 mM	–	–	[22]
	Escherichia coli PQ37, SOS Chromotest, DNA damage	354 µM	–	NT	[23]
	Bacillus subtilis M45 (rec-) and H17(rec+), spore plate method	12.5 µg/disk	+	NT	[21]
	Bacillus subtilis M45 (rec-) and H17(rec+), spore plate method	0.01M	+	NT	[20]
	Sister chromatid exchange, V79 cells	2.5 µg/ml	+	NT	[21]
	Sister chromatid exchange, human peripheral lymphocytes	1 µM	+	NT	[25]
	Comet assay, human peripheral lymphocytes	5 µM	+	NT	[28]
	Comet assay, V79 Chinese hamster cells	1 µM	+	NT	[26]
	Micronucleus test, human peripheral lymphocytes	5 µM	+	NT	[28]
	Micronucleus test, V79 Chinese hamster cells	25 µM	+	NT	[26]
	Micronucleus test, Chinese hamster ovary, human bronchial epithelial cells, human fibroblasts	>50 µM	+ (and delayed apoptosis)	NT	[27]
Antimony potassium tartrate					
	Salmonella typhimurium TA97, TA98, TA100, TA1535, reverse mutation	1 mg/plate (-S9 and TA97 +S9); 10 mg/plate (-S9)	–	–	[17,18]
	DNA damage, pBR 322 plasmid DNA	not specified	–	NT	[31]
Antimony pentoxide					
	Salmonella typhimurium TA98, TA100, reverse mutation	200 µg/plate	–	–	[21]
	Bacillus subtilis M45 (rec-) and H17(rec+), spore plate method	60 µg/disk	–	NT	[21]
	Sister chromatid exchange, V79 cells	40 µg/ml	–	NT	[21]

Antimony pentachloride					
	Salmonella typhimurium TA98, TA100, reverse mutation	864 µg/plate	–	–	21
	Bacillus subtilis M45 (rec-) and H17(rec+), spore plate method	65 µg/disk	+	NT	21
	Bacillus subtilis M45 (rec-) and H17(rec+), spore plate method	0.03 ml	+	NT	20
	Sister chromatid exchange, V79 cells	35 µg/ml	–	NT	21
Trimethylantimony dichloride					
	Chromosome aberrations, Chinese hamster ovary cells	1 mM	–	NT	29
	Sister chromatid exchange, Chinese hamster ovary cells	1 mM	–	NT	29
	Micronucleus test, Chinese hamster ovary cells	1 mM	–	NT	29
	DNA damage, pBR 322 plasmid DNA	not specified	–	NT	31
Potassium antimonate					
	Micronucleus test (with FISH), human peripheral lymphocytes	240 µM	+[c]	NT	30
Potassium hexahydroxoantimonate					
	DNA damage, pBR 322 plasmid DNA	not specified	–	NT	31
Stibine					
	DNA damage, pBR 322 plasmid DNA	200 µM (6.0 g/m^3)	+	NT	31
Trimethylstibine					
	DNA damage, pBR 322 plasmid DNA	200 µM (10.0 g/m^3)	+	NT	31

a LED = lowest effective dose; HID = highest ineffective dose
b + = positive; – = negative
c FISH: no clear preference for clastogen or aneuploidogen

In vivo genotoxicity data.

Test system	Type of assay	Dose[a] (LED or HID)	Result [b]		Reference
			exogenous metabolic activation		
			without	with	
Antimony trioxide					
	Chromosome aberrations, mouse bone marrow	1000 mg/kg bw	–		33
	Chromosome aberrations, mouse bone marrow	21x 400-1000 mg/kg bw [c]	+?		33
	Chromosome aberrations, rat bone marrow	21x 1000 mg/kg bw	–		34
	Micronucleus test, mouse bone marrow	1x 5000 or 7-21x 1000 mg/kg bw	–		19
	Micronucleus test, rat bone marrow	21x 1000 mg/kg bw	–		34
	Unscheduled DNA repair, rat hepatocytes	5000 mg/kg bw	–		19
	Sperm morphology, mouse	21x 1000 mg/kg bw	–		33
	Sister chromatid exchange and micronucleus test, human peripheral lymphocytes	0.12 µg/m^3	–		35
	Comet assay (enzyme (Fpg)-modified), human peripheral lymphocytes	0.12 µg/m^3	+		35
Antimony trichloride					
	Chromosome aberrations, mouse bone marrow	70 mg/kg bw	+		32

a LED = lowest effective dose; HID = highest ineffective dose
b + = positive; – = negative
c LED cannot be specified as historical control values are not present

Annex **H**

Carcinogenic classification of substances by the Committee

The Committee expresses its conclusions in the form of standard phrases:

Category	Judgement of the Committee (GRGHS)	Comparable with EU Category	
		67/584/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to man. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	1	1A
1B	The compound is presumed to be carcinogenic to man. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, the mechanism of action is not known.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	Not applicable	Not applicable
(4)	The compound is probably not carcinogenic to man.	Not applicable	Not applicable

Source: Health Council of the Netherlands. Guidline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.