



International
Antimony Association

**Scientific opinion, provisional read-across justification,
and further research opportunities**

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Human Health: Genotoxicity

31 July 2018

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1. Introduction

Annex XI of the REACH Regulation opens the possibility of predicting properties of substances for which no data is available, on the basis of data available on other related substances, by applying analogue or category approaches and read-across (so-called 'read-across approach') between one or more source and target substances.

Genotoxicity studies have been performed for only a few of the various antimony (Sb) substances that are registered under REACH. Overall, around 15 studies have assessed the genotoxicity of Sb substances since 1991. Most of the information was generated using a small number of trivalent Sb substances, and the in vitro and in vivo studies do not yield comparable results. This dataset is used as the starting point to perform the hazard and classification assessments, as well as to identify further research needs. The related read-across assessment is built upon the similarities and differences that can be observed among the Sb substances in studies seeking to define the circumstances under which genotoxic activity may be expressed.

This "scientific opinion, provisional read-across justification, and further research needs" document outlines the approach followed to predict the genotoxicity properties of Sb substances under REACH. It implements the recommendations and principles laid down in the 2017 ECHA Guidance on grouping of substances, and ECHA's Read-Across Assessment Framework (RAAF), its read-across approaches and scenarios, and the respective assessment elements, in order to facilitate the examination of the read-across justification and any pending evidence able to validate it.

When describing the resulting opinion and read-across approach in the last section of this document, where necessary, the concepts and terms used in the RAAF are adapted to have greater compatibility with the unique properties of metals and metalloid substances.

This document refers to evidence which is available in the REACH Registration dossier of the Sb substances and therefore avoids repetition of detailed description of evidence which is available in the dossier and/or the Chemical Safety Reports. Reading this document in conjunction with the REACH dossiers and/or Chemical Safety Reports will hence bring a more complete picture to the reader.

With the submission of this document to the German REACH Competent Authorities (BAuA) in charge of the Substance Evaluation of five of the ten Sb substances in scope of i2a's product stewardship program, i2a requests that the conclusions reached in this document and the further research opportunities that are outlined, are taken into account by BAuA in preparing their Substance Evaluation decision(s). Accordingly, this document will be attached to the next REACH Dossier updates.

About i2a

The mission of the International Antimony Association is to inspire product stewardship along the antimony value chain. This mission is accomplished by generating and sharing information concerning the environmental and health safety and societal benefits of antimony and antimony compounds. Through a common evidence base, i2a promotes a harmonized risk management and continued safe use of antimony and antimony substances across the value chain and geographical borders.

For further information: www.antimony.com.

2. Available genotoxicity data for antimony source and target substances

Tables 1.1, 1.2 and 1.3 below provide an overview of the genotoxicity data that is available for the Sb substances considered for grouping and read-across, and the response observed after treatment with different antimony compounds. Studies in prokaryotic systems are presented first, followed by results in eukaryotic systems in vitro and in vivo. Within each subgroup, test results are ordered by relevance – tests for mutations are presented first, followed by results from “indicator assays”.

Indicator assays are assays that suggest genotoxic activity but do not themselves provide direct evidence of mutagenicity. For example, gene mutations or chromosome aberrations are indicative of a change in the primary sequence of DNA (i.e. are evidence for a mutagenic response). In a weight of evidence evaluation, mutagenic responses are generally accorded greater significance than responses in indicator assays (e.g. sister chromatid exchanges) that suggest impacts such as DNA damage may have occurred but do not determine if mutations have taken place in viable cells). Indicator assays can yield positive responses via mechanisms that do not produce mutagenic changes.

Sb (III) compounds have been tested most frequently – tests of Sb (V) compounds have been conducted less frequently and are in the shaded table rows. To further assist in data interpretation, positive assay responses indicative of genotoxicity are indicated in red. “Klimisch scores” indicative of study quality are presented in each table. Klimisch scores were derived in accordance with Klimisch et al. (1997) and compliance with international guidelines for genotoxicity testing. The recommendations of the International Workshop on Genotoxicity Testing (IWGT) often provided the most rigorous protocols defining acceptable testing methods and are cited as appropriate. In most instances, only studies with Klimisch scores of 1) Reliable without restriction and 2) Reliable with restrictions are included in this review. As regards in vitro studies, a number of low quality studies have been published, and are summarized in the CSRs.

Table 1.1. Overview of genotoxicity responses to Sb substances in prokaryotic systems

Assay	Compound Tested	Concentration Tested	Response	Reference and Klimisch Score
Mutation Assays				
Ames Mutation	SbCl ₃	625 – 5000 µg/plate	Negative	Kuroda, 1991 2: Reliable with restriction
Ames Mutation	Sb ₂ O ₃	0.43 – 1.71 µg/plate	Negative	Kuroda, 1991 2: Reliable with restriction
Ames Mutation	Sb ₂ O ₃	100 – 5000 µg/plate	Negative	Elliot, 1998 2: Reliable with restriction
Ames Mutation	SbCl ₅	54 – 864 µg/plate	Negative	Kuroda, 1991 2: Reliable with restriction
Ames Mutation	Sb ₂ O ₅	50 – 200 µg/plate	Negative	Kuroda, 1991 2: Reliable with restriction
Ames Mutation	NaSb(OH) ₆	31.6 – 5000 µg/plate	Negative	Spruth, 2015 2: Reliable with restriction
Escherichia Coli	NaSb(OH) ₆	31.6 – 5000 µg/plate	Negative	Spruth, 2015 2: Reliable with restriction

Assay	Compound Tested	Concentration Tested	Response	Reference and Klimisch Score
Indicator Assays				
B. subtilis rec	SbCl ₃	6.3 – 23 µg/disk	Positive	Kuroda, 1991 2: Reliable with restriction
B. subtilis rec	Sb ₂ O ₃	0.3 – 1.1 µg/disk	Positive	Kuroda, 1991 2: Reliable with restriction
B. subtilis rec	SbCl ₅	65 - 260 µg/disc	Positive	Kuroda, 1991 2: Reliable with restriction
E. Coli SOS chromotest	SbCl ₃	11 – 707 µM	Negative	Lantzsch, 1997 2: Reliable with restriction

In vitro genotoxicity responses to Sb compounds are generally weak, inconsistent and often result from the use of high compound concentrations that sometimes exceed the solubility limits of sparingly soluble Sb compounds. The response profile that emerges from *in vitro* tests is mixed. Mutation assays in prokaryotic (bacterial) test systems are mostly negative, and indicative of no mutagenicity (Table 1.1). Paradoxically, prokaryotic indicator assays (e.g. the *B. subtilis* rec test for DNA damage) generally provides positive responses. For a variety of technical reasons explained in the Technical Annex to this paper, results from prokaryotic systems can be unreliable when applied to metals in general and to Sb in particular.

Table 1.2. Overview of genotoxic effects of Sb substances in cultured mammalian cells

Assay	Compound Tested	Concentration	Test Result	Reference and Klimisch Score
Mutation Assays				
Mouse Lymphoma Cell Mutation	Sb ₂ O ₃	6 – 50 µg/ml	Negative	Elliot 1998 2: Reliable with restriction.
Mouse Lymphoma Cell Mutation	NaSb(OH) ₆	2.2 – 570 µg/ml	Negative	Stone, 2010 1: Reliable without restriction
Human Lymphocyte Chromosomal Aberrations	Sb ₂ O ₃	10 – 100 µg/ml	Positive	Elliot 1998 2: Reliable with restriction.
MN CHO cells	SbCl ₃	50 - 400 µM	Positive	Huang 1998 2: Reliable with restriction.
MN Human Fibroblasts	SbCl ₃	50 - 400 µM	Positive	Huang 1998 2: Reliable with restriction,
MN Human bronchial epithelial	SbCl ₃	50 - 400 µM	Positive	Huang 1998 2: Reliable with restriction,
MN V79 cells	SbCl ₃	0.1 – 50 µM	Positive	Gebel 1998 2: Reliable with restriction.
MN Human Lymphocytes	SbCl ₃	0.1 – 25 µM	Positive	Schaumloffel 1998 2: Reliable with restriction.

Assay	Compound Tested	Concentration	Test Result	Reference and Klimisch Score
MN Human Lymphocytes	NaSb(OH) ₆	64 – 540 µg/ml	Negative	Whitwell, 2010 1; Reliable without restriction
MN Human Lymphocytes/FISH staining	KSbO ₃	240 – 600 µM	Positive	Migliore, 1999 2: Reliable with restriction.
Indicator Assays				
SCE Human Lymphocyte	SbCl ₃	0.1 – 10 µM	Weak Positive	Gebel, 1996 2: Reliable with restriction.
SCE Human Lymphocyte	Sb ₂ O ₅	0.1 – 10 µM	Weak Positive	Gebel, 1996 2: Reliable with restriction.

A somewhat more consistent response profile emerges in tests using mammalian cells cultured *in vitro*. Two gene mutation tests have been done using a mouse lymphoma cell line (Table 1.2) and failed to detect mutagenic activity. A number of studies have evaluated the ability of Sb compounds to induce chromosome aberrations or micronucleus (MN) induction. Micronuclei are usually from fragmented chromosomes so tests for chromosome aberrations and micronucleus induction are expected to yield similar results. Positive assay results for the induction of micronuclei are the most consistent indication of genotoxic activity for Sb compounds reported in the scientific literature. However, as detailed in the Technical Annex, recent studies have reported potential experimental artefacts in the staining procedures applied for the detection of micronuclei in cells treated with metals or metalloids (Wedel et al., 2013; Cohen et al., 2013).

Table 1.3. Genotoxic effects of Sb Trioxide in vivo

Assay	Compound	Dose	Result	Reference and Klimisch Score
Mutation Assays				
Chromosome Abb. Mouse Bone Marrow	Sb ₂ O ₃	400 – 1000 mg/kg single oral dose	Negative	Gurnani, 1992 3, not reliable
Chromosome Abb. Mouse Bone Marrow	Sb ₂ O ₃	400 – 1000 mg/kg 21-day oral dose	Equivocal Positive	Gurnani, 1992 3, not reliable.
Chromosome Abb. Rat Bone Marrow	Sb ₂ O ₃	250 – 1000 mg/kg 21-day oral dose	Negative	Kirkland, 2007 1: Reliable without restriction
MN mouse bone marrow	Sb ₂ O ₃	3200 and 5000 mg/kg oral gavage, one dose	Negative	Elliot 1998 2: Reliable with restrictions
MN mouse erythrocytes	Sb ₂ O ₃	One-year daily inhalation exposure to 3, 10 and 30 mg/m ³	Equivocal Positive	NTP, 2017 2: Reliable with restrictions.
MN rat erythrocytes	Sb ₂ O ₃	One-year daily inhalation exposure to 3, 10 and 30 mg/m ³	Negative	NTP, 2017 1: Reliable without restriction

Assay	Compound	Dose	Result	Reference and Klimisch Score
MN rat bone marrow	Sb ₂ O ₃	250 – 1000 mg/kg 21 day repeated oral dose	Negative	Kirkland, 2007 1: Reliable without restriction.
Genotoxicity Indicator Assay Data				
UDS rat liver	Sb ₂ O ₃	5000 mg/kg oral gavage, single dose	Negative	Elliot 1998 2: Reliable with restrictions.
Comet Assay mouse lung	Sb ₂ O ₃	One-year daily inhalation exposure to 3, 10 and 30 mg/m ³	Equivocal Positive	NTP, 2017 3: Not reliable.
Comet Assay rat lung	Sb ₂ O ₃	One-year daily inhalation exposure to 3, 10 and 30 mg/m ³	Negative	NTP, 2017 3: Not reliable.

Studies of the genotoxic effects of Sb compounds *in vivo* have focused upon the impacts of antimony trioxide (Sb₂O₃ or ATO). The results of testing in experimental animals are summarized in Table 1.3 and provide a mixed display of effects that lacks the consistency evident in the *in vitro* studies. Given the importance of *in vivo* findings for genotoxicity evaluations, low quality studies have been retained in the table. Several of the low-quality studies have been extensively cited in the peer-reviewed literature and their inclusion in this section of the evaluation is intended as a demonstration of the value of considering study quality in weight of evidence evaluations.

Gurnani et al. (1992) reported that chromosome aberrations would result in the bone marrow cells of mice repeated oral exposures to ATO. Although single (acute) exposures to ATO did not produce effects, exposure of mice for up to 21 days produced chromosome changes that increased in frequency as a function of exposure duration and intensity. Interpretation of this positive study is problematic due to a number of technical deficiencies and deviations from Good Laboratory Practice (GLP) protocols. Kirkland et al., (2007) attempted to replicate the findings of Gurnani et al. (1992) in a study that strongly adhered to GLP guidelines and possesses technical rigor superior to other *in vivo* studies evaluating clastogenic effects of Sb compounds. No treatment related increases in chromosome aberrations or micronuclei were observed.

The National Toxicology Program of the United States recently conducted inhalation cancer bioassays upon rats and mice, exposing animals to 3, 10 and 30 mg/m³ Sb₂O₃ for two years (NTP, 2017). After one year of inhalation exposure, a subgroup of rats and mice were evaluated for the presence of micronuclei in red blood cells. No impact was seen in rats whereas a slight increase in micronucleus induction was reported in mice. As detailed in the technical annex, this increase is most plausibly a reflection of hypoxia and not actually an indication of induced chromosome damage.

DNA damage in lung cells was also evaluated via the Comet assay – no DNA damage was observed in rat lung cells whereas NTP (2017) indicated DNA damage could be detected in mouse lung cells. The detection of DNA damage in lung tissues exhibiting a toxic response (as occurred in the lung tissues of mice) is technically challenging (Speit et al., 2015) and NTP did not conduct any of the experimental controls needed to guard against false positive results in the Comet assay.

Thus, the NTP studies did not observe genotoxicity in rats, and produced at best equivocal findings in mice. Lung tumors were subsequently observed in rats and mice, but the role of genotoxicity in the etiology of these tumors is unclear.

Other studies evaluating the genotoxic impacts of Sb *in vivo* followed protocols limited in scope. Elliot et al. (1998) examined the impacts of a single 5000 mg/kg oral gavage Sb₂O₃ dose upon micronucleus induction. No evidence was obtained for micronucleus induction. The same authors also examined the induction of unscheduled DNA synthesis in rat liver after a single dose of Sb₂O₃ administered by oral gavage at doses of 3200 and 5000 mg/kg. No treatment related impacts upon unscheduled DNA synthesis were observed.

Table 1.4 below presents an overview of the available genotoxicity studies per Sb substance (except the indicator assays). The table shows that although there is a good amount of information for trivalent substances, there is no *in vivo* genotoxicity data for pentavalent ones. *In vivo* mutagenicity testing was not required under REACH since no positive results were obtained from *in vitro* mutation assays (cf. Annex VII and VIII, Section 8.4 Column 2 of Regulation (EC) 1907/2006) and because the absence of mutagenicity *in vivo*, for Sb (III) substances, where Sb 3+ is known to be more toxic than Sb 5+, allowed to presume that no *in vivo* genotoxicity would be observed for pentavalent substances either. **ASSESSMENT NOTE 1: *If possible, if further in vivo testing is considered necessary, it should ideally also address the lack of in vivo genotoxicity data on pentavalent Sb substances.***

ASSESSMENT NOTE 2: *While 6 out of 17 in vitro genotoxicity studies have reported positive responses for genotoxicity, 5 out of the 7 in vivo genotoxicity studies in rodents have failed to observe genotoxic effects.* The positive or equivocal assay responses which have been observed are restricted to studies with significant technical deficiencies.

Table 1.4 Overview of genotoxicity data (except indicator assays) available for Sb substances considered for grouping and read-across for genetic toxicity endpoints (x = Klimisch score 1 or 2, x = Klimisch score 3 or 4).

Name	CAS #	in vitro				in vivo	
		Ames, (OECD 471)	Chromosomal Aberrations (OECD 473)	Micronucleus (OECD 487)	Mouse Lymphoma Assay (OECD 490)	Chromosomal Aberrations (OECD 475)	Micronucleus (OECD 474)
Sb metal							
Sb –powder	7440-36-0						
Sb – massive	7440-36-0						
Trivalent Sb compounds							
Diantimony trioxide	1309-64-4	x, x	x		x	x, x, x	x, x, x, x
Antimony sulfide	1345-04-6						
Antimony tris(ethylene glycolate)	29736-75-2						
Antimony trichloride	10025-91-9	x		x, x, x, x, x			
Pentavalent Sb compounds							
Sodium hexahydroxoantimonate	33908-66-6	x, x	x	x	x		
Sodium antimonate	15432-85-6						
Antimony pentachloride	7647-18-9	x					
Antimony pentoxide	1314-60-9	x					
Potassium hexahydroxoantimonate	12208-13-8						

3. Considerations around mode of action and classification of Sb substances for genotoxicity

The mechanism(s) by which Sb compounds produce positive response in some in vitro test systems is unclear. Direct covalent interaction of Sb with DNA has not been detected, leading to suggestions that genotoxicity responses may be mediated by indirect mechanisms. De Boeck et al. (2003) suggest that the generation of oxygen radicals constitute an indirect pathway for inducing genotoxic responses. **ASSESSMENT NOTE 3: *If reactive oxygen species mediate most in vitro observations of genotoxicity, robust in vivo antioxidant systems could explain why most in vivo studies have not observed genotoxicity.*** Anti-oxidant system in an intact animal are robust and would mitigate against oxidative damage. Expression of genotoxicity would be absent in vivo or exhibit a threshold with genotoxicity only resulting when the protective capacity of anti-oxidant systems is exceeded (Kirkland et al., 2015).

Sb has also been suggested to interfere with DNA repair processes (Beyersmann and Hartwig, 2008) and this may facilitate genotoxic responses. Inhibition of repair of double strand DNA breaks (Takahashi et al., 2002) and excision repair (Grosskopf et al., (2010) have both been reported. However, **ASSESSMENT NOTE 4: *the relevance of these observations to in vivo exposure scenarios is further uncertain since the concentrations required to produce effects in vitro are significantly higher than plausible systemic levels of Sb in vivo.***

Most studies have been conducted using soluble Sb in the form of trivalent Sb trichloride and the assumption made that any activity observed in various test systems could be attributed to the release of the Sb ion via hydrolysis to yield $Sb(OH)_3$. The behavior of trivalent Sb compounds in solution is likely to be complex and involve the sequential formation of Sb oxide chloride ($SbOCl$), Sb oxide hydroxide ($SbO(OH)$) and ultimately the formation of Sb trioxide (Sb_2O_3) (Hashimoto et al., 2003). The chemical moiety responsible for producing a possible genotoxic response is thus uncertain.

The pentavalent Sb pentachloride is a strong oxidizing agent which, as a function of pH, will similarly undergo a series of hydrolytic transformations to oxychlorides and oxide hydroxides that result in the formation of Sb_2O_5 (Zheng, Zhi and Chen, 2006). Once again, hydrolysis products are the likely mediator for positive test responses but the chemical moiety responsible for effects remains unknown.

As oxyanions, Sb hydrolysis products would also be expected to undergo electrophilic interactions with cellular constituents such as thiol rich proteins. Such interactions provide the mechanistic basis for intracellular inclusion body formation. Documented to form after exposure to metals and metalloids, such inclusion bodies can be mistaken for micronuclei unless staining procedures are employed that are specific for the presence of DNA (Wedel et al, 2013; Cohen et al., 2013). Studies of micronucleus induction after treatment with Sb compounds have not routinely employed such high affinity staining procedures. There is thus an element of uncertainty now associated with the interpretation of existing micronucleus studies.

Mechanistic aspects of Sb genotoxicity have recently been evaluated in the “ToxTracker” assay, a novel test system that employs mammalian stem cell lines containing fluorescent reporters that respond to the expression of genes expected to be induced following the direct induction of DNA damage, oxidative stress and protein damage (Hendriks, 2017). Although still in validation, the ToxTracker assay has generated a response profile for Sb compounds consistent with the induction of genotoxicity though indirect mechanisms that entail oxidative stress or protein damage. None of the nine Sb compounds tested (antimony powder, sodium antimonate, diantimony pentoxide, diantimony trioxide, sodium hexahydroxoantimonate, antimony (III) sulfide, antimony glycolate, antimony (III) chloride and antimony (V) chloride) yielded responses indicative of direct DNA damage. However, all but two compounds (diantimony pentoxide and sodium hexahydroxoantimonate) were found to be strong inducers of oxidative stress. All but three (sodium

antimonate, diantimony pentoxide and sodium hexahydroxoantimonate) exerted protein damage that could reflect interference with DNA repair functions. **ASSESSMENT NOTE 5: *The ToxTracker assay findings confirm that the genotoxicity observed in some of the test items is most probably the result of indirect mechanisms, and that Sb (III) compounds will generally tend to exert such mechanisms in a stronger manner than Sb (V) compounds. Based on this, it can be assumed that if most in vivo studies with Sb (III) compounds have not observed genotoxicity, it is very unlikely that the same studies conducted with Sb (V) compounds would produce genotoxic effects.***

Inorganic Sb compounds have been evaluated for genotoxic potential in in vitro and in vivo test systems. The available data suggest that Sb hydrolysis products do not induce point mutations but that clastogenic events result from in vitro exposures. In vivo assessments of genotoxicity have generally produced negative or, at best, equivocal results. Several negative studies possess the highest technical rigor – those with equivocal findings have significant technical deficiencies. Thus, whereas in vitro studies suggest genotoxic properties, there is little evidence that this is expressed in vivo. **ASSESSMENT NOTE 6: It is therefore concluded that the tested Sb substances are not genotoxic in vivo.**

4. Identity, characterization, physico-chemical properties, and bioavailability of the source and target antimony substances

Tables 2 and 3 list and describe the Sb substances considered for grouping and read-across. Information on their identity and characterization (identifiers, counter-ion, impurities, physical form, structure and size) are provided in Table 2, whereas Table 3 summarizes the physico-chemical properties which may influence their bioavailability and toxicity properties (water solubility, bio-accessibility, cytotoxicity, etc.).

Basic physicochemical characteristics

Metal and metalloid compounds are typically categorized on the basis of the valence or oxidation state of the ion contained in the substance. The oxidation state (IUPAC Definition: the charge of the atom after ionic approximation of its heteronuclear bonds) will dictate the affinity and potential for interaction and chemical bonding of a given metal/metalloid substance with biological systems.

In Table 2, the Sb substances and their corresponding CAS number are listed in order of valence state, namely 0, 3+ and 5+. ***Considering the specificity of interactions between a chemical and a cell, the difference in valence provided in Table 2 may need to be considered for the purpose of read-across for genotoxicity evidence.***

The table also provides information on the physical form (powder, particle size) of each Sb substance. The physical form, and particle size, is relevant to the consideration of the exposure routes through which the various Sb substances may enter the body under realistic use conditions. Genotoxicity would require the systemic uptake of a relatively large amount of a given substance, able to interact directly or indirectly¹ with the genetic material or genetic processes of the cells. In this context, the oral exposure route offers the main physiological entry point for Sb substances into the human body; physical forms (and sizes) that can be ingested are those of relevance to genotoxicity (in theory, any of the Sb substances in Table 2). The ingestion route is also the route through which consumers will be more likely to be exposed to Sb substances. ***The differences in physical form and particle sizes provided in Table 2 can be omitted for the purpose of read-across for genotoxicity evidence.*** The fact that a substance is ingestible however, does not imply it can then be absorbed systemically and reach the relevant cells and have a genotoxic effect. Even if the substance is able to reach the relevant cells, it may express no genotoxic effect at all.

Table 2 also provides information on the moiety (functional group) of each Sb substance that will normally influence the physico-chemical properties, and the bio-availability of the substance. As explained by Hashimoto et al. (2003) and Zheng, Zhi and Chen (2006), Sb will be taken up as the (oxyan)ion after release from the parent compound due to hydrolysis of ionic bonds. The functional groups will dictate the ease with which Sb oxyanions are released from a substance and made available for systemic uptake. The primary impact of the moiety will be in determining the dissolution rate of compounds in the gastrointestinal tract (US EPA, 2007). The actual uptake of Sb ions/oxyanions from the gastrointestinal tract appears to be mediated by saturable carrier protein transport systems and will occur with low (<1 %) efficiency (ATSDR, 2017). This low uptake rate combines with the emetic properties of antimony compounds (ATSDR, 2017) to limit the systemic levels of antimony that can be achieved via oral exposure.

¹ When genotoxicity is caused by other means than by direct interaction with DNA, e.g. induction of aneuploidy, oxidative stress, inhibition of DNA synthesis or cytotoxicity presents, the mode of action is assumed to be an indirect one. In short, indirect mechanisms of genotoxicity correspond to interactions of mutagens with non-DNA targets.

The released moieties, many of which are essential nutrients or Essential Trace Elements (ETEs), may also be subject to independent gastrointestinal uptake (in many instances regulated by homeostatic control mechanisms). Given their essential nature, the anticipated genotoxicity impact of these moieties, compared to the possible genotoxicity impact of Sb, is expected to be negligible. Other moieties are normal metabolites (NM), often of carbohydrate metabolism, and are expected to be rapidly metabolized.

The chemical nature of the ligand moiety may exert its own toxicity in rare cases, but this is the exception and not the rule, and particularly not for the moieties reported in Table 2. The notable exception to this generalization will be moieties (e.g. chlorides) which, when administered in pure or concentrated doses, will have corrosive or irritant properties that serve to limit substance administration due to local effects that disrupt essential functions such as food ingestion or breathing. But again, this is not a reasonable and foreseeable situation under REACH. ***The difference in moieties provided in Table 2 can be omitted for the purpose of read-across for genotoxicity evidence.***

As regards the formula weight and structure of each substance, the information in Table 2 shows that there are no specific trends or patterns among the molecular weight or structure that can inform the read-across approach. ***Considering the specificity of interactions between a chemical and a cell however, the difference in molecular weight provided in Table 2 may need to be considered for the purpose of estimating dosimetry in read-across comparisons of genotoxicity evidence.***

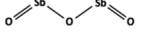
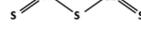
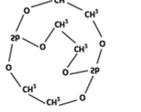
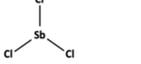
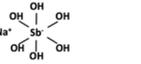
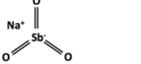
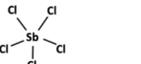
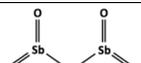
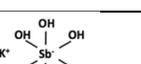
Impurities in Sb substances are commonly arsenic and lead (in the relevant speciation)², but typically in concentration levels below 0,1% or their respective Specific Concentration Limits (SCL)³. This means that the assessment and read-across of the toxicity hazard and effect of the Sb substance will be driven by the Sb, and **not** by the impurities in the substances; and that the various *pure* Sb substances do not need to be distinguished on the basis of their impurities for the purpose of read-across. Table 2 however confirms that ***the impurity profile is relatively comparable across the various Sb substances, and that there is no reason to discriminate between these on the basis of (im)purity for purposes of read-across for genotoxicity evidence.***

The content of Table 2 shows that on the basis of identity or characterization, beyond valency and molecular weight, there are no major differences between the Sb substances subject to REACH that would challenge a grouping or read-across approach. In short, any Sb substance which does not contain a moiety with a more severe systemic toxicity than that of the moieties in Table 2 could in principle be part of the Sb substances provisional read-across group for genotoxicity.

² Because of the geological affinity there is between the antimony, arsenic and lead in the predominant natural source of Sb (stibnite), arsenic and lead will typically be present as impurities in any Sb substance. Indeed, even following the transformation of stibnite into Sb “metal”, and then into subsequent Sb compounds, these impurities will remain, albeit in controlled quantities. In Sb metal, the impurities will be present in metallic form whereas in e.g. Sb oxides or sulfides, they will be present in oxidic or sulfidic form, respectively.

³ For carcinogens category 1A such as As oxides or acid the cut-off level is 0.1 %. For reprotoxicants category 1A such as Pb oxides the cut-off is 2.5 %, for Pb metal massive the SCL is 0.3 %, and for Pb metal powder the SCL is 0.03%.

Table 2. Identity and characterization of Sb substances considered for grouping and read-across, or as sources of read-across relevant information.

Name	CAS #	Form and typical particle size	Molecular weight (g/mol)	Chemical formula	Structure	Moiety	Purity (% w/w)	Impurities
“Metallic” Sb								
Sb – powder	7440-36-0	Powder (< 1 mm)	121.76	Sb	Sb	--	>89.45 - <100	As: <2.5
Sb – massive	7440-36-0	Massive (> 1 mm) ⁽³⁾	121.76	Sb	Sb	--		Pb: <9
Trivalent Sb substances								
Diantimony trioxide	1309-64-4	Powder 0.2-0.44 μm	291.5	Sb ₂ O ₃		--	>97 - <100	As ₂ O ₃ : <0.1 PbO: <2.5
Antimony sulfide	1345-04-6	Powder D ₅₀ : 32.7 μm	339.7	Sb ₂ S ₃		SO ₄ ²⁻		
Antimony tris(ethylene glycolate)	29736-75-2	Crystal D ₅₀ : 1600 μm	495.7	Sb ₂ (C ₂ H ₄ O ₂) ₃		(C ₂ H ₄ O ₂) ²⁻	>99	n.s. ⁽²⁾
Antimony trichloride	10025-91-9	Crystal D ₅₀ : 897 μm	190.7	SbCl ₃		Cl ⁻	>99	n.s. ⁽²⁾
Pentavalent Sb substances								
Sodium hexahydroxantimonate	33908-66-6	Powder MMAD: 26.2 μm ⁽¹⁾	246.8	Na(Sb)(OH) ₆		Na ⁺	>94.8 - <99.75	PbO: <2.5
Sodium antimonate	15432-85-6	Powder/Crystals: 1-180 μm	192.7	NaSbO ₃		Na ⁺	>95 - <99.9	n.s. ⁽²⁾
Antimony pentachloride	7647-18-9	Liquid	299,02	SbCl ₅		Cl ⁻	>98	SbCl ₃ : <1 As: <0.1 Pb: <0.1
Antimony pentoxide	1314-60-9	Powder/colloidal suspension D ₅₀ : 24.4 μm	323.5	Sb ₂ O ₅		--	>87 - <99.9	As ₂ O ₃ : <0.1 PbO: <0.25
Potassium hexahydroxoantimonate	12208-13-8	Crystal	262.9	K(Sb)(OH) ₆		K ⁺	> 94 - < 97	n.s. ⁽²⁾

⁽¹⁾ Mass Median Aerodynamic Diameter; ⁽²⁾ Non-specified impurities for which the individual composition does not exceed 0.1% and/or which are not classified; ⁽³⁾ Cf. Guidance on the Application of the CLP Criteria Version 5.0 – July 2017, page 600, Section IV.5.5 Particle size and surface area.

Solubility and bioavailability

Table 3 provides information on the release and behavior of Sb species in water and a number of physiologically relevant media. Information on the solubility in media mimicking physiological fluids, as an estimation of bioavailability potential, is known to be more informative of possible absorption and systemic distribution of metals than plain water solubility.

For metals, inorganic metal compounds, or metal-containing complex materials, the bioavailable metal ion is usually considered to be responsible for the systemic toxicity. The systemic toxicity of most metals and metalloids is associated to a large degree with the release of soluble metal ions and their uptake by the body and/or interaction at their target organ sites (i.e. the bioavailability of the metal ions). It is the bioavailability of the released metal at the site of action (for local effects) or uptake (for systemic effects) in the organism that can be the most important determining factor modulating the toxicity of metals and minerals.

For metals under homeostatic control, uptake is regulated and bioavailability can be of secondary importance. For others, such as Sb, uptake is not necessary linear in function of dose since sites of uptake (carrier systems) can saturate. There are indications that this occurs with Sb uptake from the gastrointestinal tract. Considering only bioavailability data could hence be seen as a conservative approach in the assessment of Sb uptake and hazard. ***In reality, through normal exposure routes, low Sb uptake rates will limit the maximum systemic Sb levels that can be attained in the body.***

Information on bioavailability can be derived from *in vivo* sources such as toxicokinetic or toxicological test data or predicted using *in vitro* models that seek to simulate processes that govern uptake rates *in vivo*. Indeed, the amount of released metal “available for absorption” may be measured using *in vitro* methods. Bioelution refers to these *in vitro* methods, which are used to measure the degree to which a substance is released (e.g. as metal ions) into simulated biological fluids. Such tests are thus used to assess a substance’s metal bioaccessibility in the form of released metals under physiological conditions. Bioelution enables parallel and comparative determinations of the bioaccessibility of various substances without using laboratory animals.

The basic premise is that the systemic absorption and toxicity of metal-containing materials are related to bioavailability and the release of metal ions that are then available for systemic absorption. A relationship can thus be assumed between *in vitro* bioaccessibility in an artificial biological fluid and relative *in vivo* bioavailability.

There are artificial fluids for every relevant route of exposure to be assessed. For oral route, which is the most relevant for Sb substances and genotoxicity, fluids exist which simulate the stomach and the intestinal conditions. A key difference between these two fluids is the pH they mimic: pH of 1.5 for gastric, and pH of 7.4 for intestinal. Although the uptake of metals is known to occur in the intestine, at neutral pH, the highest release of metal can be expected to take place in the stomach, at acidic pH. Because of this, the gastric fluid is often selected for bio-accessibility testing, as it represents ‘worst-case conditions’ for release. Bioaccessibility methods are generally considered to overestimate absolute bioavailability and toxicity of inorganic compounds since bioelution tests do not assess absorption after release. Based on this overestimation, it can be safely assumed that a (worst-case) relationship can be defined between *in vitro* bioaccessibility in the artificial fluid and relative *in vivo* bioavailability of the ions that are also absorbed systemically.

As shown in Table 3, bioelution information is not available for all Sb substances. It does not allow a true comparison or ranking between the various Sb substances at this point in time. It does not show much

correspondence with water solubility data either. While additional solubility and bioaccessibility information is produced, for the purpose of the provisional read-across, a worst-case assumption can be made that all Sb substances would be equally bioavailable via ingestion. This would mean that **ASSESSMENT NOTE 7: any Sb substance, provided it has a comparable valency and molecular weight, able to release soluble Sb ions to be taken up by the body at levels comparable to those of the most water-soluble ones can provisionally be part of the same read-across group for the genotoxicity endpoint.**

This appears to be an over-conservative approach but it is the proposed way forward for the provisional read-across justification. In order to perform a more refined comparison, additional information would be needed to complete Table 3. This provisional read-across justification will be revisited once the additional information becomes available, probably in late 2019.

Table 2. Solubility and bio-accessibility data of Sb substances considered for grouping and read-across.

Name	CAS #	Solubility in water	Bioaccessibility in GMB (artificial lung fluid)	Bioaccessibility in (artificial saliva)	Bioaccessibility in GST (artificial stomach fluid)	Bioaccessibility in ASW (artificial sweat)	Bioaccessibility in artificial intracellular fluid	Extraction in culture medium
Metallic Sb								
Sb – metal powder	7440-36-0	18.2 µg/ml	60 µg Sb/ml		13 µg Sb/ml			60 µg Sb/ml
Sb – massive metal	7440-36-0							
Trivalent Sb substances								
Diantimony trioxide	1309-64-4	19.7-28.7 µg/ml	4.3 µg Sb/ml					0,8 µg Sb/ml
Antimony sulfide	1345-04-6	0.944 µg/ml 0.677 µg Sb/ml	2 µg Sb/ml		2 µg Sb/ml	2 µg Sb/ml		5.6 µg Sb/ml
Antimony tris(ethylene glycolate)	29736-75-2	0.0004-0.0012 µg/ml	0 µg Sb/ml		0.7 µg Sb/ml			32 µg Sb/ml
Antimony trichloride	10025-91-9	Technically not feasible						30 µg Sb/ml
Pentavalent Sb substances								
Sodium hexahydroxoantimonate	33908-66-6	594 µg/ml 293 µg Sb/ml	16 µg Sb/ml		46 µg Sb/ml	29 µg Sb/ml		30 µg Sb/ml
Sodium antimonate	15432-85-6	247 µg/ml						2.5 µg Sb/ml
Antimony pentachloride	7647-18-9	Decomposes in water						29 µg Sb/ml
Antimony pentoxide	1314-60-9	453 µg/ml 341.2 µg Sb/ml						4.7 µg Sb/ml
Potassium hexahydroxoantimonate	12208-13-8	20,000 µg/ml ⁽¹⁾						

⁽¹⁾ Taken from available SDS

Toxicokinetics, Absorption, Distribution, Metabolization and Excretion

There is little data detailing speciation or changes in valency following uptake of Sb into mammalian systems (ATSDR, 2017). The following information may however provide an indication of general trends:

Absorption	Distribution	Metabolization	Excretion
<p>The valence state of Sb compounds has been suggested to impact uptake from the gastrointestinal tract but differences reported are generally small (ATSDR, 2017). As a generalization, uptake efficiency is very low (less than 1%) and likely nonlinear with saturation of uptake processes as administered doses increase.</p>	<p>The impact of valency (and changes in valency) of systemic Sb once taken up into mammalian systems, has not been well characterized but appears to influence Sb distribution within the body and subsequent excretion. Systemic Sb (V), after ingestion or injection, tends to partition to blood plasma and the spleen whereas Sb (III) is preferentially found with the erythrocyte and the liver (Edel et al., 1983; Poon et al., 1998; Coelho et al., 2014). Sb ions cross the placenta, but there is no comparative data that documents the impact of valence state upon placental transfer. The half-life of systemic Sb appears to be on the order of 10 days.</p>	<p>Metabolism of systemic Sb is not known to occur. Although Sb is not metabolized, conversion of 23% of Sb (V) to Sb (III) has been documented in studies of substances administered via intramuscular injection to humans (Vasquez et al., 2006). This conversion appears to be thiol mediated (Ferreira et al., 2003) and not enzymatically controlled. Conversion from Sb (III) to Sb (V) in vivo is theoretically possible but has not been reported. From a toxicological perspective, systemic Sb (III) could be responsible for, or contribute to toxic responses after the administration of Sb (V) compounds but there is little data to suggest the converse is true. Poorly soluble Sb compounds seem to be retained unchanged in the lungs after inhalation (Newton et al., 1994; NTP 2017). Poorly soluble material deposited within the deep lung of humans exhibits a half-life on the order of 600 days or longer, a retention time that can be significantly increased by lifestyle factors such as smoking (Garg et al., 2003).</p>	<p>The impact of valency, and changes in valency, of systemic Sb once taken up into mammalian systems has not been well characterized but appears to influence Sb distribution within the body and subsequent excretion. Valency does affect excretion routes with Sb (V) exhibiting urinary excretion while fecal metabolism predominates for Sb (III).</p>

The information above shows that Sb (III) and Sb (V):

- Have a comparable gastrointestinal absorption of around 1%;
- Will follow different distribution paths after absorption, with Sb (III) found mainly in erythrocyte and the liver, while Sb (V) found in blood plasma and the spleen;
- Will not metabolize but possibly convert (from Sb (V) to Sb (III)), especially where they are soluble (insoluble forms will remain unchanged); and



- Will follow different excretion paths, with Sb (III) being excreted via feces and Sb (V) via urine.

ASSESSMENT NOTE 8: *From an ADME potential viewpoint (beyond Sb substance-specific systemic bioavailability and uptake), there is no striking need to distinguish Sb substances for the purpose of grouping and read-across for genotoxicity.*

5. Provisional hazard, classification and read-across assessment, and further research opportunities

Provisional hazard, classification and read-across assessment

The assessment notes recorded along the document are used to inform the provisional hazard, classification and read-across assessment, and further research needs:

1. If further in vivo testing is considered necessary, it should ideally address the lack of in vivo genotoxicity data on pentavalent Sb substances.
2. While 6 out of 17 in vitro genotoxicity studies have reported positive responses for genotoxicity, 5 out of the 7 in vivo genotoxicity studies in rodents have failed to observe genotoxic effects.
3. If reactive oxygen species mediate most in vitro observations of genotoxicity, robust in vivo antioxidant systems could explain why most in vivo studies have not observed genotoxicity.
4. The relevance of these [in vitro] observations to in vivo exposure scenarios is further uncertain since the concentrations required to produce effects in vitro are significantly higher than plausible systemic levels of Sb in vivo.
5. The ToxTracker assay findings confirm that the genotoxicity observed in some of the test items is most probably the result of indirect mechanisms, and that Sb (III) compounds will generally tend to exert such mechanisms in a stronger manner than Sb (V) ones. Based on this, it can be assumed that if most in vivo studies with Sb (III) compounds have not observed genotoxicity, it is very unlikely that the same studies conducted with Sb (V) compounds would produce genotoxic effects.
6. It is therefore concluded that the tested Sb substances are not genotoxic in vivo.
7. Any Sb substance [in scope of this document], provided it has a comparable valency and molecular weight, able to release soluble Sb ions and to be taken up by the body to levels comparable to those of the most water-soluble ones can provisionally be part of the same read-across group for the genotoxicity endpoint.
8. Except for valency, [and perhaps also] molecular weight, and bioavailability, from an ADME, and systemic bioavailability and uptake potential viewpoint, there is no striking need to distinguish Sb substances for the purpose of grouping and read-across for genotoxicity.

Based upon the preceding, the following provisional conclusions can be drawn:

- Sb (III) compounds can produce positive results when tested for genotoxicity in vitro. Clastogenic events, usually the formation of micronuclei, appear to be most commonly observed endpoint. Sb (V) compounds have been tested with less frequency and available data are inconsistent, but it is expected that they would produce no or lower genotoxic effects than Sb (III) compounds.
- Reliance upon genotoxicity data that is heavily weighted towards the observation of micronuclei is potentially problematic. Recent studies have observed that some metals and metalloids (see Technical Annex) can form cytoplasmic inclusion bodies that can be mistaken for micronuclei if stains used for conduct of the assays are not highly specific for DNA (Wedel et al, 2013; Cohen et al., 2013). It cannot be precluded that some reports of micronucleus induction by Sb compounds may reflect staining artefacts.
- In vitro genotoxicity, assuming it occurs, is likely mediated by indirect mechanisms (e.g. induction of oxidative stress or interference with DNA repair processes). The available data do not permit discrimination between alternative mechanisms – nor do the mechanisms need to be mutually exclusive. According to ToxTracker, Sb (V) compounds do not interfere with DNA repair processes nor are they (as) strong inducers of oxidative stress (as Sb (III) compounds).
- There is no technically sound evidence to suggest expression of genotoxic potential in vivo. This difference may reflect factors such as defense mechanisms against oxidative stress that block genotoxic insults. Alternatively, or in addition, the poor uptake efficiency and rapid excretion of Sb may prevent the attainment of systemic Sb concentrations capable of producing a genotoxic impact in vivo.

- Based upon the negative in vivo data, there is no need to apply classification for mutagenicity for Sb substances.
- Despite the lack of in vivo data for Sb (V) compounds, it can be safely assumed that Sb (V) in scope of this document will not exert any genotoxicity in vivo. Other properties than valency may influence this grouping approach but have not shown to influence observations in any specific manner.

Further research opportunities

For predicting the (absence of) genotoxicity properties among Sb substances, the hazard, classification and read-across assessment could potentially be further informed by and justified with the following considerations:

- **Complete solubility and bio-accessibility information:** Complete Table 3 with comparative in vitro solubility and bioaccessibility (also with proteins) for all REACH Sb substances. This will inform, in the event of a genotoxic effect, the likelihood of absorption and uptake of each Sb substance.
- **Verify possible Micronucleus staining artifacts:** The possible formation of inclusion bodies in vitro following treatment with Sb and its compounds should be evaluated with priority for its potential impact upon micronucleus test data. The conduct of micronucleus tests with staining procedures with high specificity for DNA could provide a rapid means of determining whether staining artifacts have impacted the existing genotoxicity data base.
- **Verify proposed mode of action for in vitro genotoxicity:** Oxidative stress and reactive oxygen species are thought to potentially mediate genotoxicity that might be expressed by Sb and its compounds – a suggestion inconsistent with the observation that Sb compounds do not induce point mutations. Given that point mutations should result from oxidative stress, additional mutagenicity studies in mammalian cells could be conducted. Such studies might be conducted using cells lines containing gene inserts (e.g. gpt) that can be retrieved and sequenced should mutations be induced. The genetic fingerprint for potential mutagenic events could help elucidate molecular mechanisms of genotoxic action (e.g. oxidative damage) and also compared to the point mutations that activate oncogenes in antimony trioxide induced lung tumors. Given the negative mutagenicity testing conducted to date, protocol amendments (e.g. extended treatment times) would likely be required to detect mutagenic activity.
- **Generate missing in vivo data for Sb (V) compounds:** It is often assumed that Sb(III) compounds are genotoxic and that Sb(V) compounds are not. The testing literature for Sb (V) compounds is limited with only a small number of in vitro tests having been conducted. Indeed, in vitro testing of Sb(V) compounds for micronucleus induction has yielded both positive and negative results. Pending resolution of the two points above, additional testing of Sb (V) compounds may be needed to support read-across assumptions.
- **Compare ToxTracker results with tailored in vitro testing:** Recently generated data from the ToxTracker assay has suggested compound-specific differences in the ability to induce oxidative stress and/or protein damage. It remains to be seen if the in vitro genotoxic properties of these compounds exhibit differences concordant with their ToxTracker response profiles. Testing for point mutation induction (establishing any mutation fingerprint) and micronucleus induction in vitro could be indicated as a function of the three points above.

6. Genotoxicity provisional read-across approach justification, as per RAAF scenarios

Analogue or category approach?

As information is available from more than one source substance, and used for more than one target substance, the provisional read-across approach applied to fill in genotoxicity data gaps for Sb substances is a category one, and not an analogue one.

Substances share structural similarity or similarity in precursor or (bio)transformation products?

Although structural similarity could be claimed on the basis of the common presence of Sb atoms in all substances, for the purpose of genotoxicity read-across justification, the substances are actually grouped in two subgroups which are each releasing a valence-specific soluble metal (oxyan)ion (III or V). In each subgroup, a comparable uptake by the body (i.e. a comparable bioavailability and potential to produce systemic toxicity) can be assumed. The release of the specific Sb ion can be considered a common transformation product within each subgroup (no matter how the transformation occurs).

Although two types of Sb ions can be released by Sb substances: Sb (III) and Sb (V) forms, on the basis of the information on the “metabolization” of Sb substances in vivo, it is possible that all forms of Sb will convert at least partially to a Sb (III) form in vivo. Ultimately, systemic exposure to a pentavalent form of Sb will also yield some exposure to a trivalent form of Sb, no matter whether the original form administered was a metallic, trivalent or pentavalent Sb substance. Still, for purposes of read-across, it is preferred to retain two subgroups: one for Sb (III) substances, and one for Sb (V) ones.

A regular pattern/trend across systemic toxicity properties or general similarity of systemic toxicity properties?

The genotoxicity data available on the Sb substances reported in Tables 1.1-1.3 reveal a general trend of no (in vivo) genotoxicity. Mechanistic information shows that in vitro genotoxicity observed in some cases can be the result of indirect mechanisms, and that such mechanisms differ between Sb (III) and Sb (V) substances. Whereas the former appear to be able to induce oxidative stress or interfere with DNA repair processes, the latter are not. The trends observed are hence different for Sb (III) and Sb (V) substances, which identified two subgroups.

Resulting read-across approach scenario

The provisional read-across approach applied to the Sb substances corresponds to scenario 5, because there is an absence of relevant quantitative variations in the predicted property across the substances in each subgroup (because of the assumption that all Sb (III) and Sb (V) substances, respectively, will have a comparable (non-)interaction with genetic material and processes).

7. Common assessment elements for category approach applied to antimony substances

Table 4 below summarizes the evidence available for each one of the common and specific assessment elements to be considered to assess the read-across approach and its justification.

Table 4. Assessment elements and evidence provisionally justifying the ECHA RAAF read-across approach scenario 5 for Sb substances.

Assessment Element/Details	Supporting evidence
Characterization of source and target substances	
Identity and characterization of all substances in category	<p>Subgroup Sb (III): Source substances: Any Sb (III) substance releasing Sb (III) ion and having moieties or impurities which do not have a more toxic systemic toxicity profile than Sb (III). Target substances: Other Sb (III) substances releasing Sb (III) ion and having moieties or impurities which do not have a more systemic toxicity profile than Sb (III).</p> <p>Subgroup Sb (V): Source substances: Any Sb (V) substance releasing Sb (V) ion and having moieties or impurities which do not have a more toxic systemic toxicity profile than Sb (V). Target substances: Other Sb (V) substances releasing Sb (V) ion and having moieties or impurities which do not have a more systemic toxicity profile than Sb (V). More information in Section 4 of this document.</p>
Structural similarity and dissimilarity within the category (category description)	
The structural similarities and differences identified for all category members	<p>Subgroup Sb (III) or Sb (V): All substances in each provisional category have in common that they have one or more Sb atoms bond through ionic or covalent bonding with moieties, many of which are essential nutrients or Essential Trace Elements (ETEs), with none or negligible genotoxicity, or normal metabolites (NM), which are expected to be rapidly metabolized. More information in Section 4 of this document.</p>
Structural differences that are allowed within the category are specified	<p>Subgroup Sb (III): Differences in molecular weight, moieties and release rates are allowed as long as there is evidence that the final speciation of the released (oxyan)ions, i.e. Sb (III), remains comparable.</p> <p>Subgroup Sb (V): Differences in molecular weight, moieties and release rates are allowed as long as there is evidence that the final speciation of the released (oxyan)ions, i.e. Sb (V), remains comparable. More information in Tables 1 (and 2) of this document.</p>
Link of structural similarities and structural differences with the proposed regular patterns (presence of hypothesis) - It is explained why and how the category members should behave in a predictable manner	
Formation of common (identical) and non-common compounds	<p>Subgroup Sb (III): All substances in the provisional category have in common that they release a common Sb (oxyan)ion (III) form in vivo. This Sb (III) form can be considered as a common transformation product. Systemic exposure will be to this common transformation product, no matter the original form of the substance originally present and/or administered.</p>

Assessment Element/Details	Supporting evidence
	<p>Subgroup Sb (V): All substances in the provisional category have in common that they release a common Sb (oxyan)ion (V) (which may partially convert into a Sb (III) form in vivo). The Sb (V) form can be considered as a common transformation product. More information in Section 4 of this document.</p>
Degradation, bioaccumulation and impact of non-common compounds	<p>Subgroup Sb (III) or Sb (V): The moieties of the various Sb substances of the group will be absorbed for essential functions in the body or metabolized as any other normal metabolite. More information in Section 4 of this document.</p>
Impact of impurities on the prediction	
The identified impurities have an impact on the prediction	<p>Subgroup Sb (III) or Sb (V): All Sb substances will typically have some levels of As and/or Pb as impurities, because of the geological affinity/common primary origin of these three elements in nature. These impurities are not expected to have an impact on the (predicted) effect as long as they are present in concentrations below the classification threshold. More information in Section 4 of this document.</p>
Consistency of properties in the data matrix	
A data matrix with experimental data for source and target substances is needed to support the read-across	<p>Subgroup Sb (III): The genotoxicity dataset available for Sb (III) substances contains only one endpoint for which comparable studies are available on more than one Sb (III) substance: Ames Test. Overall there is test data for two Sb (III) substances, and the ToxTracker assay provides a comparative overview for all other Sb (III) substances. When comparing available data:</p> <ul style="list-style-type: none"> - Most in vitro studies yield positive genotoxicity results for clastogenic events but not point mutations, - ToxTracker shows a comparable induction of oxidative stress and interference with DNA repair processes, and - All high quality in vivo studies yield negative genotoxicity results. <p>Subgroup Sb (V): The genotoxicity dataset available for Sb (V) substances contains only one endpoint for which comparable studies are available on more than one Sb (V) substance: Ames Test. Beyond Ames, there is mutation test data (i.e. excluding indicator assays) for one Sb (V) substance, but the ToxTracker assay provides a comparative overview for all other Sb (V) substances. When comparing available data:</p> <ul style="list-style-type: none"> - Most in vitro studies yield negative genotoxicity results (cf. interpretation!), - ToxTracker shows a comparable absence of induction of oxidative stress and interference with DNA repair processes, and - There are no in vivo genotoxicity results. <p>More information in Sections 2 and 3 of this document.</p>
Reliability and adequacy of the source data	
The source study(ies) needs to be reliable and adequate as requested for any other key study	<p>Only adequate and reliable data has been used to support the read-across justification. More information in Section 2 of this document.</p>

8. Resulting hazard assessment and classification for the substances of the category

Table 5 provides, for each Sb substance, the result of the hazard assessment and classification constructed on the basis of the provisional read-across approach.

Table 5. Classification resulting from provisional read-across approach for Sb substances.

Name	CAS #	Mutagenicity classification	Further testing needs
Subgroup Sb (III)			<ul style="list-style-type: none"> • Complete solubility and bio-accessibility information • Verify Micronucleus staining artifacts • Verify proposed mode of action for in vitro genotoxicity • Generate missing in vivo data for Sb (V) compounds • Compare ToxTracker results with tailored in vitro testing
Sb –powder	7440-36-0	Not classified	
Sb – massive	7440-36-0	Not classified	
Diantimony trioxide	1309-64-4	Not classified	
Antimony sulfide	1345-04-6	Not classified	
Antimony tris (ethylene glycolate)	29736-75-2	Not classified	
Antimony trichloride	10025-91-9	Not classified	
Subgroup Sb (V)			
Sodium hexahydroxoantimonate	33908-66-6	Not classified	
Sodium antimonate	15432-85-6	Not classified	
Antimony pentachloride	7647-18-9	Not classified	
Antimony pentoxide	1314-60-9	Not classified	
Potassium hexahydroxoantimonate	12208-13-8	Not classified	

9. Technical Annex

Prokaryotic Test Systems

Tests using prokaryotic systems generally provide negative responses for mutagenicity, but interpretation of this negative finding must be qualified by recognition that uptake of ions for metalloids such as Sb by prokaryotic organisms is generally considered to be limited (Kuroda et al., 1991). Genetic resistance to antibiotics, often carried by DNA plasmids transmissible from one bacterial strain to another, can also impart properties of a “metalloid pump” that actively reduces intracellular concentration of Sb ions (Xu et al., 1998). The presence of such a gene in a bacterial test strain would predispose to false negative test results. Given these caveats, assays for reverse gene mutation in (e.g. the Ames test) have generally produced negative results (Table 1) for Sb(III) and Sb(V) compounds.

Although gene mutations were not observed in bacterial mutation test, positive response were observed for Sb compounds in the *B. subtilis* rec assay for DNA damage (Table 1). This “indicator assay” assesses increases in recombination events that are most likely the result of DNA damage induced by chemical treatment. Sb₂O₅ did not produce a response but also seemed to lack toxicity as evidenced by lack of a zone of inhibition resulting from Sb₂O₅ treatment. The authors attributed this to limited solubility of the pentoxide but data to substantiate this are not presented. Independent of the reasons, the rec assay results for Sb₂O₅ do not appear to have resulted in significant exposure to Sb ions. The authors further hypothesized that the difference in response in the two bacterial test systems might have been produced by differences in compound uptake or toxicity in the two bacterial strains. False negatives would result in the Ames test if inadequate Sb uptake occurred, whereas false positives can occur in the rec assay if cytotoxicity results in lysosomal nuclease release. In the absence of information that discriminates between these alternate hypotheses, response inconsistency between the bacterial test systems, and between compounds in the rec assay, make it difficult to derive definitive conclusions regarding mutagenicity or genotoxicity from studies using bacteria.

In Vitro Tests with Mammalian Cells

Two studies have evaluated Sb compounds for forward mutation at the thymidine kinase (TK) locus of cultured L5178Y mouse lymphoma cells (Elliot *et al.*, 1998; Stone 2010; Table 2). Sb₂O₃, tested in the presence and absence of S9 for metabolic activation, failed to induce mutation after 4 h exposures. Tested concentrations were nominal (i.e. not measured in the cell culture medium) and may have exceeded the aqueous solubility of the test compound. Little cytotoxicity was observed, further suggesting limited release of Sb(III) ions. Finally, the 4 h treatment time employed was shorter than the 24 h exposure duration currently recommended by international guidelines (Moore *et al.*, 2002). Thus, while Sb₂O₃ was not mutagenic, positive responses might have been induced by longer duration of chemical exposure or the study of more soluble Sb compounds that would yield higher Sb concentrations. Similarly, negative results were obtained in the testing of NaSb(OH)₆ in the presence and absence of S9 using the microtiter fluctuation technique for the assay.

Elliot *et al.* (1998) also examined the induction of chromosomal aberrations in cultured human lymphocytes. at nominal Sb₂O₃ concentrations that ranged from 10 to 100 µg/ml. Setting aside concerns over possible exceedance of solubility limits, a dose dependent increase in chromosome aberrations was observed in the absence of cytotoxicity. The nature of the aberrations was not explicitly described except to note that chromosome gaps had been excluded.

Given the finding of chromosome aberrations, it is not surprising that studies have reported that treatment with Sb compounds (usually SbCl₃) is associated with micronucleus (MN) induction in a variety of different cell types. Huang *et al.* (1998) observed MN induction in a series of studies using Chinese hamster ovary cells, human bronchial epithelial cells and human fibroblasts. MN induction was concentration dependent and, at higher concentrations, associated with significant cytotoxicity. The authors further observed an influx of calcium into cells after SbCl₃ treatment followed by time-delayed apoptosis and DNA fragmentation. Calcium

influx was noted to potentially be an indication of oxidative stress and to provide a mechanistic pathway for DNA damage via indirect pathways. Induction of apoptosis was similarly noted to provide an additional pathway for DNA damage to occur independent of direct Sb ion interaction with DNA. Both mechanisms of actions would be expected to exhibit non-linear dose response functions (i.e. thresholds).

Similar dose dependent increases in MN induction were observed in V79 cells (Gebel et al., 1998) and cultured human lymphocytes (Schaumloffel and Gebel, 1998). Finally, Migliore et al. (1999) observed strong dose dependent induction of micronuclei in cultured lymphocytes from two human volunteers following in vitro treatment with KSbO_3 (potassium antimonate). Fluorescence in situ hybridization was used to examine micronuclei for the presence of centromeres – micronuclei in Sb treated cells generally lacked centromeres suggesting the occurrence of clastogenic events as opposed to aneuploidy. The concentrations tested (240 – 600 μM) are within the range expected for a moderately soluble compound but higher than others have reported as being possible in cell culture medium.

The absence of centromeres in Sb induced MN, although consistent with chromosome breakage, also raises technical concerns with respect to the majority of the micronucleus studies conducted of Sb compounds. Studies conducted to date have primarily relied upon Giemsa staining for micronucleus detection, a staining method that lacks specificity for DNA (Nersesyan et al., 2006). Metalloids such as arsenic have recently been reported (Wedel et al, 2013; Cohen et al., 2013), presumably due to electrophilic interaction with thiol groups on proteins and other macromolecules, to produce cytoplasmic inclusion bodies that can be mistaken for micronuclei if non-DNA specific stains (e.g. Giemsa) are used. There thus remains the possibility that inclusion body formation by Sb may have produced staining artifacts misinterpreted as micronuclei. Further research would be required to determine if this potential source of experimental artifact is applicable to antimony.

The study of Sb compounds in indicator assays yields positive results (Table 3). Sister chromatid exchange induction and Comet assay results have been generated most frequently but the quality of most studies is low. Both assays require careful monitoring of, and control for, cytotoxicity, terminal differentiation and/or apoptosis to permit meaningful interpretation of results. Most studies have failed to implement proper controls for these sources of experimental artifact and have been excluded from consideration here. Moreover, given the preponderance of positive micronucleus data, indicator assay data adds little to a weight of evidence evaluation. Indicator assay data considered but excluded from evaluation here are summarized in the CSRs.

In vivo test results

Gurnani et al. (1992) evaluated the effects of single and repeated doses of Sb_2O_3 chromosome aberrations in mouse bone marrow. Oral gavage of 400 -1000 mg/kg in a single dose, followed by analysis of chromosome aberrations after dosing did not detect an increase in aberration frequency. In a repeated dosing protocol, mice were exposed to 400, 667 and 1000 mg/kg Sb_2O_3 by oral gavage for up to 21 days and animals sacrificed at 7, 14 and 21 days for evaluation of chromosome aberrations. Day 21 evaluations were restricted to the 400 and 667 mg/kg dosing group since lethality occurred on day 20 in the 1000 mg/kg treatment group. The authors reported a variety of chromosome alterations including chromatid gaps and breaks, polyploid cells and “centric fusions” that increased as a function of dose through day 7 and 14 and then declined at day 21. Presentation of the data is less than straight forward and statistical evaluations were conducted after pooling of data for aberration types that should have been evaluated independently (e.g. chromatid breaks and polyploid cells should have been evaluated separately). Kirkland et al. (2007) have noted a number of deviations from GLP protocols in the conduct of the study of Gurnani et al. (1992), questioned the purity of the test substance used and noted irregularities in the nature of the chromosomal changes observed (i.e. breaks and centric fusions should have been associated with chromosome fragments but were not). The study deficiencies are significant and indicate a need for validation from other studies. A later publication by Gurnani et al. (1993) would at first seem to provide confirmation of Gurnani et al. (1992) but, as also noted by Kirkland et al. (2007), is merely republication of the data originally published in 1992. Gurnani et al. (1993) has thus been excluded from Table 4 since it is not a new study.

Kirkland et al. (2007) mirrored the protocols of Gurnani et al. (1992) in a study of male and female rats administered 250, 500 and 1000 mg/kg Sb_2O_3 by oral gavage for 21 days. Six male and six female rats were included in each treatment group and the protocol included a positive control treatment group (lacking in the Gurnani et al., 2002 study). Treatment with Sb_2O_3 produced few signs of clinical toxicity other than a modest reduction in weight gain in the highest dosing group. Additional toxicokinetic studies confirmed both the uptake of Sb into the blood and the presence of Sb in bone marrow. Animals were then evaluated for the induction of both bone marrow chromosome aberrations and micronuclei in polychromatic erythrocytes on day 22. No treatment related increases in chromosome aberrations or micronuclei were observed. This study strongly adhered to GLP guidelines and possesses technical rigor superior other *in vivo* studies evaluating clastogenic effects of Sb compounds.

Other studies evaluating the genotoxic impacts of Sb *in vivo* followed protocols limited in scope. Elliot et al. (1998) examined the impacts of a single 5000 mg/kg oral gavage Sb_2O_3 dose upon micronucleus induction. No evidence was obtained for micronucleus induction but the use of only a single treatment and one dose limits the significance of this negative finding. The same authors also examined the induction of unscheduled DNA synthesis in rat liver after a single dose of Sb_2O_3 administered by oral gavage at doses of 3200 and 5000 mg/kg. No treatment related impacts upon unscheduled DNA synthesis were observed.

The National Toxicology Program of the United States recently conducted inhalation cancer bioassays upon rats and mice, exposing animals to 3, 10 and 30 mg/m^3 Sb_2O_3 for two years (NTP, 2017). The NTP also conducted studies to evaluate the genotoxic effects of exposure to antimony trioxide after one year of inhalation exposure. Sensitive flow cytometric procedures were also applied to enumerate induction of micronuclei in the erythrocytes and white blood cells from rats and mice. Increased micronuclei were not observed in cells from rats but a low level of micronucleus induction was observed in mouse erythrocytes. The incidence of micronuclei increased in both male and female mice generally increased in a dose-dependent fashion but the response magnitude was small. For example, normochromatic erythrocytes exhibited an average of 1.04 micronuclei per 1000 cells in controls, increasing to a maximum of 1.38 per 1000 cells in female mice exposed to 30 mg/m^3 of antimony trioxide. This level of response is statistically significant by virtue of 1,000,000 cells having been scored but would not have been detectable or significant without the application of flow cytometry to screen large numbers of cells. While the response observed may be statistically significant, the biological significance of the response is unclear.

Other laboratories have observed that conditions which accelerate or perturb erythropoiesis produce small increases in erythrocyte micronuclei. Thus, induction of anemia by blood loss or dietary iron restriction causes modest increases in micronucleus incidence - generally accompanied by the appearance of immature reticulocytes in the blood (Tweats et al., 2007; Molloy et al, 2012). The pulmonary toxicity of antimony trioxide produced hypoxia and bone marrow hyperplasia that perturbed erythropoiesis as evidenced by increased prevalence of immature reticulocytes in the blood of mice. Although NTP (2017) interprets the induction of micronuclei in mice as evidence of genotoxicity, the small magnitude of the response and evidence of disturbed red blood cell production indicates that designation of this as a positive response is not inappropriate. Indeed, as acknowledged by NTP (2017) an independent Peer Review Panel had evaluated the genotoxicity study results and indicated that evidence of genotoxicity was lacking in the NTP studies.

Lung tissues from a separate cohort of rats and mice exposed to antimony trioxide for 12 months were analyzed for DNA damage by the Comet assay. No DNA damage was observed in exposed rats while positive assay responses are reported for cells within mouse lung tissue. Although the NTP report does not attribute great significance to the positive Comet assay results, it must be noted that the protocols employed for conduct of the Comet assay do not meet current minimal quality standards. (Speit et al., 2015). Application of the Comet assay to intact tissues must carefully control for natural process that can produce DNA fragmentation and false positive assay outcomes. Cytotoxicity, apoptosis and terminal differentiation must all be carefully assessed for their impact upon assay outcomes. The study controlled for none of these sources of artifact, casting doubt upon the significance of the modest positive response observed in mice. Lack of genotoxicity

in rats remains a significant observation since the uncontrolled sources of experimental artifact would create false positive assay response and would not mask genotoxicity to create a false negative response.

Potential Mechanisms of Action

The mechanism(s) by which Sb compounds exert genotoxic effects *in vitro* remain to be determined. There is no evidence that Sb ions undergo covalent interaction with DNA - genotoxicity is thus believed to involve indirect mechanisms. De Boeck et al. (2003) suggest that the generation of oxygen radicals constitute an indirect pathway for inducing genotoxic responses. Supportive evidence for this is derived from the calcium influx studies of Elliot et al. (1998) noted earlier. The cytotoxic effects of potassium antimony tartrate upon cardiomyocytes also appears to be associated with the generation of oxygen radicals (Tirmenstein *et al.*, (1995). Finally, Jiang *et al.*, (2016) have observed that apoptosis induced by Sb appears to be a response to the generation of active oxygen species. If reactive oxygen species mediate most *in vitro* observations of genotoxicity, this could explain why most *in vivo* studies have not observed genotoxicity. Anti-oxidant system in an intact animal are robust and would mitigate against oxidative damage. Expression of genotoxicity would with be absent *in vivo* or exhibit a threshold with genotoxicity only resulting when the protective capacity of anti-oxidant systems is exceeded (Kirkland et al., 2015).

Not all evidence supports oxidative stress as a mechanism for Sb genotoxicity. Shaumlöffel and Gebel (1998) did not observe attenuation of Sb induced Comet assay responses by the exogenous addition of superoxide dismutase or catalase, but it is not clear whether the positive Comet assay results reported were artifacts of cytotoxicity or apoptosis. The NTP inhalation cancer bioassays of antimony trioxide (NTP, 2017) observed activation of the EGFR oncogene in a number of mouse lung tumors and “fingerprinted” the DNA sequence changes presumed to be responsible for activation. The observed changes were not characteristic of DNA sequence changes associated with oxygen radicals. A high frequency of G to T transversions was not observed in activated oncogenes, the DNA sequence alteration that is most commonly associated with interaction of active oxygen species with DNA to form 8-hydroxyguanine and a subsequent G to T transversion (Tchou *et al.*, 1991; Hong et al., 2016). Oncogene activation in mouse lung tumors may thus result from events unrelated to oxidative stress and/or may not be the critical event by which antimony trioxide induces mouse lung tumors.

Sb has also been suggested to interfere with DNA repair processes (Beyersmann and Hartwig, 2008) and this may facilitate genotoxic responses. Both antimony trichloride and antimony potassium tartrate appear to inhibit the repair of radiation induced double strand DNA breaks in cultured CHO cells (Takahashi et al., 2002). Grosskopf et al., (2010) have similarly observed that antimony trichloride will inhibit excision repair of lesions induced by UV irradiation or chemical treatment with activated polycyclic aromatic hydrocarbons in A549 human lung carcinoma cells. Such effects upon DNA repair processes could contribute to genotoxic responses associated with exposure to Sb. However, the relevance of these observations to *in vivo* exposure scenarios is uncertain due to the concentrations (100 mM or higher) of Sb required to produce effects.

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