



International
Antimony Association

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

-

Human Health: Lung toxicity and carcinogenicity

31 July 2018

Contents

1. Introduction	3
2. Available lung toxicity and carcinogenicity data for antimony source and target substances	4
3. Considerations around mode of action and classification of Sb substances for lung toxicity and carcinogenicity	9
Antimony and (lung) carcinogenicity	9
Antimony and lung toxicity	12
Hypothesis mode of action and lung toxicity and carcinogenicity classifications.....	14
4. Identity, characterization, physico-chemical properties, and bioavailability of the source and target antimony substances	15
Basic physicochemical characteristics.....	15
Solubility and bioavailability	18
5. Provisional hazard, classification and read-across assessment, and further research opportunities.....	21
Provisional hazard, classification and read-across assessment	21
Further research opportunities	2
6. Lung toxicity and carcinogenicity provisional read-across approach justification, as per RAAF scenarios.....	24
7. Common assessment elements for category approach applied to antimony substances	5
8. Resulting hazard assessment and classification for the substances of the category ...	27
9. References.....	28

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.

From the various antimony (Sb) substances which are registered under REACH, lung toxicity and carcinogenicity studies have been performed only for a few. Overall, very few studies have assessed the lung toxicity and carcinogenicity properties of Sb substances. Most of the information is available on a few trivalent Sb substances, and recent animal data is only available for antimony trioxide. This dataset is used as 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 scope, which may influence a possible lung toxicity or carcinogenicity.

This "scientific opinion, provisional read-across justification, and further research opportunities" document outlines the approach followed to predict the lung toxicity and carcinogenicity 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 better fit the specificities 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 lung toxicity and carcinogenicity data for antimony source and target substances

Tables 1.1, 1.2 and 1.3 below provide an overview of the Klimisch score 1 or 2 lung toxicity and carcinogenicity data that is available for the Sb substances considered for grouping and read-across. Typical effects reported are pneumoconiosis or stibiosis (synonyms¹) without significant pathological changes (e.g. fibrosis) in humans; and inflammation, fibrosis and cancer in rodents.

Table 1.1 Overview of antimony-related observations on lung toxicity in humans

Cohort Studied	Results	Remarks	Reference
51 males (aged of 31 – 54) employed in a smelter and exposed to dust containing predominantly antimony trioxide with small amounts (2.1 – 7.8%) of antimony pentoxide. Arsenic co-exposure documented. Duration of employment was from 9-31 years. All workers exhibited symptoms of pneumoconiosis.	X-rays confirmation small, dense, roundish or polygonal opacities typical of pneumoconiosis in the majority of workers but little evidence for fibrosis. Clinical respiratory symptoms observed included permanent or periodic breathlessness in effort, coughing and wheezing Pulmonary function tests showed obstructive changes that were mild in most cases.	2 (reliable with restrictions)	Potkonjak V., Pavlovich M. (1983b)
274 men at an antimony processing plant with exposure to antimony trisulfide, antimony trioxide, process slags	X-ray examination revealed simple pneumoconiosis in 97 workers. No clinically significant changes in lung function tests.	2 (reliable with restrictions)	McCallum RI (1967)

Several long-term historical occupational inhalation exposures to Sb compounds have been associated with impairments of lung function resulting from chronic inflammation and fibrosis. Two studies, relatively good quality (Table 1.1), and other studies of lower quality (summarized in the CSRs) document such effects in Sb-exposed workers. In general, however, the pneumoconiosis observed in workers tends to be benign with only little evidence of pathological changes (e.g. fibrosis) that would indicate reactivity of the Sb burden in the lungs of workers.

Studies confirm the historical incidence of pneumoconiosis in workers employed at Sb processing facilities, with an exposure exceeding the current Occupational Exposure Limit of 0.5 mg/m³ by a factor of 10 or more. This is consistent with medical surveillance data reported to the International Antimony Association by its membership and with the decrease of the incidence of pneumoconiosis at an antimony trioxide production facility after the implementation of OELs (Figure 1).

¹ Pneumoconiosis is the term for lung diseases caused by inhalation of mineral or inorganic dust. When it is associated with inhalation of Sb dusts, it is called stibiosis.

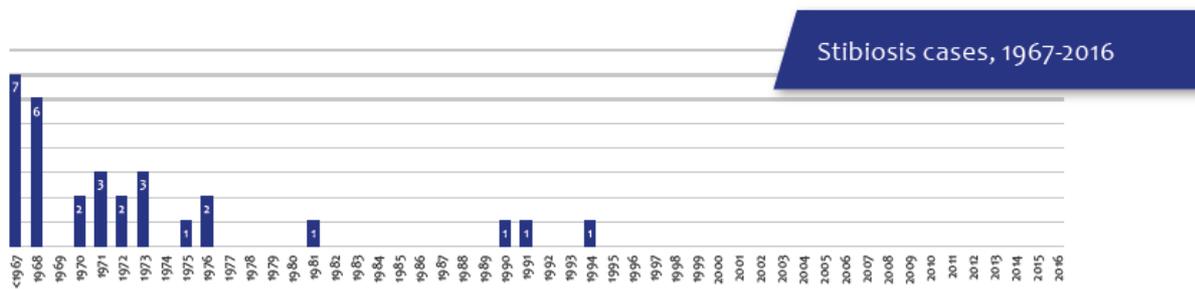


Figure 1. Decrease in pneumoconiosis/stibiosis cases at an antimony trioxide production facility following the implementation of the OEL

Table 1.2. Summary of epidemiology studies workers of occupationally exposed to antimony trioxide

Cohort	Results	Remarks	Reference
Prospective cancer mortality study of 1420 men employed at an antimony smelter which converted to antimony trioxide production in 1973. Selection criteria entailed 3 months of employment between 1961 and 1992	Elevated lung cancer risk (37 observed vs. 23.9 expected). Lung cancer excess (32 cases) confined to workers with employment history prior to 1961. Significant exposures to arsenic and lead were present until the early 1970s, precluding assumptions of causality between antimony exposure and lung cancer. No data on cigarette smoking rates	2 (reliable with restrictions)	Jones, R.D. (1994)
Mortality study of 1014 men employed at an antimony smelter between 1937 and 1971. Worker population was largely Hispanic and an appropriate referent group was difficult to assemble. Occupational exposures included antimony ores, metal and antimony trioxide.	Elevated incidence of lung cancer (SMR 1.39) when it was assumed that Hispanics have a lower incidence of smoking. Pneumoconiosis also elevated (SMR 1.22) but ethnic specific rates were not available for comparison. Significant co-exposure to known lung carcinogens such as arsenic precludes attribution of health risk to antimony. No data available on cigarette smoking rates.	2 (reliable with restrictions)	Schnorr et al., 1995
Mortality study of 1,462 male workers employed at a tin smelter over an employment period of 1937 - 2001	Elevated risk of lung cancer in the overall smelter population. However, co-exposures to lead, arsenic and cadmium were documented in air sampling conducted from 1972 – 1991. Personal exposure measurements for exposure to antimony trioxide not available for lung cancer cases– air samples from different process areas indicated relatively low Sb (presumable antimony trioxide) levels in occupational aerosols ranging from 0.01 to 0.11 mg/m ³ . Median exposure prior to 1972 estimated at 0.63 mg/m ³ . No control for	2 (reliable with restrictions)	Jones et al., 2007

Cohort	Results	Remarks	Reference
	smoking or other lifestyle confounders. Attribution of lung cancer to antimony exposure not possible.		

Although epidemiology studies in smelter environments have reported small increases in lung cancer in workers occupationally exposed to antimony trioxide (Table 1.2) attribution to Sb compounds has not been possible due to significant levels of co-exposure to known lung carcinogens such as arsenic and cadmium.

Table 1.3. Overview of inhalation exposure impacts upon experimental animals.

Method	Results	Remarks	Reference
Rat (Fischer 344) male and female exposed (65/sex/group) 6 h per day, days per week for 52 weeks to 0.0, 0.055, 0.511 or 4.50 mg/m ³ antimony trioxide followed by 12 months of observation. MMAD of particles was 3.76 microns.	Interstitial inflammation, fibrosis and granulomas in treated animals and controls. No induced benign or malignant tumors observed. Evidence of particle overload being attained at highest dose tested. LOAEC of 4.5 mg/m ³ based upon 80% inhibition of particle clearance. NOAEC of 0.51 mg/m ³ based upon diminished clearance impacts.	2 (reliable with restrictions)	Newton et al., (1994)
Rat (Wistar) male and female exposed in groups of 90 /sex/substance to 45.0 mg/m ³ Sb ₂ O ₃ (MMAD 2.8 microns) or 36.0 mg/m ³ Sb ore (MMAD 4.78 microns) for up to 52 weeks. (7 h/d and 5 d/wk) and then held for observation. Purity of Sb ₂ O ₃ only 80% with significant lead and arsenic contamination.	Lung tumors in 25% of female rats exposed to Sb ₂ O ₃ and 27% of female rats exposed to ore. No lung tumors in exposed males or controls. Lung pathology showed interstitial fibrosis hyperplasia and metaplasia in response to treatment	2 (reliable with restrictions)	Groth et al., (1986)
Groups of 20 female CDF rats exposed to 0, 1.9 and 5.0 mg/m ³ Sb ₂ O ₃ 6 h/day, 5 days /week for one year followed by one year of observation. MMAD of 5.06 microns	Focal fibrosis at 3 months in high dose group that increased with exposure duration. Evident in the low dose group at 12 months. No malignant lung tumors in controls or low dose group but scirrhous carcinoma of the lung found in 9 out of 18 high dose rats and squamous cell carcinomas in 2 out of 18 animals.	2 (reliable with restriction)	Watt (1983)
Rats (Wistar Han) male and female exposed in groups of 60 per sex per treatment concentration to antimony trioxide (MMAD 0.9 – 1.5 microns) at concentrations of 0, 3, 10 and 30 mg/m ³ 6 h per day, 5 days per week for up to two	Reduced body weight gain and survival at all treatment levels indicating MTD approached or exceeded. Dose dependent increase in benign and malignant lung tumors and adrenal pheochromocytomas in both sexes. Significant lung inflammation and fibrosis accompanied by abnormal breathing and	1 (reliable without restriction)	NTP (2017)

Method	Results	Remarks	Reference
years.	cyanosis indicative of hypoxia		
Mice (B6C3F1) male and female exposed in groups of 60 per sex per treatment concentration to antimony trioxide (MMAD 0.9 – 1.5 microns) at concentrations of 0, 3, 10 and 30 mg/m ³ 6 h per day, 5 days per week for up to two years.	Reduced body weight gain and survival at all treatment levels indicating MTD approached or exceeded. Dose dependent increase in benign and malignant lung tumors in both sexes. Dose dependent lymphoma increase (predominately B cell) especially in female mice. Benign and malignant skin neoplasms also observed. Significant lung inflammation, fibrosis and abnormal breathing.	1 (reliable without restriction)	NTP (2017)

The animal studies (Table 1.3) using exposures of sufficient duration and respirable particle aerosols with a small size facilitating penetration to the deep lung (< 7 microns) confirmed that antimony trioxide can have toxic impacts upon the lung. This affirmation needs to be properly evaluated regarding to the workers exposed in industrial facilities as the occupational aerosols possess a larger particle size distribution (Hughson, 2005) with larger particles (the inhalable fraction) preferentially depositing in the nose, throat and upper airways. The respirable fraction is, on average, only about one third the size of inhalable fraction.

Animal inhalation studies are thus designed to maximize the likelihood of damage to tissues of the deep lung. Although three initial experimental inhalation studies deviate from standard protocols (one year of exposure opposed to the two years specified by most cancer bioassay guidelines), it has been demonstrated that antimony trioxide could impair particle lung clearance. More recently, a two-year cancer bioassay (NTP, 2017) reported evidence of a relationship between exposure to respirable diantimony trioxide and lung tumors in the mouse and, to a lesser extent, the rat.

Table 1.4 Overview of lung toxicity and carcinogenicity data available for Sb substances considered for grouping and read-across for lung toxicity and carcinogenicity endpoints (only Klimisch 1 or 2 studies).

Name	CAS #	Human		Rodent	
		Workplace observations	Epidemiology studies	Rat	Mice
Sb metal					
Sb –powder	7440-36-0		x		
Sb – massive	7440-36-0				
Trivalent Sb compounds					
Diantimony trioxide	1309-64-4	x, x	x, x, x	x, x, x, x	x
Antimony sulfide	1345-04-6	x	x		
Antimony tris(ethylene glycolate)	29736-75-2				
Antimony trichloride	10025-91-9				
Pentavalent Sb compounds					
Sodium hexahydroxoantimonate	33908-66-6				
Sodium antimonate	15432-85-6				
Antimony pentachloride	7647-18-9				
Antimony pentoxide	1314-60-9				
Potassium hexahydroxoantimonate	12208-13-8				



Table 1.4 above presents an overview of the available lung and carcinogenicity toxicity studies per Sb substance. The table shows that evidence is only available for Sb metal, Sb trioxide, and Sb trisulfide. There is no information on the potential to cause lung toxicity or cancer for any other antimony substance.

ASSESSMENT NOTE 1: *If further testing is considered necessary, it should ideally also address the lack of lung toxicity data on pentavalent Sb substances.*

ASSESSMENT NOTE 2: *While all studies have reported some degree of lung toxicity, only one reports clear evidence of cancer (in mice). Evidence in rat is either less clear or can be attributed an overload response.*

3. Considerations around mode of action and classification of Sb substances for lung toxicity and carcinogenicity

Antimony and (lung) carcinogenicity

Chronic inhalation of diantimony trioxide by rats and mice can produce damage to the lungs characterized by the progressive development of pulmonary inflammation, tissue damage and fibrotic changes. These dose-dependent changes, at sufficiently high exposures, can produce significant impairment of pulmonary function and severe systemic hypoxia that induces adaptive physiological changes (e.g. erythroid hyperplasia).

According to the experimental studies, Sb compounds might pose a carcinogenic risk to the lungs of rats through particle overload (Newton *et al.*, 1994; Schroeder, 2003). Rat's lungs do not have the capacity to remove excessive quantity of respirable particle and this triggers a cascade of inflammatory responses leading to a tumor formation, by accumulation of inert particles. This response to particle overload is not observed in mice or humans. Rat lung tumors induced by particle overload are thus of questionable significance for hazard classification or risk assessment.

NTP (2017) studies conclude that overload does not occur at an antimony trioxide exposure of 3 mg/m³ and therefore that pulmonary overload is not required for the induction of neoplasms. The rationale for this conclusion is tenuous in that 3 mg/m³ is indeed associated with impaired clearance in the rat in the NTP studies – the departure from modeled clearance rates is just not sufficient to attain the lung burden levels that meet an arbitrary criterion for overload. Moreover, significant impairment of clearance has been reported at levels much lower than those used in the NTP studies (e.g. Newton *et al.*, 1994). Finally, the incidence of lung neoplasms in both male and female rats is not statistically elevated over that in controls at by 3 mg/m³ antimony trioxide exposures. The lack of both overload and a carcinogenic response in the rat at 3 mg/m³ antimony trioxide cannot be taken as an indication that tumors produced in the rat lung at higher levels of exposure were not the result of the pulmonary overload observed to occur at these higher doses. **ASSESSMENT NOTE 3: Particle overload and the subsequent cascade of inflammatory responses leading to a tumor formation can be retained as a possible mode of action for lung cancer in rats.**

The impacts of antimony trioxide exposure upon the overall health status of rats and mice should not be neglected and may explain other adverse effects observed in the NTP studies. Exposure of rats to 3, 10 or 30 mg/m³ antimony trioxide was associated with end of study body weight suppression of 7, 8 and 20% in male rats and 10, 20 and 28% in female rats, respectively. Corresponding body weight suppression in male mice was 8, 11 and 25% and 3, 8 and 21% in female mice. Much of the data generated by the NTP bioassays reflects effects near, or in excess of, the maximum tolerated dose for antimony trioxide. This conclusion is bolstered by the observations of labored breathing, hypoxia and premature mortality due to pulmonary inflammation in exposed animals. These observations do not negate the induction of pulmonary lesions but indicate that care must be exercised in the interpretation of other systemic effects that might be associated with inhalation exposure to antimony trioxide.

There is a significant body of evidence that adrenal gland neoplasms (pheochromocytomas) lesions are to be expected under conditions of pulmonary inflammation and hypoxia. As reviewed by Greim *et al.* (2009), the association of this adrenal lesion with pulmonary impairment is sufficiently robust that, within the context of the EU REACH process, pheochromocytomas secondary to pulmonary impairment are not considered as relevant for cancer classification or risk assessment. The adrenal lesions are most properly regarded as a response to pulmonary damage induced by antimony trioxide and not a direct substance-specific effect of antimony trioxide. Indeed, they can be interpreted as confirmation that maximum tolerated doses have been exceeded in the rat.

The NTP studies further detected the presence of activated EGFR oncogenes in tumors from treated but not control animals and suggested that these tumors may have been induced by antimony trioxide. However, the mechanisms responsible for the appearance of activated EGFR oncogenes in lung tumor are far from straightforward. Activated EGFR oncogenes typically arise during later stages of neoplastic progression and not as initiating events for carcinogenesis. Moreover, activated EGFR oncogenes appear to facilitate the growth of neoplastic cells under hypoxic conditions. It thus becomes difficult to ascertain whether antimony trioxide induced changes in EGFR or if the pulmonary toxicity and hypoxia induced by antimony trioxide treatment selected for, or otherwise facilitated, the clonal expansion of neoplastic cells containing spontaneous or pre-existing activated EGFR oncogenes.

Antimony trioxide exposures in mice were also associated with an increase in lymphomas. Interpretation of this increase in the incidence of lymphomas in female mice poses diagnostic challenges that were not addressed by NTP's histopathological analysis. Whereas lymphomas induced by chemicals are usually T cell in origin (Ward, 2005), those associated with antimony trioxide exposure were predominantly B-cell or mixed B- and T-cell in origin and many appeared to be reactive lesions responding to antimony trioxide cytotoxicity. Mouse B-cell lymphomas are further difficult to interpret due to their high spontaneous incidence and complex etiology that likely includes endogenous retrovirus activity. In NTP inhalation studies, the average historical control incidence of lymphomas in B6C3F1 female mice is 25.2% (range 14 – 36%). Thus, lymphoma incidence at 10 and 30 mg/m³, but not 3 mg/m³, is significantly elevated over historical controls. The complex and diverse mechanisms for B-cell lymphoma induction have prompted the development of histopathological diagnosis and classification strategies to distinguish between spontaneous and induced lesions (Ward, 2005). Unfortunately, none of these diagnostic criteria were applied in the NTP study. Based upon the limited data provided, the excess lymphomas associated with antimony trioxide exposure appear to be similar to the naturally occurring lesions in the B6C3F1 mouse; it can be plausibly postulated that the chronic inflammation and hypoxic conditions in the antimony trioxide exposed lung produced adaptive responses in the lung and spleen that promoted the development of what is already a high incidence spontaneous neoplasm in the female mouse. As such, the increased incidence of lymphomas would not provide clear evidence of carcinogenicity.

Neoplastic skin lesions were also observed in mice exposed to antimony trioxide and different types of skin lesions were pooled to yield statistical significance. Given the high level whole body inhalation exposures employed by NTP, the appearance of histiocytomas (a benign skin lesion) is mostly likely an immunological response, as opposed to neoplastic response, and not a precursor lesion to fibrosarcoma (malignant tumors of fibrous tissues). Histiocytomas are not generally known to be precursor lesions to fibrosarcoma and there appears to be no legitimate scientific rationale to support data pooling. The observation of two squamous cell carcinomas in antimony trioxide treated female mice is unusual but is similarly difficult to interpret in the absence of preneoplastic precursor lesions. Moreover, no other study has suggested skin as a target organ for antimony trioxide carcinogenesis. There is no legitimate scientific rationale to support that skin tumors are induced by antimony trioxide.

ASSESSMENT NOTE 4: *The primary target organ of inhaled Sb substances appears to be the lung, and mode of action considerations should look at local effects in the lung rather than systemic effects. The inhalation exposure route is the only route of exposure relevant for the assessment of carcinogenicity properties.* Exposure route specificity (the lung by inhalation exposure) is further evidenced by lack of pulmonary changes after sub-chronic oral exposures to high doses of diantimony trioxide (Hext et al., 1999) and high sub-chronic i.p. dosing with the antimony (III) potassium tartrate (Dieter, 1992).

As regards the lung tumors observed in antimony trioxide treated rats and mice (NTP, 2017), it is important to note that the presence of activated oncogenes in tumors can be the result of a myriad of direct and indirect processes. Focusing on the mice lung tumors, which were observed with far higher frequency, permits more

robust analysis of the “molecular pathology” responsible for activated oncogenes in spontaneous and induced neoplasms. Spontaneous lung tumors were found to contain altered Kras genes with the activating mutations generally mapping to established “hot spots” (i.e. G to A transitions in codon 12). Altered Kras oncogenes were detected in 43% of the tumors observed in antimony trioxide treated animals. NTP notes that tumors in antimony trioxide treated animals possessed base sequence changes in hot spots similar to those observed in spontaneous tumors and suggests that the Kras altered genes observed in the tumors of antimony trioxide treated animals were the result of spontaneous lesions permitted to undergo clonal expansion by the pulmonary toxicity of antimony trioxide. This suggestion is consistent with the observation that spontaneous activated oncogenes are now known to be present in the normal tissues of animals used in cancer bioassays (Parsons *et al.*, 2009), exhibiting both tissue and animal strain specificity with respect to the prevalence of different activated oncogenes.

In addition to Kras alterations, 46% of lung tumors in antimony trioxide treated mice were observed to contain altered Egfr oncogenes. The high prevalence of tumors with Egfr alterations in exposed animals can be interpreted as evidence of mutagenic oncogene alterations induced by antimony trioxide. However, the origin of Egfr alterations is potentially more complex than is described. In humans, lung cancer tumors are increased in subjects with disease syndromes (e.g. chronic obstructive pulmonary disease) that impair lung function and lead to hypoxic conditions. Signaling pathways involving EGFR appear to play a role in the growth of such tumors under hypoxic condition (Karoor *et al.*, 2012). Egfr alterations are further linked to the ability of cancer cells to survive in hypoxic microenvironments (Murakami *et al.*, 2014). The prevalence of Egfr alterations in antimony trioxide treated animals may thus be a result of selection for tumors capable of undergoing rapid clonal expansion under the hypoxic conditions associated with the pulmonary toxicity produced by antimony trioxide. The activated Egfr oncogenes may thus be spontaneous in origin or produced by a variety of indirect processes during tumor progression (e.g. ROS generation, error prone DNA repair) with an increased prevalence in tumors that is more indicative of the conditions that permitted clonal expansion of neoplastic lesions. The mere observation of an activated oncogene in a tumor in and of itself confers little information that permits determination of the mechanism(s) that may have produced it.

As discussed in the i2a dedicated assessment of the genotoxicity dataset available on Sb compounds, (in vitro) Sb genotoxicity is likely mediated by indirect mechanisms, such as 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, but **ASSESSMENT NOTE 5: there is relatively high confidence that the lung carcinogenicity is not a result of direct genotoxicity of Sb. Excess tumors observed may reflect the clonal expansion of pre-existing preneoplastic cells with activated oncogenes in the absence of genotoxicity (direct or indirect). If lesions are induced, it is most likely via a local indirect genotoxic mode of action. The most probable indirect modes of action (e.g. overload in the rat, inflammation and ROS generation in the mouse) would be expected to exhibit effect thresholds that produce neoplastic response only above a given exposure threshold.**

In conclusion:

- The summary of cancer induction includes lesions at tissue sites (adrenal, lymphoma and skin) that are likely side effects of pulmonary toxicity or the irritant properties of antimony trioxide. These lesions are not relevant to an evaluation of the carcinogenic properties of antimony trioxide.
- There is no statistically significant increase in rat lung tumors at antimony trioxide concentrations (3 mg/m³) that do not produce overload. Rat lung tumor incidence at higher exposure levels is low, lacking in dose-response and most likely the result of pulmonary overload. As such, the rat pulmonary lesions are not reflective of human risk.
- NTP suggests that many of the lung tumors in mice originate from cells with spontaneous Kras oncogene activation that are permitted to undergo clonal expansion in response to the pulmonary toxicity induced by antimony trioxide.



- Mouse lung tumors with Egfr lesions most probably reflect selection for, and clonal expansion of, cells with enhanced proliferative capacity under hypoxic conditions. It is not possible to ascertain whether Egfr alterations are spontaneous or induced.
- **ASSESSMENT NOTE 6: *The etiology of Kras and Egfr oncogene alterations observed in lung tumors merits investigation to determine if they are pre-existing spontaneous lesions, lesions induced by Sb via indirect mechanisms of genotoxicity and/or lesions selected for clonal expansion as a consequence of pulmonary toxicity and hypoxia.*** Given the above, data from experimental animal studies do not yield compelling evidence of cancer risk at exposure levels, or via mechanisms, that are likely to be relevant to present occupational or consumer exposure scenarios.
- Plausible mechanisms are noted for genotoxicity, particularly via indirect mechanisms, but there is little quality data that suggests these mechanisms produce genotoxic impacts *in vivo*.
- Epidemiological studies have failed to demonstrate elevated cancer risk that can be attributed to antimony trioxide exposure.
- According to the ECHA Guidance on the Application of CLP criteria, the present evidence satisfies, and likely exceeds, that required for a Category 2 cancer classification. However, the criteria for a Carcinogenicity Category 1B classification are not met.
- **ASSESSMENT NOTE 7: *Pending the outcome of the studies noted in above, maintaining a Category 2 carcinogenicity classification for antimony trioxide via inhalation is warranted.***
- As regards the other Sb substances, the below sections contribute to the development of a provisional read-across approach, pending the validation of the hypothesis mode of action, on the basis of the further evidence that can be generated.

Antimony and lung toxicity

The historical medical surveillance literature, documenting the impacts of inhalation exposure to diantimony trioxide in occupational setting, is concordant with the animal studies in that impairments of pulmonary function (e.g. spirometry deficits and radiographic indications of mild pulmonary fibrosis) were associated with occupational exposures experienced prior to the adoption of modern OELs (McCallum, 1967; Potkonjak and Pavlovich, 1983). Although the pulmonary changes associated with occupational exposures were much less severe than those evident in rats and mice, they confirm that the human lung can be adversely impacted by inhalation exposure to diantimony trioxide and ore materials containing diantimony trisulfide (stibnite). **ASSESSMENT NOTE 8: *The combined animal and human exposure data support a STOT RE classification for impacts upon the lung after repeated inhalation exposure to diantimony trioxide and diantimony trisulfide.***

STOT RE classifications are further assigned either category 1 or category 2 (ECHA, 2017). Category 1 classifications are indicative of high potency for the product of significant to severe health effects whereas a category 2 classification indicates moderate potency to induce significant health effects. Although the ECHA Classification and Labelling guidance indicates that Category 1 designations are often indicated when there is “good quality evidence from human case or epidemiology studies”, it is further noted that “In exceptional cases human evidence can also be used to place a substance in Category 2” (ECHA, 2017). Category assignment of a STOT RE substance thus entails a weight of evidence evaluation that results in a classification that accurately conveys both the potency of the substance and the severity of the health effects observed.

STOT RE Category 1 designations are triggered by the observation of significant or severe impacts in rats at aerosol concentrations less than 0.02 mg/liter/6h/day (20 mg/m³) in a 90-day exposure study whereas category 2 is indicated for effects induced between 0.02 and 0.2 mg/liter/6r/day (20 – 200 mg/m³). These values are not intended as strict demarcation points but as general guidance to be used in conjunction with expert judgement. Adjustment of these values in accordance with Haber’s rule is also suggested – thus the

demarcation value of 20 mg/m³ would be reduced by a factor of at least 4 (to < 5 mg/m³) in comparing results of a 90-day study and to those from a 1 – 2-year inhalation study.

The comprehensive two-year inhalation studies of NTP (2017) observed a LOAEL for pulmonary impacts of 3 mg/m³, just below the Haber's law adjusted Category 1 demarcation value. However, the NTP (2017) studies utilized experimentally generated respirable diantimony trioxide aerosols capable of deep lung penetration and deposition. Studies of real-world occupational aerosols indicate that their particle size distribution has a relatively low content of respirable particles. On average, for exposed humans, inhalable aerosols capable of yielding pulmonary deposition fractions comparable to those produced by experimentally generated diantimony trioxide aerosols used in rodent inhalation studies would require a 5-fold higher concentration of diantimony trioxide in air (Hughson, 2005; Vetter, 2018).

Using the Multiple-Path Particle Dosimetry Model (v. 3.01) described by Ashgarian and Price (2009), one can further compare the pulmonary deposition of the experimental aerosols used by NTP (2017) with those of the real-world occupational diantimony trioxide aerosols measured by Hughson (2005). Whereas the NTP aerosols (MMAD 1.2 µm +/- 1.9 GSD) would yield a pulmonary deposition rate in rats of 7.6%, the average particle size distribution of the aerosols sampled by Hughson (2005), as calculated by Vetter (2018) had an MMAD of 17.2 µm with a GSD of 2.7. This would yield a pulmonary deposition rate in the rat of 0.3%. In terms of potency for the rat, a real-world diantimony trioxide occupational aerosol of 75 mg/m³ would be required to produce the pulmonary impacts observed by NTP (2017) at their airborne LOAEL of 3 mg/m³. This total aerosol diantimony trioxide concentration is significantly above the 5 mg/m³ demarcation point for chronic exposure potency in establishing category 1 vs. category 2 in STOT RE inhalation classifications.

Granulometry studies are described in the CSRs for antimony metal powder and diantimony trisulfide and predict the characteristics of the aerosols each would produce. Antimony metal powder would be expected to generate an occupational aerosol with an MMAD of 19.05 µm +/- 2.75 GSD. A bimodal distribution is predicted for diantimony trisulfide aerosols with 11% of the particle mass having an MMAD of 2.69 µm +/- 2.38 GSD and 88.7% of the aerosol mass with a MMAD of 28.48 µm +/- 1.56 GSD). MPPD modelling predicts that pulmonary deposition rates of 0.05% and 0.22% would result from aerosols of antimony metal powder and diantimony trisulfide, respectively. Pulmonary deposition rates equivalent to those for rats exposed to 3 mg/m³ of diantimony trioxide in the NTP studies would thus require metal powder aerosols of approximately 100 mg/m³ and diantimony trisulfide aerosols of 450 mg/m³. Real-world aerosols of diantimony trioxide, diantimony trisulfide and antimony metal powder would be judged to have a moderate to low potency as pulmonary toxicants when viewed from the perspective of deposition rates in the lung regions that are the targets for pulmonary toxicity.

Historical exposures capable of producing human pulmonary impacts after years of chronic exposure, although not precisely defined, were most likely in significant excess of 10 mg/m³ (ECHA, 2008). The historical exposure levels associated with changes to lung pathology and function confirm that diantimony trioxide has only moderate potency for inducing pulmonary impacts in humans. The nature of the pulmonary alterations associated with exposure of humans to diantimony trioxide provides further indications that diantimony trioxide has only moderate potency as a pulmonary toxicant.

Inhalation of diantimony trioxide by rats and mice produced severe impairment of both pulmonary structure and function (NTP, 2017). The severity of the impacts in rodents, contrasts with the observed impacts in humans. Although impacts upon human lung function are judged as clinically significant, the pulmonary function impacts observed are generally mild. The underlying alterations to human lung tissue that mediate these modest functional changes are in turn associated with comparatively modest inflammatory responses and rather benign and generally non-progressive fibrotic changes.

ASSESSMENT NOTE 9: *The concentrations of diantimony trioxide associated with pulmonary toxicity in both humans and rodents indicate moderate potency that is consistent with a STOT RE category 2 classification.* The relatively benign and non-progressive nature of the structural alterations documented in workers with high-level historical occupational exposures similarly indicates relatively mild potency consistent with STOT RE category 2 classification for lung toxicity from inhalation exposure. **ASSESSMENT NOTE 10:** *Modelling of the alveolar deposition fractions predicted for rats exposed to aerosols of antimony metal powder and diantimony trisulfide further indicates that the potency of these substances would be lower than diantimony trioxide and thus also consistent with a category 2 STOT RE classification.*

Hypothesis mode of action and lung toxicity and carcinogenicity classifications

ASSESSMENT NOTE 11: *Available animal evidence suggests a hypothesized mode of action according to which lung toxicity or lung carcinogenicity (whichever the exact sequence of processes they result from or into), could only be caused by exposure to:*

- *respirable² sizes of Sb, capable of deep lung deposition, and*
- *insoluble or poorly soluble³ forms of Sb, capable of accumulation at target tissue sites*

which would then trigger some sort of (deep) lung toxicity as those observed in cases of pneumoconiosis in humans, overload and cancer in rats or lung cancer in mice. While pulmonary toxicity has been observed after exposure to respirable aerosols of several highly insoluble Sb compounds, carcinogenic responses have thus far been restricted to antimony trioxide.

On the basis of this hypothesis, particle size, solubility and intrinsic toxicity information on the Sb substances can be used to identify which other substances besides should carry a Carcinogenicity and a Lung STOT RE classification.

² Particles < 50 µm for example, could be inhaled but most would deposit in the nose and throat, those < 25 µm could reach the thoracic region, and only the particles < 7 µm are deemed respirable and could reach the alveoli and deep lung.

³ To be interpreted as solubility under specific conditions of the lung/alveoli environment. Various sources of solubility data (water solubility, bio-accessibility in artificial alveolar fluid, in vitro lung cell/tissue testing conditions, in vivo tests, etc.) need to be taken into account to assess this aspect.

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.). Particle size, solubility and intrinsic toxicity (possibly related to the valency) are the three most relevant parameters to be considered and compared when assessing the potential lung toxicity and carcinogenicity of Sb substances.

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+. **ASSESSMENT NOTE 12: *Considering the specificity of interactions between a chemical and a cell or tissue, differences in Sb valence state for compounds described in Table 2 may need to be reconsidered in due course for the purpose of read-across for lung toxicity and carcinogenicity 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. As per the hypothesis mode of action, lung toxicity and carcinogenicity would require the local presence and accumulation of a relatively large amount of a Sb substance, able to interact with the lung cells and respiratory processes. In this context, the inhalation exposure route constitutes the main physiological entry point for Sb substances into the lung; physical forms (and sizes) that can be respired, and reach the deep lung, are those of relevance to lung toxicity and carcinogenicity (in theory, only Sb metal, Sb trioxide, Sb trisulfide in Table 2).

As regards the Sb chlorides, the trivalent form is supplied in the form of crystals, and the pentavalent one is supplied as a liquid. Although these are not respirable 'particles', their corrosive nature may cause respiratory irritation, which is why these chlorides carry a harmonized STOT SE3 classification already. This differs from the lung toxicity and carcinogenicity effects and mode of action investigated in this document. The chlorides should hence be considered as a separate subgroup for the purpose of read-across.

The inhalation route is also the route through which workers will be more likely to be exposed to Sb substances. Consumers would not be expected to have significant exposures to Sb substances via inhalation. The differences in physical form and particle sizes provided in Table 2 are hence relevant for the purpose of read-across for lung toxicity and carcinogenic potential. As a general principle, the fact that a substance is respirable however, does not imply it can then accumulate in and damage the lung. It would need to be insoluble too. Even if the substance is able to reach the relevant alveoli, it may express no (cyto)toxic effects, for example if the rate of dissolution and bioavailability preclude accumulation of compounds in the deep lung. It is important to note this when assessing each Sb substance against the hypothesis mode of action for lung toxicity described above.

Table 2 also provides information on the moiety (functional group) of each Sb substance that will normally influence the physico-chemical properties, including solubility and the bio-availability, of the substance. In the context of Sb mediated lung toxicity, insoluble or poorly soluble/poorly bioavailable forms will have a slow dissolution and reside longer in the lung. The forms which are more soluble or bioavailable will be taken up as the (oxyan)ion after release from the parent compound due to hydrolysis of ionic bonds (Hashimoto et al. 2003, and Zheng, Zhi and Chen 2006). The primary impact of the moiety will be in determining the dissolution rate of compounds in the lung; the moieties will influence the solubilization and transformation processes the Sb substance could be subject to. This aspect is addressed later below.

As regards the toxicity, given their essential nature, the anticipated lung toxicity impact of these moieties, compared to the possible lung toxicity impact of Sb, is expected to be negligible. The most common 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, as addressed above in this section) 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 breathing. ***The difference in moieties provided in Table 2 can be omitted for the purpose of read-across for lung toxicity and carcinogenic potential.*** Instead, the solubility and bioavailability (although influenced by the moiety), will be more specifically relevant to consider.

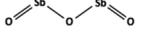
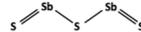
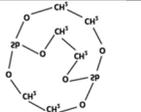
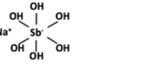
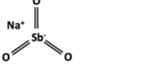
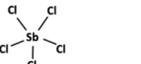
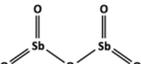
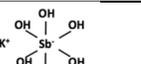
As regards the formula weight and structure of each substance, the information in Table 2 shows that there are no specific trends or patterns among the molecular weight or structure that can inform the read-across approach. ***Without further information on the actual mode of action behind lung toxicity, it cannot be fully ruled out that the difference in molecular weight and structure provided in Table 2 may need to be considered for the purpose of read-across for lung toxicity and carcinogenicity evidence.***

Impurities in Sb substances are commonly arsenic and lead (in the relevant speciation)⁴, but typically in concentration levels below 0,1% or their respective Specific Concentration Limits (SCL)⁵. 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 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 lung toxicity and carcinogenicity evidence.***

⁴ 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.

⁵ 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.

The content of Table 2 is of informative nature. It generally shows that on the basis of identity or characterization, beyond particle size (and valency and molecular weight/structure?), there are no major differences between the Sb substances subject to REACH that would challenge a grouping or read-across approach for lung toxicity or carcinogenicity. **ASSESSMENT NOTE 13: So far, three subgroups can be identified based on Table 2:**

- 1) the two chlorides classified as STOT SE3 due to their intrinsic corrosivity;**
- 2) the Sb substances having particle sizes < 7 µm and/or yielding respirable aerosols, capable of reaching the deep lung (Sb metal powder, Sb trioxide, and Sb trisulfide); and**
- 3) the other Sb substances not meeting this particle size criterion.**

Within each subgroup, no difference needs to be made on the basis of the moieties or impurities.

Solubility and bioavailability

Table 3 provides information on the release and behavior of Sb species in water and a number of physiologically relevant media. This information is important as the hypothesis mode of action behind lung toxicity and carcinogenicity involves 1) inhalation of a given small sized Sb substance, and 2) that the substance is capable of residing and accumulating in the deep lung, i.e. that the substance is poorly soluble or insoluble (under lung conditions). Various sources of solubility data (water solubility, bio-accessibility in artificial alveolar fluid, in vitro lung cell/tissue testing conditions, in vivo tests, etc.) need to be taken into account to assess this latter aspect referred to as 'solubility' or 'bioavailability' in this document.

For metals, inorganic metal compounds, or metal-containing complex materials, the bioavailable metal ion is considered to be responsible for the systemic or the local toxicity. In this case of lung toxicity and carcinogenicity, it is the bioavailability of the released metal at the site of action (i.e. the deep lung) in the organism that can be the most important determining factor to understand the likely toxic effect of metals and minerals.

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 releases of ions *in vivo*. 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 one or more substances' 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 toxicity of metal-containing materials is related to bioavailability and the release of metal ions that are then available for absorption or other local accumulation. 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 the inhalation route, which is the most relevant for Sb substances and lung toxicity and carcinogenicity, fluids exist which simulate the alveoli conditions.

Bioaccessibility methods are generally considered to overestimate absolute bioavailability and toxicity; indeed, a simple extraction step is all that is necessary, and so bioelution does not take into account actual absorption after release, or accumulation. The basic premise however, is 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 released locally. For the purpose of determining the potential to cause lung toxicity and carcinogenicity,

bioaccessibility data obtained in simulated alveolar fluid may assist in further discriminating between the Sb substances assessed here. Among the Sb substances being supplied in particle sizes < 7 µm or capable of generating respirable aerosols, those yielding the lowest bioaccessibility would be considered as those having the highest potential to reside and accumulate. Those with very high bioaccessibility would unlikely cause lung toxicity, as they would dissolve, be absorbed/taken up and excreted.

As shown in Table 3, bioelution information is not available for all Sb substances. Also, the data was not generated in parallel. 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, only particle size and the potential of being respired can be considered. Once bioaccessibility data becomes available, the three subgroups identified on the basis of Table 2 data, will be refined to:

- 1) the two chlorides classified as STOT SE3 due to their intrinsic corrosivity;
- 2) the Sb substances having particle sizes < 7 µm and/or yielding respirable aerosols, capable of reaching the deep lung (Sb metal powder, Sb trioxide, and Sb trisulfide) **and showing lack or poor solubility/bioavailability under lung/alveoli exposure conditions**; and
- 3) the other Sb substances not meeting this particle size **and solubility/bioavailability** criteria.

This would mean that **ASSESSMENT NOTE 14: while further bioaccessibility information becomes available, the solubility criteria mentioned in the hypothesis mode of action cannot yet be utilized to identify the Sb substances which may be able to reside (deep and) long enough in the lung to yield local toxicity.** Instead, the cut-off particle size of 7 µm is the only criteria able to inform the different subgroups in which to allocate the ten Sb substances in scope.

Once the Sb substance has been able to penetrate the deep lung and accumulate in the alveoli, it is important to understand what interaction the Sb substance or ion will have with the lung tissue and cells. This will depend on criteria beyond those of particle size and solubility or bioaccessibility and be influenced by other intrinsic properties of the Sb substance, such as its lung (cyto)toxicity potential. This information is not available for most Sb substances. If it is generated, it would be most valuable if it would be assessed under representative conditions of local exposure to the lung. **ASSESSMENT NOTE 15: For the read-across approach to be more robustly developed, the (cyto)toxicity and capability of Sb substances to interact with or damage lung cells, alter pulmonary tissue structure and impair pulmonary function, should be assessed.**

In order to perform a more refined comparison, additional information would be needed to complete Table 3, and a table 3.2 on (cyto)toxicity should be produced. Once bioaccessibility and lung (cyto)toxicity data become available, the three subgroups identified on the basis of Table 2 data, will be refined to:

- 1) the two chlorides classified as STOT SE3 due to their intrinsic corrosivity;
- 2) the Sb substances having particle sizes < 7 µm and/or yielding respirable aerosols, capable of reaching the deep lung (Sb metal powder, Sb trioxide, and Sb trisulfide), showing lack or poor solubility/bioavailability under lung/alveoli exposure conditions, **and exerting toxic effects on lung cells and tissues**; and
- 3) the other Sb substances not meeting this particle size, solubility/bioavailability **and toxicity** criteria.

This provisional read-across justification will be revisited once the additional information becomes available, probably in late 2019.

Table 3. Main 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

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 testing is considered necessary, it should ideally also address the lack of data on pentavalent Sb substances.
2. While all studies have reported some degree of lung toxicity, only one reports clear evidence of cancer (in mice). Evidence in rat is either less clear.
3. Particle overload and the subsequent cascade of inflammatory responses leading to a tumor formation can be retained as a possible mode of action for lung cancer in rats.
4. The primary target organ of inhaled Sb substances appears to be the lung, and mode of action considerations should look at local effects in the lung rather than systemic effects. The inhalation exposure route is the only route of exposure relevant for the assessment of carcinogenicity properties.
5. There is relatively high confidence that the lung carcinogenicity is not a result of direct genotoxicity of Sb. Excess tumors observed may reflect the clonal expansion of pre-existing preneoplastic cells with activated oncogenes in the absence of genotoxicity (direct or indirect). If lesions are induced, it is most likely via a local indirect genotoxic mode of action. The most probable indirect modes of action (e.g. overload in the rat, inflammation and ROS generation in the mouse) would be expected to exhibit effect thresholds that produce neoplastic response only above a given exposure threshold.
6. The etiology of Kras and Egfr oncogene alterations observed in lung tumors merits investigation to determine if they are pre-existing spontaneous lesions, lesions induced by Sb via indirect mechanisms of genotoxicity and/or lesions selected for clonal expansion as a consequence of pulmonary toxicity and hypoxia.
7. Pending the outcome of the studies noted in point 6 above, maintaining a Category 2 carcinogenicity classification for antimony trioxide via inhalation is warranted. There is no sufficient data indicating a need to extend carcinogenicity classification to other antimony compounds.
8. The combined animal and human exposure data support a STOT RE classification for impacts upon the lung after repeated inhalation exposure to diantimony trioxide and diantimony trisulfide.
9. The concentrations of diantimony trioxide associated with pulmonary toxicity in both humans and rodents indicate moderate potency that is consistent with a STOT RE category 2 classification.
10. Modelling of the alveolar deposition fractions predicted for rats exposed to aerosols of antimony metal powder and diantimony trisulfide further indicates that the potency of these substances would be lower than diantimony trioxide and thus also consistent with a category 2 STOT RE classification.
11. Available animal evidence suggests a hypothesized mode of action according to which lung toxicity or lung carcinogenicity (whichever the exact sequence of processes they result from or into), could only be caused by exposure to:
 - respirable sizes of Sb, capable of deep lung deposition, and
 - insoluble or poorly soluble forms of Sb, capable of accumulation at target tissue siteswhich would then trigger some sort of (deep) lung toxicity as those observed in cases of pneumoconiosis in humans, overload and cancer in rats or lung cancer in mice. While pulmonary toxicity has been observed after exposure to respirable aerosols of several highly insoluble Sb compounds, carcinogenic responses have thus far been restricted to antimony trioxide.
12. Considering the specificity of interactions between a chemical and a cell or tissue, differences in Sb valence state for compounds described in Table 2 may need to be reconsidered in due course for the purpose of read-across for lung toxicity and carcinogenicity evidence.

13. So far, three subgroups can be identified based on Table 2:
 1. the two chlorides classified as STOT SE3 due to their intrinsic corrosivity;
 2. the Sb substances having particle sizes < 7 µm and/or yielding respirable aerosols, capable of reaching the deep lung (Sb metal powder, Sb trioxide, and Sb trisulfide); and
 3. the other Sb substances not meeting this particle size criterion.
14. While further bioaccessibility information becomes available, the solubility criteria mentioned in the hypothesis mode of action cannot yet be utilized to identify the Sb substances which may be able to reside (deep and) long enough in the lung to yield local toxicity.
15. For the read-across approach to be more robustly developed, the (cyto)toxicity and capability of Sb substances to interact with or damage lung cells, alter pulmonary tissue structure and impair pulmonary function, should be assessed.

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

- Pneumoconiosis without severe pathological changes (e.g. fibrosis) have been reported for humans exposed to fine dusts of antimony metal, antimony trioxide and antimony trisulfide. Inflammation, fibrosis and cancer has been observed in rodents exposed to antimony trioxide at high concentrations of respirable aerosols.
- The evidence suggests that the toxicity observed is the result of a local exposure through inhalation, of respirable Sb substances, which are poorly soluble or insoluble, and as such have the opportunity to reach the deep lung and reside there long enough to cause local toxicity in alveolar tissues.
- The actual mode of action behind the observed lung carcinogenicity in mice would benefit from further investigations to better understand the local (potentially indirect genotoxicity) mode of action and the origins of activated Kras and Egr oncogenes observed in lung tumors.
- Meanwhile further investigation is needed to resolve the interpretation gaps, the provisional (self-) classification applied to Sb metal powder, antimony trioxide, and antimony trisulfide is: Carcinogenicity Category 2 via inhalation, and STOT RE Category 2. The chlorides retain their existing harmonized STOT SE category 3 classification.
- There is no positive or negative evidence of lung toxicity or carcinogenicity for Sb (V) compounds. Sb (V) forms are however often reported to be less toxic than the Sb (III) forms.
- There is currently insufficient information to develop a complete read-across justification for the lung toxicity and carcinogenicity of the other Sb substances. Provisionally, because none are produced in particle sizes which are respirable, no classification for lung toxicity or carcinogenicity appears relevant for other Sb substances than the three provided above.

Further research opportunities

For predicting the (absence of) lung toxicity and carcinogenicity properties among Sb substances, the hazard, classification and read-across assessment needs to be further informed by and justified with the following research:

- **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 potentially inform the likely behavior of the substances in the alveoli, and possibly predict their potential for 'deep lung accumulation'.
- **Complete lung cytotoxicity evidence:** A dedicated in vitro inhalation toxicity study will be performed in order to assess both the influence of solubility on lung toxicity, as well as the lung (cyto)toxicity potential of the various selected Sb substances. This will start to address the gap there is on Sb (V) substances and inform a possible categorization approach, where different behaviors are observed in the in vitro system expected to reproduce the lung conditions.



- **Clarify local (indirect genotoxic?) mode of action for lung toxicity and carcinogenicity:** This could potentially be included in the above study.
- **Clarify the role of Kras and Egfr oncogene activation in lung tumor formation:** A dedicated tailored in vivo study will be required to determine the frequency and mechanism of oncogene activation in normal mouse tissues. The role of pulmonary toxicity and hypoxia in facilitating the clonal expansion of pre-existing oncogenes will be evaluated.

6. Lung toxicity and carcinogenicity 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 lung toxicity and carcinogenicity 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 the lung toxicity and carcinogenicity provisional read-across justification, the Sb substances are actually grouped in two subgroups according to particle size. One group would contain Sb substances able to reach the deep lung/alveoli, and the other group would contain all other Sb substances. Once in the deep lung/alveoli, the release of toxic Sb ion can be considered a common transformation product among the substances within the first subgroup (no matter how the transformation occurs).

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

The lung toxicity and carcinogenicity data available on the Sb substances reported in Tables 1.1-1.3 reveal a general trend of lung toxicity following high and repeated inhalation exposure for three of the ten substances. There is no evidence for any of the other Sb substances. Within the first subgroup however, a regular pattern of lung toxicity can be observed. Within the second subgroup, the absence of evidence prevents the identification of any pattern or trend, but it is assumed to be different from the one observed in the first subgroup.

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 substances in each subgroup will have a comparable (non-)interaction with lung cells and tissues, and respiratory 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 (except chlorides, which due to their corrosivity are in a subgroup of their own).

Assessment Element/Details	Supporting evidence
Characterization of source and target substances	
Identity and characterization of all substances in category	<p>Subgroup Sb substances capable of reaching the deep lung/alveoli: Source substances: Any Sb substance with respirable particle size (MMAD < 7 µm) and/or that would lead to formation of respirable aerosols or alveolar deposition in the lung, and having moieties or impurities which do not have a more toxic lung toxicity profile than Sb. Target substances: Other Sb substances with respirable particle size (MMAD < 7 µm) and/or that would lead to formation of respirable aerosols or alveolar deposition in the lung, and having moieties or impurities which do not have a more toxic lung toxicity profile than Sb.</p> <p>Subgroup other Sb substances: Source substances: Any Sb substance with non-respirable particle size (MMAD > 7 µm) and/or that would not lead to formation of respirable aerosols or alveolar deposition in the lung, and having moieties or impurities which do not have a more toxic lung toxicity profile than Sb. Target substances: Other Sb substances with non-respirable particle size (> 10 µm) and/or that would not lead to formation of respirable aerosols or alveolar deposition in the lung, and having moieties or impurities which do not have a more toxic lung toxicity profile than Sb. 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>Both subgroups: All substances in each subgroup 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 no or negligible toxicity to the lung, or normal metabolites (NM), which are expected to be rapidly metabolized. The only difference between the two subgroups (which is not related to structure) is particle size. More information in Section 4 of this document.</p>
Structural differences that are allowed within the category are specified	<p>Both subgroups: Differences in molecular weight, moieties and release rates are allowed as long as the particle size, aerosol formation or alveolar deposition behavior remain within the subgroups criteria. 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 substances capable of reaching the deep lung/alveoli: All Sb substances have in common that once in the deep lung/alveoli, they will accumulate and behave in comparable manner, possibly by slowly releasing a common Sb (oxyan)ion form or a form expressing a common (cyto)toxic effect in the lung/alveoli. This Sb form can be considered as a</p>

Assessment Element/Details	Supporting evidence
	<p>common transformation product having a common biological fate following release.</p> <p>Lung exposure will be to this common transformation product, no matter the original form of the substance originally present and/or administered.</p> <p>Subgroup other Sb substances:</p> <p>Will not reach the deep lung in the first place, and their transformation product is hence irrelevant in this context.</p> <p>More information in Section 4 of this document.</p>
<p>Degradation, bioaccumulation and impact of non-common compounds</p>	<p>Subgroup Sb substances capable of reaching the deep lung/alveoli:</p> <p>All Sb substances have in common that their moieties will be absorbed for essential functions in the body or metabolized as any other normal metabolite.</p> <p>Subgroup other Sb substances:</p> <p>Will not reach the deep lung in the first place, and the nature of their moieties is hence irrelevant in this context.</p> <p>More information in Section 4 of this document.</p>
<p>Impact of impurities on the prediction</p>	
<p>The identified impurities have an impact on the prediction</p>	<p>Subgroup Sb substances capable of reaching the deep lung/alveoli:</p> <p>All Sb substances have in common that their impurities will be the same (As, Pb) and present in concentrations below the relevant classification or hazard cut-off limits. They should not influence the likelihood to accumulate or be toxic to the lung of the Sb substances.</p> <p>Subgroup other Sb substances:</p> <p>Will not reach the deep lung in the first place, and the nature of their impurities is hence irrelevant in this context.</p> <p>More information in Section 4 of this document.</p>
<p>Consistency of properties in the data matrix</p>	
<p>A data matrix with experimental data for source and target substances is needed to support the read-across</p>	<p>Subgroup Sb substances capable of reaching the deep lung/alveoli:</p> <p>The lung toxicity and carcinogenicity dataset available for Sb metal, Sb trioxide and Sb trisulfide consistently shows a potential to cause lung toxicity under conditions of high and repeated exposure. In mice, this yields lung carcinogenicity. Although no animal evidence is available for other Sb substances than the trioxide, based on the hypothesis mode of action and the particle size criteria in particular, Sb trisulfide and Sb metal powder are provisionally assumed to be comparable to antimony trioxide.</p> <p>Subgroup other Sb substances:</p> <p>There is no evidence on any of these other substances.</p> <p>More information in Sections 2 and 3 of this document.</p>
<p>Reliability and adequacy of the source data</p>	
<p>The source study(ies) needs to be reliable and adequate as requested for any other key study</p>	<p>Only adequate and reliable data has been used to support the read-across justification.</p> <p>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 (pending the generation of additional information).

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

Name	CAS #	Lung toxicity / Carcinogenicity classification	Further testing needs
Subgroup Sb substances capable of reaching the deep lung/alveoli:			<ul style="list-style-type: none"> • Complete solubility and bio-accessibility information • Complete lung cytotoxicity evidence • Clarify local (indirect genotoxic?) mode of action for lung toxicity and carcinogenicity • Clarify the role of Kras and Egfr oncogene activation in lung tumor formation
Sb –powder	7440-36-0	STOT RE 2 Carcinogenicity 2	
Diantimony trioxide	1309-64-4	STOT RE 2 Carcinogenicity 2	
Antimony trisulfide	1345-04-6	STOT RE 2 Carcinogenicity 2	
Subgroup other Sb substances:			
Sb – massive	7440-36-0	Not classified	
Antimony tris (ethylene glycolate)	29736-75-2	Not classified	
Antimony trichloride	10025-91-9	Not classified (STOT SE 3 ⁶)	
Sodium hexahydroxoantimonate	33908-66-6	Not classified	
Sodium antimonate	15432-85-6	Not classified	
Antimony pentachloride	7647-18-9	Not classified (STOT SE 3 ⁶)	
Antimony pentoxide	1314-60-9	Not classified	
Potassium hexahydroxoantimonate	12208-13-8	Not classified	

⁶ Due to its low pH and high corrosivity.

9. References

- Asgharian B, and Price O. (2009) Multiple Path Particle Deposition Model, (MPPD Version 3.04), Software (with graphical user interface) is available for free download from Bahman Asgharian, Ph.D. at Applied Research Associates, 2009. www.ara.com (last accessed March 2018).
- Dieter, M.P., Jameson, C.W., Elwell, M.R., Lodge, J.W., Hejtmanicik, M., Grumheim, S.L., Ryan, M. and Peters, A.C. (1991). Comparative toxicity and tissue distribution of antimony potassium tartrate in rats and mice dosed by drinking water or intraperitoneal injection. *J. Toxicol. Environ. Health* 34: 51 – 82.
- ECHA (2008). European Union Risk Assessment Report – Diantimony Trioxide. Available at https://echa.europa.eu/documents/10162/13630/trd_rar_sweden_diantimony_trioxide_en.rtf/967b2892-8795-4a33-bb34-9587b8679cf9.
- ECHA (2017). Guidance on the Application of the CLP Criteria, version 5.0, ECHA-17-G-21-EN, European Chemicals Agency, Helsinki, Finland.
- Greim, H., Hartwig, A., Reuter, U., Richter-Reichhelm, H.B and Thielmann, H.W. (2009). Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. *Crit. Rev. Toxicol.* 39:695 - 718
- Groth DH, Stettler LE, Burg JR, Busey WM, Grant GC and Wong L (1986). Carcinogenic effects of antimony trioxide and antimony ore concentrate in rats. *J Toxicol Environ Health* 1986a; 18: 607-626.
- Hashimoto, H., Nishimura, T., and Umetsu, Y. (2003). Hydrolysis of antimony(III)-hydrochloric acid solution at 25° C. *Materials Transact.* 44: 1624 – 1629.
- Hext P. M., Pinot P. J. and Rimmel B. A. (1999). Subchronic Feeding Study of Antimony Trioxide in Rats. *J. Appl. Toxicol.* 19, 205-209. Testing laboratory: Zeneca Central Toxicology Laboratory.
- Hughson, G. (2005). Assessment of dermal exposures and classification of workplace aerosols for antimony trioxide production. Institute of Occupational Medicine (Edinburgh) Report No. 602-00292.
- i2a (2017). Analysis of medical surveillance data at antimony trioxide production companies A and B. Report prepared for the International Antimony Association.
- Jones, R.D. (1994) Survey of antimony workers mortality 1961 – 1992. *Occup. Environ. Med.* 51: 772 – 776.
- Jones, S.R., Atkin, P., Holroyd, E., Lutman, E., Battle, J.V., Wakeford, R. and Walker, P. (2007). Lung cancer mortality at a UK tin smelter. *Occup. Med.* 57: 238 – 245.
- Karoor, V., Merrick, D., Fagan, K.A., Dempsey, E.C. and Miller. Y.E. (2012). Alveolar hypoxia promotes murine lung tumor growth through a VEGFER-2/EGFR-dependent mechanism. *Cancer Prev. Res.* 5: 1061 – 1071
- McCallum RI (1967). Detection of Antimony in process workers' lungs by X-radiation. *Trans Soc Occup Med* 1967; 17: 134-138.
- Murakami, A., Takahashi, F., Nurwidya, F., Kobayashi, I., Minakata, K., Hashimoto, M., Nara, T., Kato, M., Tajima, K., Shimada, N., Iwakami, S., Moriyama, M., Moriyama, H., Koizumi, F., and Takahashi, K. (2014). Hypoxia increases gefitinib-resistant lung cancer stem cells through activation of insulin-like growth factor 1 receptor. *PLOS One* 9: 1 – 12 (e86459).
- Newton P. E. and Daly I. W. (1990). A one Year Inhalation Toxicity Study of Antimony Trioxide in the Rat (with a one Year Recovery Period). Testing laboratory: Bio dynamics Inc. Report no.: 83-7647. Owner company: i2a international Antimony Association (i2a), Avenue de Broqueville 12, 1150 Brussels, Belgium. Report date: 1990-02-09.
- Newton P. E., Bolte H. F., Daly I. W., Pillsbury B. D., Terrill J. B., Drew R. T., Ben-Dyke R., Sheldon A. W. and Rubins L. F. (1994). Subchronic and chronic Inhalation toxicity of antimony trioxide in the rat. *Fund. Appl. Toxicol.* 22, 561-576.
- NTP (2016). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Antimony Trioxide in Wistar HAN Rats and B6C3F1/N Mice. National Toxicology Program, National Institute of Health, U.S> Department of Health and Human Services. NTP TR 590..
- Parsons, B., Myers, M.B., Meng, F., Wang, Y. and McKinzie, P.B. (2009). Oncomutations as biomarkers of cancer risk. *Environ. Molec. Mutagen.* 51:836 – 850.
- Potkonjak V., Pavlovich M. (1983). Antimoniosis: A particular Form of pneumoconiosis. *Int Arch Occup Environ Health* 51:199-207.

- Schroeder R. E. (2003). An inhalation developmental toxicity study in rats with antimony trioxide. Testing laboratory: MPI research, Inc. 54943 North Main Street, Mattawan, Michigan. Report no.: 952-002. Owner company: i2a international Antimony Association (i2a), Avenue de Broqueville 12, 1150 Brussels, Belgium. Report date: 2003-11-17.
- Schnorr, T.M., Steenland, K., Thun, M.J. and Rinsky, R.A. (1995). Mortality in a cohort of antimony smelter workers. *Am. J. Ind. Med.* 27: 759 – 770.
- Vetter, D. (2018). Antimony metal and antimony substances: Derivation of a conversion factor for exposure levels of respirable antimony dust from measurements of the inhalable fraction. Report to i2a from EBRC consulting GmbH.
- Ward, J. (2005). Lymphomas and leukemias in mice. *Exp. Toxicol. Path.* 57: 377 – 381.
- Warheit, D.B., Kreiling, R., and Levy, L.S. (2016). Relevance of the rat lung tumor response to particle overload for human risk assessment – Update and interpretation of new data since ILSI 2000. *Toxicology* 374: 42 – 59.
- Watt W. D. (1983). Chronic inhalation toxicity of antimony trioxide: validation of the threshold limit value. Wayne State University, Detroit, Michigan.
- Zheng, G.-Q & Zhi, B & Chen, J.-Z. (2006). Hydrolysis of antimony pentachloride. *Ch. J. Nonferr. Met.* 16. 1628-1633.