

International
**ANTIMONY
OXIDE**
Industry Association

June 1, 2005

Dr. Scott A. Masten
Office of Chemical Nomination and Selection, NIEHS/NTP
P.O. Box 12233
MD A3-07
Research Triangle Park, NC 27709

Re: Nomination of Antimony Trioxide for Chronic Testing by NTP. Response to Federal Register Notice of May 5, 2005.

Dear Dr. Masten,

This letter is submitted on behalf of the industry consortium, International Antimony Oxide Industry Association (IAOIA) in response to the nomination of antimony trioxide (ATO) [CAS No.: 1309-64-4] for chronic and carcinogenicity toxicology as well as cardiotoxicology testing by the NTP.

The IAOIA is in support of the proposal to conduct appropriate long-term chronic and carcinogenicity studies in rodents. We understand the main concern as being related to the high number of individuals who are occupationally exposed to this material. We also infer from the basis for nomination that the route of exposure would logically be inhalation as this is the primary route of concern in the workplace. While we do not believe that workers are at risk for developing lung tumors as a result of occupational exposures at the current Threshold Limit Value (TLV) of 0.5 mg/m^3 , we do believe that a chronic study conducted under current guidelines could be useful in understanding the carcinogenic potential of ATO if indeed one does exist. Such a study should utilize test material of the highest purity.

As mentioned in the basis for nomination, the database on antimony (Sb) compounds is limited to three inhalation studies in rats exposed to ATO particles for one-year duration (Table 1). The most recently completed study by Newton *et al.* was conducted by the Antimony Oxide Industry Association (AOIA). This study consisted of a one-year exposure period followed by a one-year recovery period. The protocol for this study was agreed between the U.S. EPA and the AOIA pursuant to the request from EPA and the subsequent sanctioning of this protocol by the U.S. EPA through the Negotiated Testing Agreement process. This study was conducted using modern testing equipment and methods, and was in compliance with Good Laboratory Practice (GLP) standards. In this study the highest exposure concentration of 4.5 mg/m^3 resulted in impaired clearance rates but did not result in an increased incidence of tumors in the lungs of either male or female rats compared to controls. The IAOIA would like to note that even

though ATO exposures ceased at 12 months, the animals were kept and observed for another 12 months prior to their termination with still significant levels of ATO dust remaining in the lungs as ATO is very insoluble.

As pointed out in the basis for nomination, this study was not considered by the International Agency for Research on Cancer (IARC) when they concluded antimony trioxide should be classified as a group 2B, *possibly carcinogenic to humans*. There are two studies which were considered by IARC demonstrating the appearance of tumors in the lungs rats. Again both these studies only exposed animals to ATO for 12 months followed by terminal sacrifices after a recovery period of either 5 (Groth *et al.*) or 12-15 months (Watt). In the studies conducted by Groth *et al.*, animals were exposed to both ATO and an Sb-ore there was a tumor incidence rate of 27% (ATO) or 25% (Sb-ore) in females only (no tumors were seen in males) without regard to whether they were exposed to antimony as ATO (45.5 mg/m³, 80% Sb ug/g) or as an antimony ore (38 mg/m³, 46% Sb ug/g). While in the study by Watt an ATO exposure of 4.2 mg/m³ induced a tumor incidence 61.7 % in female rats (only sex studied) and 0% in swine (located in the same chamber). The fact that tumors were observed at such disparate incidence rates compared to the exposure concentrations and in only one gender is peculiar. However, it is strongly believed that the 4.2 mg/m³ exposure reported by Watt was an error and that the animals were likely exposed to significantly more test material. In addition, the fact that the third study by Newton *et al.* did not reveal the same potential indicates that ATO may not be carcinogenic or is acting by a dust overload mechanism rather than by some direct genotoxic mechanisms. Thus, the IAOIA believe that a new study conducted by the NTP following current guidelines may help elucidate the mode of action if tumors can be generated.

While industrial exposures to ATO are primarily by inhalation, the NTP should also consider conducting a study utilizing an oral route of exposure. This route is important in industrial settings following inhalation of non-respirable dust that gets transported to the GI tract and is pertinent to the general public from consumption of antimony in food and drinking water.

Enclosed you will also find two reviews on the genotoxicity of ATO written by Dr. James Parry and Dr. A.T. Natarajan. The conclusions drawn by these experts is in alignment with the conclusion drawn by the World Health Organization (WHO) that ATO should not be deemed a genotoxic hazard following *in vivo* exposures (http://www.who.int/water_sanitation_health/dwq/chemicals/antimony/en/). The attached data in Table 2 summarizes the results of several genotoxicity studies conducted on ATO assessing its ability to induce either point mutations or aberrations under both *in vitro* and *in vivo* exposure conditions. While positive results were seen in two different studies assessing mutagenicity using non *B. subtilis*, studies conducted using the more traditional Salmonella and E.coli bacterial species, as well as a study using mouse lymphoma cells, clearly demonstrate that ATO should not be deemed a mutagen. In contrast to the nontraditional studies using *B. subtilis*, the latter studies followed standardized protocols (OECD guidelines 471 and 476), were conducted under GLP assurances, and utilized test article with characterized purity. In total these data indicate ATO is unlikely to pose a mutagenic hazard.

While the results of the mutagenicity studies indicate ATO is unlikely to induce point mutations, results of an *in vitro* cytogenetics assay following OECD guideline 473 and GLP assurances indicated ATO had a potential to induce chromosomal aberrations.

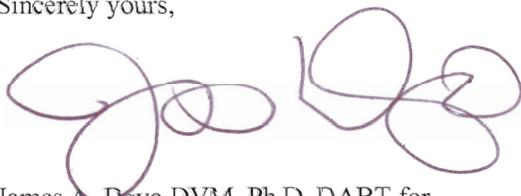
Currently there are two studies assessing the *in vivo* relevance of the aberration effects seen in the *in vitro* study. One of the *in vivo* studies followed established OECD protocols, GLP assurances, and utilized ATO of known purity. The results of this study clearly showed no evidence of genotoxicity following both acute and chronic exposures at dose levels up to 1000 mg/kg. This study was conducted to validate the reliability of two previously published studies (the same data was used in both publications) by Gurnani *et al.* 1992, 1993 whose research

mg/kg. This study was conducted to validate the reliability of two previously published studies (the same data was used in both publications) by Gurnani *et al.* 1992, 1993 whose research indicated positive aberration effects in bone marrow and reported an unexpected mortality effect. Specifically, all the mice exposed to 1000 mg/kg died on Day 20 in the context of their 21 day study. Such a finding was clearly unexpected in light of an absence of severe toxicity in rats exposed to an average dietary dose of approximately 1,700 mg/kg for 90 days (Hext *et al.* 1998). The attached reviews detail other peculiarities of the research reported by Gurnani *et al.* that questions its overall reliability. Therefore, the IAOIA does not believe that the toxicity of ATO is mediated by genotoxic mechanisms.

ATO's potential to cause cardiotoxicity is also highlighted as a basis for further investigation by NTP. These concerns originate from studies in which patients were treated with water soluble antimony-containing drugs. The IAOIA does not believe that these data are relevant to ATO due to its limited water solubility. The solubility of ATO in a simulated gastric juice is 17 mg/L (PlasticsEurope, unpublished data) while the water solubility for antimony potassium tartrate is 83,000 mg/L (HSDB). Exposure to more soluble forms of antimony would result in blood concentrations that are likely impossible to achieve from exposure to the trioxide form of antimony. Furthermore, Newton *et al.* reported no histological evidence of cardiotoxicity after one year of exposure, nor did Hext report any such effects subsequent to 90 days of dietary exposures averaging 1,700 mg/kg.

If you have any questions or other concerns please do not hesitate to phone me at 765-497-6637 or email tserex@glcc.com.

Sincerely yours,

A handwritten signature in purple ink, consisting of several loops and curves, positioned above the typed name.

James A. Deyo DVM, Ph.D, DABT for
Tessa L. Serex, Ph.D., D.A.B.T.
Toxicologist
Lead Technical Advisor to the IAOIA

Cc: Dieter Drohmann
Karine Van de Velde
James Deyo
John Biesemeier
Andrew Soiefer
Awni Sarraf

Table 1: Summaries of the Inhalation Carcinogenicity Studies on Antimony Trioxide

Study	Newton <i>et al.</i> 1994																																																																		
Test article	Antimony trioxide (purity 99.68%)																																																																		
TWA [Actual]	0, 0.06, 0.51, and 4.5 mg/m ³																																																																		
Species	Rats – F344 (Male & Female)																																																																		
Exposure Duration	6 hr/day, 5 days/wk for 12 months followed by a 12 month recovery																																																																		
Necropsy Evaluations	6 and 12 months, and 6 and 12 months post exposure																																																																		
General Observations	Ophthalmic related pathologies were noted. Neither absolute nor relative lung weights nor body weight gains were affected. Clinical chemistries were normal. There was no impact on mortality.																																																																		
Lung Tissue Concentration (ug Sb/g dry tissue)	<table border="1"> <thead> <tr> <th colspan="2"></th> <th colspan="4">Males</th> </tr> <tr> <th>Month</th> <th>0</th> <th>0.055</th> <th>0.51</th> <th>4.5 mg/m³</th> <th></th> </tr> </thead> <tbody> <tr> <td>6</td> <td>0</td> <td>19.6</td> <td>75.4</td> <td>1190</td> <td></td> </tr> <tr> <td>12</td> <td>0</td> <td>11.5</td> <td>132.0</td> <td>1420</td> <td></td> </tr> <tr> <td>18</td> <td>0</td> <td>1.4</td> <td>28.9</td> <td>991</td> <td></td> </tr> <tr> <td>24</td> <td>0</td> <td>0.4</td> <td>8.1</td> <td>554</td> <td></td> </tr> <tr> <th colspan="2"></th> <th colspan="4">Females</th> </tr> <tr> <td>6</td> <td>0</td> <td>15.1</td> <td>76.9</td> <td>1100</td> <td></td> </tr> <tr> <td>12</td> <td>0</td> <td>9.6</td> <td>107.0</td> <td>1500</td> <td></td> </tr> <tr> <td>18</td> <td>0</td> <td>2.2</td> <td>33.2</td> <td>757</td> <td></td> </tr> <tr> <td>24</td> <td>0</td> <td>0.2</td> <td>14.7</td> <td>663</td> <td></td> </tr> </tbody> </table>			Males				Month	0	0.055	0.51	4.5 mg/m ³		6	0	19.6	75.4	1190		12	0	11.5	132.0	1420		18	0	1.4	28.9	991		24	0	0.4	8.1	554				Females				6	0	15.1	76.9	1100		12	0	9.6	107.0	1500		18	0	2.2	33.2	757		24	0	0.2	14.7	663	
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Histopathology of the lung	<p>(12 mo) Chronic interstitial inflammation (min to mod) was observed in all dose groups with a slightly higher incidence rate and severity in the high dose group. Alveolar and peribronchial lymph node macrophages (min to moderate) containing foreign particulate material was seen in a dose dependent manner.</p> <p>(12 mo recovery) Chronic interstitial inflammation (min to mod) was observed in all dose groups with a slightly higher incidence rate and severity in the high dose group. Interstitial fibrosis and granulomatous inflammation was only noted in a few animals and was of slight to mod severity. Bronchiolar/alveolar hyperplasia, all min to mod, occurred either primarily or exclusively in a small number of animals during the observation period and mostly in the high dose group. Alveolar and peribronchial lymph node macrophages containing foreign particulate material were seen in a dose dependent manner at mod to moderate-severe levels with a high incidence rate.</p>																																																																		
Histopathology of other tissues	Nothing remarkable																																																																		
Tumor Incident Rate and Type	Pulmonary carcinomas were noted in 2 males (1 control and 1 high dose) and 1 female at the mid-dose.																																																																		

Study	Groth <i>et al.</i> 1986	Groth <i>et al.</i> 1986
Test article	Pure Antimony trioxide (80% Sb ug/g) (40 ppm As, 2100 ppm Sn, 2300 ppm Pb)	Antimony trioxide <u>ore</u> (46% Sb ug/g) (792 ppm As, 1600 ppm Sn, 2500 ppm Pb, 3340 ppm Fe, 4830 ppm Al, 600 ppm Cu, 530 ppm Zn)
TWA [Actual]	45.5 mg/m ³ (avg of two chambers)	38 mg/m ³ (avg of two chambers)
Species	Rat – Wistar (Male & Female)	Same
Exposure Duration	7 hr/day, 5 days/wk for 12 months followed by a 5 month recovery	Same
Necropsy Evaluations	6, 9, and 12 months and 5 months post exposure	Same
General Observations	There was no effect on survival and BW decrease at 50 weeks, was only statistically significant in males (-4.9%). Lung weight was not noted in the publication.	There was no effect on survival and BW decrease at 50 weeks, was only statistically significant in females (-6.4%). Lung weight was not noted in the publication.
Lung Tissue Concentration (ug Sb/g dry tissue)	Month 9 38,300 (Male) 25,600 (Female)	Month 9 7,140 (Male) 4,520 (Female)
Histopathology of the lung	<p>Females</p> <p>(6 mo): Evenly scattered particles in 90% of the alveoli with 10% containing particle aggregates about the size of macrophages. In some alveoli particle were within a dense pink, homogenous protein. Alveolar wall thickening, consisting of interstitial fibrosis, and alveolar-wall cell hypertrophy and hyperplasia, appearing in about 50% of the alveolar duct region, affecting about 5-10% of all alveoli. Cuboidal and columnar cell metaplasia occurred in some of these foci.</p> <p>(9-12 mo): The density of the particles, amount of dense, pink, homogenous alveolar protein, areas of interstitial fibrosis, and cuboidal and columnar cell metaplasia increased. Some foci of cholesterol clefts were noted. The first neoplasms were seen (2/5); a bronchioalveolar adenoma and a squamous cell carcinoma.</p> <p>(Terminal sacrifice): A significant decrease in was noted in the particle density and the amount of alveolar protein. The extent of the interstitial fibrosis, however, had increased affecting over 80% of the alveoli in some rats. In some rats, dense scars that appeared to be confluent areas of interstitial fibrosis were present. Neoplasms arose from these sites on occasion.</p> <p>Males</p>	<p>Females</p> <p>The histopathology was qualitatively similar to the pure Sb animals with fewer particles and less alveolar protein visible at all intervals. However, the extent of the interstitial fibrosis and cuboidal and columnar cell metaplasia was the same. Some of the particulates were birefringent under polarized light and appeared to be silicates. The tracheobronchial lymph nodes contained fewer particles than the pure SB exposed rats and contained mononuclear cell granulomas, similar to those seen in the early stages of silicosis or sarcoidosis. The first tumor was seen in an animal dying in week 41.</p> <p>Males</p> <p>The alterations were similar as that of the pure Sb. The only differences were fewer particles and the presence of some birefringent particles as well as granulomas in the tracheobronchial lymph nodes.</p>

	<p>(6 mos): Males showed a similar amount interstitial thickening as females with less alveolar protein.</p> <p>(12 mos): The severity of the interstitial fibrosis was the same as females. In some interstitial areas, there was, in addition, dense eosinophilic material with the appearance of amyloid. The cuboidal alveolar wall metaplasia was not as extensive as females, and there were fewer cholesterol clefts.</p> <p>(Terminal sacrifice): Some diminution in the amount of alveolar wall metaplasia and the metaplasia was less severe than females and again fewer cholesterol clefts. The amount of alveolar protein was less than at 12 mos and much less than that of females. In several interstitial spaces there appeared to be more mononuclear cells, lymphocytes and plasma cells than seen in females. Some of the amyloid appearing substance was still present. There did not appear to be a significant difference in the extent and severity of the interstitial fibrosis relative to females.</p>	
Histopathology of other tissues	Nothing remarkable with no increase in tumors seen in any other tissue relative to control	Same
Tumor Incident Rate and Type	<p>F: 18-73 wk 19/89 (21%) F: 41-72 wk 19/70 (27%) M: No tumors seen at any time</p> <p>Squamous cell carcinoma 9/19, bronchioloalveolar adenomas and carcinomas 11/19, scirrhous carcinomas 5/19. In 6 animals multiple tumors were seen (2-4/rat).</p>	<p>F: 18-73 wk 17/87 (20%) F: 41-72 wk 17/68 (25%) M: No tumors seen at any time</p> <p>Squamous cell carcinoma 9/17, bronchioloalveolar adenomas and carcinomas 6/17, scirrhous carcinomas 4/17. In 3 animals multiple tumors were seen (2-4/rat).</p>

Study	Watt 1983 PhD Dissertation (study conducted in 1976-77)	Watt 1983 PhD Dissertation (study conducted in 1976-77)
Test article	Antimony trioxide (purity 99.4%)(2300 ppm Pb, 210 ppm As,	Same
TWA [Actual]	0, 1.6, 4.2 mg/m ³	Same
Species	Rat – Charles River Fischer (Females only)	Swine – Sinclair S-1 (Females only)
Exposure Duration	6 hr/d, 5 days/wk for 12 months followed by a 12-15 month recovery	6 hr/d, 5 days/wk for 12 months
Necropsy Evaluations	Animals were evaluated for toxicity by blood draw at 3, 6 and 12 months. Animals were sacrificed at 3, 9, and 12 months and approx. 12 - 15 months post exposure.	Animals were evaluated for toxicity by blood draw at 3, 6 and 12 months with lung x-rays and cardiac EKG's at 6 and 12 months. Terminal sacrifice was at 12 months.
General Observations	No affect on hematology or serum enzymes were noted. There was no effect on growth. Body weights at initiation differed between the 3 dose groups. The only organ weight showing a weight change was the lung which was increased in a statistically significant manner in both low and high dose groups at 12 months.	No affect on hematology or serum enzymes were noted. No affect was noted in heart EKG's or in the lungs based on x-rays. There was no effect on growth weights. The only organ weight showing a change was that of lung which was increased (not statistically significant though).
Lung Tissue Concentration (ug Sb/g dry tissue)	Not reported	Not reported
Histopathology of the lung	A dose related focal fibrosis is seen post exposure in the low dose and in all high dose animals sacrificed at 6 months or later. A similar pattern was noted in pneumocyte hyperplasia which was noted in all but 1 animal in the high dose sacrificed prior to exposure termination, but only in 17 of 25 animals post exposure. A similar pattern was noted in the low dose group with all but two animals exhibiting hyperplasia during exposure, and 14 of 22 sacrificed post exposure. An adenomatous hyperplasia was only noted in the high dose group beginning late in exposure and present throughout the post exposure period (decreasing somewhat at the terminal sacrifice. Multinucleated giant cells and the presence of cholesterol clefts were also noted to have been induced in a dose and time related manner. The incidence rate of pigmented macrophages was approximately similar between dose levels.	Not reported
Histopathology of other tissues	Nothing remarkable when compared to control	Not reported
Tumor Incident Rate and Type	Any tumor type (12 mos sacrifice and later): control (2/28, 7.1%), low (1/31, 3.2%), high (21/34, 61.7%) High dose: squamous cell carcinoma (2/34), bronchioalveolar	None reported

	adenoma (3/34), scirrhous carcinomas (15/34)	
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Table 2: Genotoxicity Studies Conducted on Antimony Trioxide**Chromosomal Mutation Studies**

In vitro Assays	Result	Method	Purity	Ref.
Bacterial mutagenicity on <i>S. typhimurium</i> strains TA98, 100, 1535, 1537, and <i>E. coli</i> WP2P and WP2P <i>uvrA</i>	-	OECD guideline 471/472	99.9%	1
Mutagenicity assay in L5178Y TK +/- mouse lymphoma cell, +/-S9	-	OECD guideline 476 and EPA guidelines (FR50:39442-39443; and FR52:19056-19079)	99.9%	1
Bacterial mutagenicity on <i>S. typhimurium</i> strains TA98 and 100; +/-S9	-	Yahagi <i>et al.</i> , Mutation Research 48: 121-130, 1977	99.99%	2
Bacterial mutagenicity on <i>S. typhimurium</i> strains TA98, 100, 1535, 1537, and 1538, and <i>E. coli</i> B/r WP2 and WP2 <i>hcr</i> ; -S9	-	Ames <i>et al.</i> : Mutation Research, 31, 347-364, 1975	Unknown	5
Rec Assay on <i>B. subtilis</i> , -S9	+	Kada <i>et al.</i> , vol 6, 149-173 (1980) in the textbook "Chemical Mutagens" edited by de Serres and Hollaender	99.999%	2
Rec Assay, -S9	+	Kada <i>et al.</i> , Mutation Research 16: 165-174, 1972		5

Chromosomal Aberration Studies

In vitro Assays	Result	Method	Purity	Ref.
Cytogenetics assay in human lymphocytes, +/-S9	+	OECD guideline 473, EEC annex V B10, and UKEMS Recommended procedures for mutagenicity tests	99.9%	1
SCE in Chinese Hamster lung cells; -S9	+	Kuroda <i>et al.</i> , J Japan Soc. Air Pollut., 20: 342-348, 1985	99.999%	2
In vivo Assays	Result	Method	Purity	Ref.
Mouse bone marrow micronucleus test (Single dose of 1000 mg/kg)	-	OECD guideline 474	99.9%	1
Mouse bone marrow micronucleus test (Multiple doses up to 1000 mg/kg over 21 days)	-	OECD guideline 474	99.9%	1
Mouse bone marrow cytogenetics (Single dose of 1000 mg/kg)	-	Preston <i>et al.</i> , Mutation Research, 189: 157-165, 1987	Unknown	3, 4
Mouse bone marrow cytogenetics (Multiple doses up to 1000 mg/kg over 21 days)	+	Preston <i>et al.</i> , Mutation Research, 189: 157-165, 1987	Unknown	3, 4

References

1. Elliott *et al.*, Mutation Research, 415: 109-117, 1998
2. Kuroda *et al.*, Mutation Research, 264(4): 163-170. 1991
3. Gurnani *et al.*, BioMetals, 5: 47-50, 1992
4. Gurnani *et al.*, Bio Trace Element Res., 37: 281-292, 1993
5. Kanematsu *et al.*, Mutation Research, 77: 109-116. 1980

Antimony trioxide: An evaluation of genotoxicity based on the literature data

Introduction:

Genotoxicity is usually measured on the ability of a test compound to (a) induce point mutations in different bacterial strains, (b) structural and numerical chromosomal aberrations in cultured mammalian cells, (c) mutations in cultured mammalian cells and (d) structural and numerical chromosomal aberrations in somatic cells in vivo. Additional end points, such as DNA adduct formation, sister chromatid exchanges, induction of DNA repair are used to provide supporting data in evaluation of genotoxicity. It is now generally accepted that induction of micronuclei can be considered as a surrogate for induction of chromosomal aberrations. Over the years, for critical evaluation of genotoxicity, especially for regulatory purposes, standard protocols have been established by international bodies, such as European Union, OECD, EPA etc.,

The genotoxicity of antimony trioxide has been evaluated using different test systems, including bacteria, mammalian cells cultured in vitro and mammalian cells in vivo. These are summarized below:

In vitro studies:

1. Assay for induction of mutations in bacteria: Several studies using different strains of *E. coli* and *S. typhimurium* showed that antimony trioxide is negative in inducing mutations both in the presence and absence of S-9. The protocols employed are according to those approved by regulatory authorities. Using REC assay, which evaluates DNA damaging capacity of a compound, by measuring inhibition of growth in recombination repair deficient bacteria (*rec-Bacillus subtilis*) in comparison to wild type strain, antimony trioxide has been found to be positive. But this test is not a direct test for mutagenesis, and only about 70% of agents found to be positive in *rec-* assay have been found to be real mutagens (Kanzematsu et al, 1980).
2. Assay for induction of mutations in mouse lymphoma L5178Y cell line: Antimony trioxide was found to be non-mutagenic (up to 50µg/ml) in the presence or absence of S-9 metabolic activation. In this assay, large sized mutant colonies are considered as true point mutations and small sized mutant colonies are considered to be due to chromosomal changes such as, deletions, translocations etc.,
3. Assay for induction of chromosome aberrations in human peripheral lymphocytes in culture: Antimony trioxide was found to be positive in this assay at concentrations 50 and 100µg/ml both in the presence and absence of S-9 metabolic activation (Elliott et al., 1998). The raw data, which were made available to the reviewer, showed that antimony trioxide induced only chromatid breaks or fragments and hardly any rearrangements (1 in 2800 total treated cells).

4. Assay for induction of sister chromatid exchanges (SCEs) in Chinese hamster cells in culture: Antimony trioxide was found to be moderately positive in inducing SCEs (Kuroda, et al., 1991). Induction of SCEs is not an assay for mutagenicity per se and is considered only as an indicator of repairable DNA damage.

In conclusion, the available in vitro data indicate that Antimony trioxide is clastogenic mainly inducing chromatid breaks when actively dividing cells are treated. In all gene mutation tests, both in bacteria and mammalian cells, antimony trioxide was found to be negative.

In vivo studies:

In view of the positive results obtained for induction of chromosomal aberrations in vitro by antimony trioxide, it is of crucial importance to examine the results obtained in vivo. There are three papers available in the literature, two pertaining to chromosomal aberrations in bone marrow of mice (Gurnani et al, 1992, 1993) and one dealing with micronuclei in polychromatic erythrocytes of mice (Elliott et al., 1998).

Induction of chromosomal aberrations:

In the first study Gurnani et al. (1992) compared the clastogenic effects of antimony trioxide in mice following acute and chronic exposure. Following acute exposure (400, 666.67 and 1000 mg/kg) given orally, the mice were sacrificed after 6, 12, 18 and 24h of treatment and the frequency of aberrations in bone marrow cells were evaluated. The results were negative (no data are provided in the publication) For chronic exposure; mice were fed every day with antimony trioxide (same doses as for acute exposure) for 7, 14 and 21 days. They observed that the highest dose was lethal on day 20. The frequencies of aberrations increased to a statistical significance with increasing dosage but no such relationship was seen with the duration of exposure. The experimental protocol employed has several severe shortcomings. They did not use any positive control, which is mandatory in any such experiments; in order to make sure that the system employed is indeed operating properly. It is not stated as to whether the slides were coded and scored blindly. This is very important to avoid any bias, especially when the frequencies of aberrations encountered are low as in the present study. The types of chromosomal aberrations recorded were "chromatid gaps, chromatid breaks, centric fusions and polyploidy". In Table 1, the data on the aberrations are presented, as "G', G", B', B", RR and polyploids". It can be seen that only chromatid breaks (B') but no iso-chromatid or chromosome breaks (B") were observed. Similarly, only 3 iso-chromatid gaps (G") as against 127 chromatid gaps (G') were observed. No chromatid rearrangements were observed, except centric fusions. It is not stated in the table as to the nature of aberrations classified as "RR" and one has to assume that they are centric fusions. The nature of centric fusions is difficult to discern, and by the known mechanisms of centric fusions (so called Robertsonian exchanges), each such metacentric

chromosome should be accompanied by a chromosome or isochromatid fragment (Boei and Natarajan, 1996). Since no such fragments were recorded, one should assume that these observed fusions are due to stickiness and are induced only at the highest dose (1000 mg/kg) employed. Therefore they cannot be included in the quantitative evaluation. Strangely, the authors count each centric fusion as two breaks, in their calculation of "Break/cell" as well as "Frequency of aberrations" in Table 1. "Polyploidy" cannot be considered as an aberration (as presented in the Table 1) but an aberrant cell and there is no dose-related increase in the induction of polyploidy. Due to the manner the data are presented they are difficult to evaluate. The only conclusion one can derive from these data is that antimony trioxide induces chromatid breaks in bone marrow cells of mice treated chronically. The observed chromatid breaks may arise due to disturbances in DNA replication as seen in the cases of treatment with inhibitors of DNA synthesis, such as cytosine arabinoside, aphidicoline etc. which induce only chromatid breaks and not exchanges in proliferating cells.

In the second paper (Gurnani et al., 1993) the authors compare the clastogenic effects of antimony and bismuth trioxides in mice in vivo. The experimental protocol is exactly the same as in the earlier paper. The authors state (page 284) that "The same effect had been observed earlier by the authors" (Gurnani et al., 1992). However, the results presented for Antimony trioxide (see Table 2) are exactly same as those published earlier, indicating that they did not repeat the experiment with antimony trioxide for comparison with bismuth trioxide. In Table 1 (page 284) the schematic representation of the experimental protocol is given, which indicates that in each of the six experimental groups, 20 animals were used including control sets. This means, that there should be 15 control animals for the experiment with bismuth trioxide. The perusal of the data presented in Table 3 (page 286) shows that the frequencies of aberrations in control animals are exactly the same as reported for antimony trioxide, in the earlier paper as well as in the present paper. This clearly demonstrates that the authors did not run concurrent controls in this experiment. Strangely, in all the control groups, the authors recorded the same number of aberrations, namely 6 gaps (G') and 5 breaks (B') in 500 cells derived from 5 animals. Statistically, there is a very remote chance that the same frequencies can be obtained in three different experiments involving 9 groups of control animals. From the discrepancies found in these two papers, one is forced to conclude that the data presented by these authors are unreliable and should not be taken seriously for evaluating the in vivo effects of antimony trioxide.

One grave concern in this study, is the purity of the chemicals used, as antimony trioxide was lethal at the highest dose (1000 mg/kg) used, in contrast to the other study (Elliott et al, 1998) in which even higher doses were not found to be lethal.

Induction of micronuclei in polychromatic erythrocytes of mice:

Elliott et al (1998) evaluated the ability of antimony trioxide to induce micronuclei in bone marrow of both male and female mice. One of the aims of this study was to confirm the

observations of Gurnani et al (1992, 1993). Therefore, protocol employed was similar to their study, but the study was performed according to the standard guidelines of OECD. There was no evidence of toxicity in the animals even after treatment for 21 days at 1000mg/kg, in contrast to the toxicity reported in the study of Gurnani (1992,1993). In the study of Elliott et al., (1998) a positive control treatment of cyclophosphamide (65 mg/kg) was included, which increased significantly the frequencies of micronuclei in the polychromatic erythrocytes. There was no evidence that antimony trioxide was inducing micronuclei in the mouse bone marrow.

Comparison of chromosomal aberration data and micronuclei data:

The studies on in vivo clastogenicity of antimony trioxide seems to have given contradictory results. In principle, an agent which induces chromosomal breaks as revealed in metaphase analysis should also give rise to micronuclei, since most of the acentric breaks will lag during ana phase and form micronuclei. In polychromatic erythrocytes, during the maturation the main nucleus is ejected while micronuclei are retained in the cytoplasm, There could be several reasons for the reported contradictory results.

1. The studies of Gurnani et al., (1992, 1993) were conducted rather poorly and the reported results are not reliable. A positive control was not included in their studies. Only one set of control data has been used for three different (?) experiments indicating proper parallel negative controls were not included.
2. Even if we assume that the results of Gurnani et al., are reliable, they observed only a low frequency of chromatid breaks, which can be repaired by error free pathway, which will not give rise to any micronuclei. Thus, the observed chromatid breaks will not have any genetic consequence. A support for this suggestion comes from the study of Elliott et al., (1998) who observed that only chromatid breaks were induced by antimony trioxide human peripheral blood lymphocytes, but no increase in the frequencies of mutations in mouse lymphoma cells. Since small sized mutant colonies in this system arise from chromosomal aberrations, one would have expected an increase in this class of mutations if the induced breaks are not repaired before the mutations are fixed.
3. The purity of antimony trioxide in Gurnani's study was not specified, while in Elliott's study, it was specified as 99.9%. The conflicting results on the toxicity of antimony trioxide, namely, a strong lethal effect in all the 5 mice treated with 1000 mg/kg a day in Gurnani's experiments, and a non-toxicity observed in Elliott's study may be due to toxic impurities in the sample used by Gurnani et al (1992, 1993).
4. Elliott's et al., (1998) conducted an assay for unscheduled DNA synthesis in liver cells following treatment of rats with a high dose (5000mg/kg) of antimony trioxide. This assay evaluates the repair of induced lesions in DNA of cells that are not normally replicating. The results were negative, indicating that no lesions eliciting excision repair occurs following antimony trioxide treatment in vivo.

Human monitoring studies:

Cavello et al., (2002) have published the results of a biomonitoring study on workers exposed to antimony trioxide. Genotoxicity was assessed by the frequencies of micronuclei, sister chromatid exchanges and single cell gel electrophoresis ("comet assay) using peripheral blood lymphocytes. In addition to a control group, two cohorts of one high and one low-exposed group were investigated. The results employing binucleated cells (cytochalasin B blocked ones) indicated no increase in the frequencies of micronuclei in the exposed groups compared to the controls. There were no significant differences in the frequencies of sister chromatid exchanges between the control and exposed groups. Modified Comet assay employing Fpg (formamido-Pyrimidine-Glycosylase) enzyme, which recognizes oxidatively damaged bases in DNA and converts them into single strand breaks, indicated a modest increase in the tail lengths of the comets in the highly exposed group. It should be pointed out in all the three groups, the tail lengths increased after Fpg treatment, indicating oxidative damage was present in controls as well as lowly exposed group. For a validation of positive results with comet assay, they should be supported by a dose response relationship with different exposure levels (Albertini et al., 2000). This criterion is not fully filled in this study, as the number of individuals studied per group is small (e.g., 6 in group B, out of which 5 were without damage. See Table 1). The tail lengths of comets generated with no treatment with Fpg were not different between the groups. The DNA single strand breaks detected (using this technique) were obviously repaired by an "error free" pathway, leading to no genotoxic damage, as evidenced by the negative results obtained in the assays to evaluate the frequencies of micronuclei and SCEs.

General conclusions:

The available literature data on the genotoxicity of antimony trioxide indicate that:

- a) Antimony trioxide is clastogenic in human lymphocytes treated in vitro and induces SCEs in Chinese hamster cells.
- b) Antimony trioxide does not induce mutations in different strains of bacteria as well as mouse lymphoma cells in culture.
- c) The results published with regard to induction of chromosomal damage by antimony trioxide in mice in vivo are contradictory. The results were negative in bone marrow micronucleus (MN) assay, but positive in bone marrow chromosomal aberration (CA) assay. While the MN assay has been conducted according to accepted protocols by regulatory authorities, the CA studies have very many flaws and the presented results can not be considered to be reliable.
- d) Human biomonitoring studies could not detect any genotoxic damage (as assessed by the frequencies of MN and SCEs) in the lymphocytes of exposed workers.

After a careful review of the existing data available in the literature, I conclude that there is no compelling evidence to classify antimony trioxide as an *in vivo* genotoxic agent.

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An evaluation of the scientific literature pertaining to the *in vitro* and *in vivo* genotoxicity of antimony trioxide: relevance to the potential mutagenicity classification by the European Union

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Executive Summary

The range of mutagenicity studies performed with antimony trioxide to internationally accepted test standards have demonstrated that *in vitro*:

Antimony trioxide is not capable of inducing gene mutations in either bacterial or mammalian cell test systems in either the presence or absence of S9 metabolic activation systems. In contrast, antimony trioxide was capable of inducing chromosome aberrations (clastogenic activity) in human lymphocytes at concentrations of 50 and 100 micrograms /ml in both the presence and absence of S9 mix. Positive results were also observed for the induction of sister chromatid exchange.

When tested in mouse bone marrow micronucleus assays and rat liver UDS repair studies performed to OECD standards, there was no evidence that the clastogenic activity was repeated *in vivo*.

These studies indicate that antimony trioxide is not capable of inducing genetic changes *in vivo* in intact animals including humans.

There is a published study in the mouse which claims that antimony trioxide is capable of inducing chromosome aberrations in mice under conditions of repeat exposure. However, this reported positive study was not performed to a recognised international standard and there are some doubts with regard to the purity of the test material due to its high toxicity compared to other tested samples.

There is a published study which reports the presence of oxidative DNA lesions in the lymphocytes of humans exposed to antimony trioxide. However, under identical experimental conditions there was no evidence of the induction of the genetic changes of micronuclei and sister chromatid exchange.

On the basis of the currently available range of studies evaluated here I conclude that there is currently insufficient evidence to justify the classification of antimony trioxide as an *in vivo* mutagen.

Introduction

Consideration of potential factors which may influence mutagenic activity.

When evaluating genotoxicity data it is important to distinguish between those test endpoints that provide a measure of:

1. The induction of a mutagenic change, including gene (point) and chromosome structural and numerical changes.
2. The induction of indicators of the formation of lesions in DNA which may or may not be processed into mutagenic changes. These indicators can include:
 - a) the formation of measurable DNA modifications or adducts
 - b) the formation of DNA lesions detectable after further treatment such as the application of specific enzymes.
 - c) the induction of DNA repair.

The detection of the induction of endpoints in class 2 provides valuable information concerning the potential of compound treatment to produce mutagenic changes. However, there are factors such as error free DNA repair which may eliminate DNA adducts and apoptosis which may lead to the death of cells containing DNA modifications. Such factors need particular consideration when data sets are incomplete and/or confusing.

A background of DNA lesions in cells are a consequence of normal mammalian metabolism such as the production of oxidative damage. The excretion of nucleotides from the DNA which have been modified by oxidative damage demonstrates the effectiveness of DNA repair activity which results in the relatively low levels of mutagenic change observed in mammals including humans.. This background levels of DNA lesions and their repair are important considerations when assessing the mutagenic potential of compounds which function by inducing oxidative damage. For such compounds, elevations in mutagenic change will only be produced when the levels of lesions exceed the capacity of error-free DNA repair.

Factors which may influence the assessment of test data quality

When assessing the significance of mutagenicity test data published in the scientific press there are a number of factors that need to be considered:

1. Genotoxicity test data is published in a number of scientific journals of varying quality, which are generally distinguished by the standard of their refereeing. Thus “journal impact” may influence the importance the assessor may place on a specific piece of test data.
2. For many mutagenicity assay systems there are recognised national and international standards relating to test protocols e.g. such as those of the Organisation for Economic Cooperation and Development (OECD). Data produced in studies performed to these international quality standards will inevitably be considered of more value during data assessment than those performed at lower test standards
3. Because of space limitations, scientific journals will generally publish test data in an abbreviated form which may preclude the reader from assessing the significance of specific results such as those from individual test animals. Data assessment can be made with more confidence when complete data sets are available to the reader.
4. In many cases assays of the activity of specific compounds may involve the use of samples of differing purities. When assessing data sets it is important to consider the possibility that impurities may influence the results obtained.

Review of the publications discussing the potential genotoxicity of antimony trioxide

It has been clearly demonstrated that there are substantial differences in solubility between the various forms of antimony and thus their potential toxicity. This review considers only the data available on antimony trioxide and its relevance to its potential classification as a mutagen.

Mutagenicity assays

In vitro* Gene mutation assays using the bacterial cultures *Salmonella typhimurium* and *Escherichia coli

Negative results were obtained in a comprehensive study at concentrations of antimony trioxide of up to 5000µg/plate in both the presence and absence of S9 metabolic activation mix (Elliott *et al* 1998). Complete data for this study were made available for evaluation.

***In vitro* Gene mutation assay using Mouse lymphoma L5178Y cell line**

Negative results were obtained in a study at concentrations of up to 50 µg/ml of antimony trioxide in both the presence and absence of S9 metabolic activation mix. No evidence of cell toxicity was observed in this study (Elliott *et al* 1998). Complete data for this study was made available for evaluation.

***In vitro* chromosome aberration assays using cultured human lymphocytes**

Positive results were obtained in both the absence and presence of S9 metabolic activation mix at concentrations of 50µg/ml and 100µg/m of antimony trioxide (Elliott *et al* 1998).

Complete data for this study were made available for evaluation.

***In vitro* sister chromatid exchange assays using cultured human lymphocytes and Chinese hamster V79 cells**

Positive results were obtained in this **indicator** test over a low concentration range of 0.03 to 1.5µg/ml in human lymphocytes and 0.09 to 0.34µg/ml in Chinese hamster V79 cells. (Kuroda *et al* 1991, Gebel *et al* 1997).

The *in vitro* studies reviewed here indicate that antimony trioxide is capable of inducing chromosome aberrations *in vitro*. However, there was no evidence that antimony trioxide was capable of inducing gene (point) mutations *in vitro*. The critical question now concerns whether the *in vitro* chromosome aberration inducing activity of antimony trioxide is also produced *in vivo* in mammals including humans.

***In vivo* cytogenetic assays**

Gurnani and colleagues have published data in 2 papers (1992 and 1993) describing what appears to be a single chromosome aberration study in the bone marrow of mice following both single and repeat dose exposures to antimony trioxide and antimony trichloride. It should be noted that these studies were not performed to a recognised international guideline and no positive control chemical exposures were used. In the absence of positive control data it is not possible to estimate the sensitivity of the methodologies used or to relate the data to other published studies.

The study of Gurnani *et al* (1992 1993) demonstrated that positive increases in chromosome aberrations obtained in a repeat dose study at 7, 14 and 21 days using concentrations of 400, 667 or 1000 mg/kg in male mice. The authors state that no increases were observed in chromosome aberrations following single exposures to antimony trioxide. However, this negative data does not appear to have been published. The authors report that exposure at 1000 mg/kg antimony trioxide resulted in animal toxicity by day 21(i.e. the death of all 5 animals).

Dose related positive results were obtained by Gurnani *et al* (1992,1993) for chromosome aberrations at 667 and 1000mg/kg and for breaks per cell at 400, 667 and 1000 mg/kg of repeat dose exposure in male mice over 14 days.

The authors also evaluated the potential effects of antimony trioxide upon the frequency of sperm head abnormalities. The authors state that no increases in abnormalities were observed. However, these data were not reported in full and could not be adequately evaluated.

There has been a basic assumption in the preparation of guidance documents (e.g. COM 2000) that the analysis of chromosome aberrations and micronuclei in the bone marrow of mice provides similar information on the mutagenic potential of a chemical *in vivo*. However, the scientific literature on antimony trioxide includes contradictory results concerning the quantification of the induction of chromosome aberrations and micronuclei. Such differences in the responses of apparently similar test systems require careful examination and consideration of factors such as differences in test compound purity, solvents and standards of sample evaluation.

To further investigate and clarify the potential *in vivo* genotoxicity of antimony trioxide, Elliott *et al* (1998) have evaluated the ability of antimony trioxide to induce micronuclei in the bone marrow of both male and female mice using a protocol similar to that of Gurnani *et al* (1992 and 1993). This study was performed to the OECD guideline standard and involved oral gavage administration for a single dose of 5000 mg/ml and repeat dosing of up to 1000 mg/kg (400, 667 and 1000 mg/kg) up to 21 days with sampling on days 8, 15 and 22. Evidence of bone marrow toxicity was only observed in female mice in the 5000 mg/kg single dose study. There was no evidence of toxicity in the animals even when treated for 21 days at 1000 mg/kg in the Elliott *et al* (1998) study. This lack of toxicity was in marked contrast to the report of significant toxicity of the sample of antimony trioxide used in the reports of Gurnani *et al* (1992 1993) .

The Elliott *et al* (1998) study utilized a positive control treatment of 65 mg/kg cyclophosphamide which produced significant increases in the induction of micronuclei in the polychromatic erythrocytes from the bone marrow in both the single and repeat dose study. The positive result obtained with cyclophosphamide demonstrated that the assay system used by Elliott *et al* (1998) was functioning correctly. There was no evidence in this comprehensive study that antimony trioxide was capable of inducing micronuclei in mouse bone marrow following oral exposure by either single at repeat dosing. The complete data set of Elliott *et al* (1998) was made available for evaluation.

Thus, studies of the activity of antimony trioxide in the bone marrow of mice have produced contradictory results; i.e. positive results for chromosome aberrations and

negative results for micronuclei. The studies of Gurnani *et al* (1992 1993) and Elliott *et al* (1998) can be compared to determine whether there are any differences, which may account for the discrepancies in the results.

1. The Gurnani *et al* (1992 1993) study was performed to an “**in house**” standard whereas the Elliott *et al* (1998) study was performed to an OECD guideline. The lack of positive chemical control data in the Gurnani *et al.* (1992 1993) study lowers its credibility when used in chemical classification.
2. The purity of the antimony trioxide used in the Gurnani *et al* (1992 1993) study was not specified, however it was supplied by a reputable chemical supplier. The purity of the antimony trioxide used in Elliott *et al* (1998) study was specified at 99.9%.
3. Antimony trioxide at 1000 mg/kg was toxic in the Gurnani *et al* (1992 1993) study resulting in the death of all 5 treated mice at 21days. In contrast, no toxicity was observed in the Elliott *et al* (1998) study under apparently similar treatment conditions. The differences in specification of purity and toxicity raises the possibility that the Gurnani *et al* (1992 1993) positive results may be the result of an impurity which produced toxicity at 21 days.
4. The Elliott *et al* (1998) study involved the assessment of chromosome damage at the interphase stage of cell division in anucleate bone marrow cells whereas the Gurnani *et al* (1992 1993) study assessed chromosome aberrations at the metaphase stage of cell division in nucleated bone marrow cells. There is no reason to believe that these cell types differences could explain the discrepancies in the results of the two groups of authors.
5. The Gurnani *et al* (1992 1993) study they used Swiss Albino mice whereas the Elliott *et al* (1998) study used CD-1 mice. However, these mouse strains are closely related and would not be expected to produce any significant differences in their responses to genotoxic chemicals.

In situations where a compound produces some positive results *in vitro* and negative results in bone marrow assays it is usual to evaluate activity further *in vivo* in a different tissue. In the case of antimony trioxide, Elliott *et al* (1998) have undertaken

an *in vivo* rat liver study measuring the induction of unscheduled DNA synthesis as a measure of DNA lesion repair following oral gavage treatment at 5000 mg/kg. There was no evidence of the induction of DNA repair or animal toxicity in this indicator study. The complete data set of Elliott et al (1998) was made available for evaluation.

Conclusions which can be derived from the standard genotoxicity studies performed with antimony trioxide.

Antimony trioxide was shown to be capable of introducing chromosome aberrations *in vitro*. However, this activity was not reproduced *in vivo* in either the mouse bone marrow micronucleus assay or rat liver DNA repair assay performed to OECD guideline standards. On the basis of this data set, I conclude that there is inadequate data to classify antimony trioxide as a category 3 mutagen.

However, the positive and contrasting data of Gurnani *et al* (1992 1993) in mouse bone marrow indicate that consideration should be made of any available studies that may clarify the potential activity of antimony trioxide. To this end, evaluations have been made of both the published studies on human workers potentially exposed to antimony trioxide and mechanistic studies on antimony salts using *in vitro* methods.

Studies to investigate the potential mechanisms of the genotoxicity of antimony trioxide.

In this section I have also considered some studies with antimony salts other than trioxide as an aid to clarifying potential mechanisms of action .

A number of studies have demonstrated that trivalent arsenic is capable of inhibiting some of the steps of nucleotide excision repair (Hartwig *et al* 1997) and of post-replication repair (Lee Chen *et al* 1992). Such observations have stimulated research to determine whether similar mechanisms can be produced by antimony salts.

Schaumloffel and Gebel (1998) have undertaken a range of studies comparing the activity of NaAsO₂ and SbCl₃ using a range of assays in an attempt to determine the potential mechanisms which may explain the observed genotoxicity of trivalent arsenic. In these studies antimony trichloride was shown to induce both micronuclei and chromosome damage (measured in the COMET assay) in human lymphocytes.

However unlike trivalent arsenic, the damage and genetic changes induced by antimony trichloride were not influenced by treatment with superoxide dismutase or with catalase in the micronucleus assay or by proteinase K in the COMET assay.

The treatments used modified the response of exposures with trivalent arsenic but there was a lack of response with trivalent antimony .These observations do not provide evidence to support the concept of similar mechanisms of action for the genotoxicity of arsenic and antimony. I conclude that the use of experimental data derived from studies on the mechanisms of action of arsenic salts provide no information useful for investigating potential genotoxic mechanisms of antimony salts.

Genotoxicity studies on workers exposed to antimony trioxide

Cavallo *et al* (2002) have studied a number of endpoints in two groups of industrial workers exposed to antimony trioxide at estimated concentrations of $0.12 \pm 0.11 \mu\text{g}/\text{m}^3$ (highest exposure group) and $0.05 \pm 0.038 \mu\text{g}/\text{m}^3$ (lower exposure group).

The authors failed to demonstrate any elevation in the frequencies of the genetic endpoints of micronucleus induction or sister chromatid exchange in the lymphocytes of the exposed groups compared with controls. However, the authors were able to demonstrate increases in damage detected by indicator COMET assays in lymphocytes from the exposed groups when DNA was treated with the formamide-pyrimidine glycosylase enzyme (Fpg). This enzyme nicks DNA at sites modified by oxidative damage. The authors indicate that the increases in oxidative damage detected were classified by them as moderate and were statistically significant in the highest exposure ($0.12 \pm 0.11 \mu\text{g}/\text{m}^2$) group of workers.

An increased level of oxidative damage was observed in workers who were exposed to levels of antimony trioxide of $0.12 \pm 0.11 \mu\text{g}/\text{m}^3$ which were extremely low when compared to the OSHA recommended exposure levels ($500 \mu\text{g}/\text{m}^3$). However, no significant increases were observed in the genotoxic endpoints of micronuclei and sister chromatid exchange. The sister chromatid exchange endpoint has been shown to be particularly sensitive to induction by antimony trioxide *in vitro* by Kuroda *et al* (1991) and Gebel *et al* (1997) .

The lack of correlations between the detection of effects in an indicator of DNA modifications i.e. in the COMET assay and genetic changes such as micronuclei suggest that the modifications observed by Cavallo *et al*(2002) can be repaired by processes such as base excision repair and thus do not lead to genetic changes.

The use of indicator assays to aid in the assessment of the potential genotoxicity of antimony trioxide

The potential genotoxicity of antimony trioxide has been assessed in some indicator tests in which the relative sensitivity of DNA repair proficient and deficient *Bacillus subtilis* strains to antimony trioxide was determined.

Positive results were obtained demonstrating increased lethality to the repair deficient strains when discs containing up to 1.1 µg of antimony trioxide were added to bacterial culture plates. These data indicate that antimony trioxide induces repairable DNA damage to *Bacillus subtilis* cells.

Overall, the mechanistic and indicator studies suggest that antimony trioxide is capable of inducing repairable DNA damage. Although the data are limited, the strongest current evidence is that the DNA damage observed is the result of oxygen radicals which are produced by an as yet undefined mechanism(s) De Boeck *et al* (2003). The failure to demonstrate the induction of genetic changes under conditions where DNA damage is produced suggests that there are levels of exposure to antimony trioxide where DNA lesions are fully repaired and do not produce gene or chromosome mutations.

Overview and Conclusions

In this review I have only considered the published data concerning antimony trioxide except in some specific situations where other information could aid in understanding mechanisms of action. In view of the substantive differences in solubility and valency and thus the potential bioavailability of antimony salts, I considered it appropriate to only utilize data on the specific chemical under consideration for classification purposes i.e. antimony trioxide.

The available data demonstrate that, although antimony trioxide is capable of inducing toxicity in DNA repair deficient bacterial cultures, there was no evidence to suggest that the events which produce this differential toxicity are capable of inducing gene mutations in studies which used a comprehensive range of bacterial tester strains. Neither was there any evidence that antimony trioxide was capable of

inducing gene mutations at the thymidine kinase locus in cultured mouse lymphoma L5178Y cells.

Antimony trioxide was shown to be an *in vitro* clastogen capable of inducing chromosome aberrations in cultured human lymphocytes.

The clastogenic activity of antimony trioxide was not reproduced *in vivo* in a comprehensive and well reported single and repeat dose mouse bone marrow study performed to an OECD Guideline. Negative results were also obtained in a second tissue i.e. the rat liver in an unsheduled DNA synthesis indicator test. In contrast, positive results were reported in a repeat dose study in which mice were exposed to antimony trioxide for up to 21 days. These positive results were obtained in a study which did not comply with OECD Guidance . For example, no positive control chemical was used in the study. In view of these protocol differences, I would conclude that the negative bone marrow study performed to an OECD Guideline standard should be considered of higher value in a classification exercise. I consider on the basis of the results seen in a package of mutagenicity assays performed to internationally accepted standards, that antimony trioxide cannot be considered to be an *in vivo* somatic mutagen.

In a group of workers exposed to low levels of antimony trioxide there was evidence of the production of oxidative damage induced lesions as demonstrated by the Comet assay indicator test. However, in the same study there was no evidence of increases in the induction of micronuclei or sister chromatid exchange in the lymphocytes of antimony trioxide exposed workers.

These data suggest that at the levels of antimony trioxide to which the workers were exposed the oxidative damage induced DNA lesions were fully repaired by an error free repair process . Thus, the oxidative damage induced DNA lesions produced at the reported levels of antimony trioxide exposure (up to 0.12 micrograms/cubic metre) do not elevate mutation frequency.

Overall, the currently available information does not provide a convincing basis for the classification of antimony trioxide as a somatic cell mutagen *in vivo*. However,

there are a number of studies such as those as Gurnani et al (1992,1993) in the bone marrow of mice and Cavallo et al (2002) in the lymphocytes of exposed workers which raise questions concerning the potential genotoxicity of some samples of antimony trioxide under some exposure conditions which indicates the need for further clarifying studies.

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