Comments on the draft Report on Carcinogens Monograph on antimony trioxide (ATO)

(R. Cortvrindt, of November 29, 2017)

I. Introduction

Campine[1] is in the lead and antimony business for more than 100 years. Since 1935 we produce antimony trioxide (ATO). With a production of 10.000 tonnes per year, Campine is market leader for ATO in Europe of witch 15% of the endproducts are intended for the US market. Next to this conversion of antimony metal to ATO and the extrusion of ATO in masterbatches, we recycle lead acid batteries (including antimonial lead) and started recently to recycle antimony.

Rita Cortvrindt (author) is related to Campine as a toxicologist, European registered (ERT) supports with toxicological advice.

II. General comments

The draft report (November 29, 2017) on Carcinogens Monograph on Antimony Trioxide comes to the conclusion that there is no evidence based on human information, but that there is sufficient evidence from experimental animal studies to consider Antimony Trioxide (ATO) as a potential human carcinogen. This conclusion is also supported with some fragmentary mechanistic data mainly from in vitro studies.
This preliminary listing recommendation was based on the application of the RoC listing criteria to the body of scientific evidence as provided in the Peer-Review draft and is stated as:

Antimony(III) trioxide increased the incidences of malignant tumors or the combined malignant and benign tumors at two tissue sites in rats (lung and adrenal gland) and three sites in mice (lung, skin, and lymphoid system).

Biological effects associated with carcinogenicity include increases in oxidative stress and oxidative damage, impairment of DNA damage repair, and possibly inhibition of cell differentiation.

Antimony (III) trioxide is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting data from mechanistic studies.

[1] http://www.campine.biz/

Upon critical review of this draft document and the related scientific peer reviewed literature, the available animal cancer evidence is very week and there is no evidence of cancer in humans.

Campine disagrees that antimony trioxide (ATO) is reasonably anticipated to be a human carcinogen.

An independent health service monitors Campine's (ATO exposed) personnel for several decennia and did not report any significant adverse effects, nor cancer for the ATO workers over all these years.

Furthermore we like to express following concerns:

1. Next to the results obtained from studies with antimony trioxide (ATO), also results from studies with other antimony compounds are included. All are grouped as 'antimony' and observed effects are subsequently allocated to properties of ATO.
   Antimony compounds have quite different characteristics in terms of solubility and bio-accessibility upon exposure to living organisms, which can result in different bioavailability, toxicokinetics and subsequent downstream toxic effects. **It would be good to make a clear statement in the document that the final conclusions on carcinogenic properties are only valid for ATO, and remove all other references and quotes made to other compounds.**

2. The main data for human exposure are from very old studies (mainly seventies and earlier). At those times, the exposure levels were much higher than today. Especially for workers, the exposure levels have been drastically reduced, thanks to modernized production processes and implementation of personal protection measures for the workers**. The observations made at earlier times are no longer relevant and they might best be excluded from the report or at least clearly state their limited relevance for the actual situation.**

3. There is evidence, within this review document and in the open literature that the toxicokinetic and toxicodynamic behavior of ATO is species specific. From studies it is clear that the absorption and distribution profile of ATO in rats and mice is quite different. In the rat, Sb-blood concentrations are exposure concentration dependent and increase over time upon continuous exposure. In the mice, the ATO absorption rates are significant lower compared to these of the rats and do not increase with time of exposure as in the rat.
   The species specificity (rats/mice ≅ human?) should be taken into account during the assessment of the carcinogenic properties of ATO and at least adds to the uncertainty of the in the monograph proposed conclusion.

4. The selected and evaluated animal studies are restricted to inhalation studies, with local ATO exposure to the lung tissue. **It is best to specify that the conclusions drawn would only apply for the inhalation route only.**

5. Mechanistic data are most often obtained from in vitro studies. The results of these in vitro studies need to be assessed with utmost caution. **In vivo relevance and transposition of in vitro effective concentrations to in vivo exposure (dosing) effects is not straightforward, especially for targets like nuclear receptors, which have often non-monotonic concentration response profiles.**

6. Despite the wealth of epidemiology studies performed on workers, none have ever attributed cancer incidence to the exposure to ATO. **Concluding that ATO can be reasonably anticipated to cause human cancer is void of proper justification and goes against available evidence.**

III. Document specific comments

Our main comments are systematically formulated following the layout of the draft document, with focus on ATO and those section relevant for the conclusion.

1. Chemical identification and properties

P2.
Please note that in Pentostam (as Glucantime = Megulumine antimonate) is a drug that is mainly based on the availability of Sb(V) and should not be classified as Sb(III) compound as stated on p2 and in table 1-2.
Information on antimony substances other than ATO do not bring value to the discussion and should be removed from the Monograph to avoid confusion.

It might be important also to include some information on the large variability in particle size and distribution within a defined ATO product. Particle size is in a large part determining the fate upon inhalation and uptake into the body upon exposure.

2. Human Exposure (p30/31)

As already mentioned above, human exposure has declined with time, especially for workers, with the improvement and automatization of mining, refining and manufacturing processes and the dedicated personal protection equipment. All these factors also contribute to a more 'clean' environment in the surroundings of factories. One can easily confirm that the airquality in the surroundings of an ATO exploitation (Campine) are much lower than shown in the current Monograph (see chapter IV below).
One might wonder whether it is still opportune and efficient to base the exposure considerations on old and outdated/obsolete studies which are no longer representative of today's situation.

Instead, reference to recent environmental , occupational and biomonitoring values which are in line with currently applicable safety values, would be more appropriate.
This would at least help avoiding misconception for the Sb-innocent reader.

3. Disposition and Toxicokinetics

Please note:
On p28 it is stated that in the rat inhalation study of Newton et al. (1984), the Sb concentration in the red blood cells of the exposed rats did not increase with time of exposure. However, in table B-1, one can read that the concentration at 12 months is double the concentration of that at 6 months of exposure (eg: 4,5mg/m3: 6 mo/12 mo (76 /120 µg/g RBC).
This is in agreement with the observation made in the NTP study (eg: 3mg/m3: day 61/ Day 124 (7/16 µg/g blood).

In the next paragraph it is stated that in the NTP (2016c) study in both the rats and mice of both sexes exposed to ATO, blood levels increased with exposure concentration for both exposure periods. This is not according to the data provided in table B-2!

From table B-2 and figure 3-1, one can read that the blood concentration in mice are dose dependent, but remain constant over the whole treatment period. The small variations between the different sample days are clearly due to biological variations and the variability of the analytical measurement and not dependent on duration of exposure.
Data provided in the NTP 20017a study*, also show that mice accumulate more Sb in the lung tissue compared to rats (Table 1).

(* within this draft document the NTP TR590 study is referred to the Peer Review draft version of 2016c and to the Draft version of December 2017 (= 2017a); somewhat confusing ☹ , maybe use only the latest version one for clarity ?)

Table 1 - ATO Lung tissue burden in mice and rats (From table G8/ G3 - NTP 2017a)

	µg Sb/g lung	Female mice	Female rats
3mg/m3	Day 61	561 ± 12	437± 14
	Day 124	683 ± 59	689 ± 49
	Day 271	802 ± 22	838 ± 41
	Day 369	979 ± 54	765± 179
	Day 551	1,472 ± 116	978 ± 86
10mg/m3	Day 61	1,233 ± 42	1,203± 52
	Day 124	1,476 ± 33	1,571 ± 59
	Day 271	2,678 ±135	1,983 ± 92
	Day 369	3,798 ± 232	1,976 ± 93
	Day 551	4,188 ± 609	1,801 ± 278
30 mg/m3	Day 61	2,687± 56	2,895 ± 87
	Day 124	2,954 ± 95	3,751 ± 145
	Day 271	4,133 ± 667	4,610 ± 363
	Day 369	3,892 ± 248	4,256 ± 345
	Day 551	8,398 ± 609	3,262 ± 535

From all these data it is clear that there is a differential profile between mice and rats in terms of ATO disposition and toxicokinetics. In the rat ATO (Sb) absorption is dose dependent and increases with exposure duration.
Similar observations were made in the (NTP study- 1992[2]), where mice blood levels were below detection in contrast to the rats' blood levels upon oral exposure.
We can therefore, given this information, conclude that only one toxicological profile (rats) can be a model for human toxicity but not both.

Species specificity of Sb blood levels is also referred to on p 33 (eg : …in rabbits and dogs were less than 1% of those in rats (Tylenda and Fowler 2015))

To our opinion this section on toxicokinetics does not cover the complete actual knowledge on the topic. More detailed information is available within the peer reviewed literature (also from human

[2] *Dieter, M. P. (1992). Toxicity studies of antimony potassium tartrate in F344 / N Rats and B6C3F 1 Mice (Drinking Water and intraperitoneal injection studies) National Toxicology Program.*

studies) allowing to get a better insight in the distribution, metabolisation, long-term storage and excretion profile of both SbV and SbIII compounds.

4. Human Cancer Studies

As can be deduced from the Monograph and supported by our own findings (digging in the literature) good quality relevant human (epidemiological) studies are scarce. However, available studies do not allow to assign observed effects to defined Sb exposure levels, and to correlate exposure to ATO with any human cancer.

Exposure levels for the general population never have been high and are since decreasing with time (ATDSTR 2017[3]), and information on single element (Sb) exposure virtually nonexistent.
As in the early days, working without personal protection, nor adequate technology, when ATO exposure was high in mining and process industry, it was impossible to prove that ATO inhalation is a potential source of carcinogenicity, one can hardly expect to find evidence now, when less people are involved and working conditions are rightfully overly conservative to prevent exposure.

The lack of evidence and general controversy around exposure to ATO in publications might also be an indication that there is no overt cancer risk upon ATO (antimony) exposure.

5. Studies of Cancer in Experimental Animals

The 4 studies selected for evaluation are solely inhalation studies on rats, of which only one study also used mice.

As stated above, the toxicokinetic ATO (Sb) profile of rats and mice is different. The profile of rats is closer to the human toxicokinetics, urging to be very cautious to use mice data to predict human toxic effects.

Out of the 4 studies, two studies suffered from technical flaws. In the study of Groth et al (1986) the ATO was contaminated with arsenic and lead, and results are thereby irrelevant for the assessment of ATO. The study of Watt (1993) was performed with only 10 females per group and therefore can merely be used as supportive evidence. It is not enough to state that As and Pb did not add to the observed effects, but it has to be shown, for example with experiments without As and Pb (p64). So this way combined effects could be excluded.

Thereby this evaluation of the potential carcinogenic properties of ATO is based on only 2 studies of which one did not demonstrate any signs of carcinogenicity. In the study of Newton et al (1994) in either sexes, no tumor incidence was observed at the highest exposure dose of (4,5 mg/m3).

Only in the NTP study, using a dose range causing lung overload at the 2 highest dose ranges (10 mg/m3 and 30mg/m3) in the rat, a condition that is not relevant for humans[4], a number of adverse

[3] ATSDR (Agency for Toxic Substances and Diseases Registry). (2017). Toxicological PRofile For Antimony and Compounds. *Agency for Toxic Substances and Disases Registry U.S. Pubblic Health Service*, (draft-april), 136. https://doi.org/10.3109/15569529909037564

effects could be triggered. **Even when taking these high doses into account, there was no clear evidence that ATO could induce carcinogenesis in the rats. Leaving, the sole 'evidence' that ATO could have carcinogenic properties to the assessment of mice data.**

From the raw data in the NTP 2017a study (and also from the NTP 1992[5] study, assessing antimony potassium tartrate in drinking and by intraperitoneal injection in rats and mice) it is clear that the absorption rate of antimony in the mice is extremely low compared to rats (and human) and that there is no evidence of Sb accumulation in the blood with time of exposure contrary to the rats (and human). Moreover, in the mice the accumulation of ATO in the lung tissue increases more rapidly compared to the rats, resulting in a higher local tissue burden (Table 1) and reaching more quickly the lung overload stage, which is again not relevant for the human situation.

In the assessment of NTP, and followed by the reviews for RoC, the difference and discrepancy between rats and mice is not taken into account. They assigned the same criteria for increased clearance half-live to the mice (p63), although they do not fit to the raw data generated in the study. The unjustified conclusions in the mice (lung alveolar/bronchiolar carcinoma at 3mg/m3 in male and female mice), being most probably due to lung overload, triggered also the assignment of a higher level of importance to the observed non-neoplastic effects in the rats for the classification.
This interpretation is not defensible and should not be taken into account to support the RoC listing criteria.
As far as for the pheochromocytoma's observed, those are known to be stress induced and cannot be considered to be a direct effect of ATO, and these cannot be taken into account to support the RoC listing criteria either.

The incidences of malignant lymphoma, in mice increased the historical controls at the overload doses of 10 and 30mg/m3. It is known that mice are sensitive to develop lymphomas and that the variability between the control groups is high (Graeves (2008)[6]. As also stated in the draft RoC review, in the Newton study, no non-lung neoplasms were observed.

Synthesis

In contradiction to the synthesis stated on p 67, the evidence to assign a carcinogenic potential from inhalation to ATO in experimental animals is weak.

From the 4 studies presented in the Monograph, one study (Groth et al 1986) is not valid for the assessment of ATO, as it was contaminated with arsenic and lead. One study (Watt (1993), which detected lung cancer, was only performed on a small number of female rats and suffered from some technical shortcomings (particle size, limited dose range, 1 year exposure…) .

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[4] ECETOC. (2013). *Poorly Soluble Particles / Lung Overload, Technical Report No. 122; http://www.ecetoc.org/technical-reports*. https://doi.org/ISSN-2079-1526-122

[5] Dieter, M. P. (1992). *Toxicity studies of antimony potassium tartrate in F344 / N Rats and B6C3F 1 Mice. (Drinking Water and intraperitoneal injection studies) National Toxicology Program*. NTP tox 11 NIH publication No. 92-3130.

[6] Greaves P (ed) Histopathology of Preclinical Toxicity Studies, Fourth Edition: Interpretation and Relevance in Drug Safety Evaluation. Elsevier 2008.

In the Newton study (1994), no tumors were detected, however the dose range did not reach the maximum tolerated dose.

The recent inhalation NTP study (2017), was performed according to high standards. For the rats they concluded: some evidence upon inclusion of pheochromocytoma in the adrenal, which is not human relevant, and needs to be excluded, weakening this conclusion even more.

The effects observed in the mice are questionable, due to the fact that the ATO absorption and distribution profile in the lung differs substantially form the mechanism observed in the rats and might not be relevant for human. Also it does not support he weak observations in the rats. **Conclusion: there is at the moment not sufficient evidence to classify ATO as a potential human carcinogen upon inhalation and there is no evidence that ATO can induce cancer by systemic exposure**. Especially as it is known that in the rats, upon absorption of ATO, Sb (III) is taken up by the RBC, transported and accumulated with time to all tissues. In NTP 2017 study, in the highest 30mg/m3 dose the rat blood levels raised steadily form 44 at day 61 up to 232 µg/g blood at day 551 and no neoplastic lesions were observed in any of the organs.

6. Mechanistic and Other Relevant Data

In this section interesting and valuable information is reviewed, which can contribute to a better comprehension of the molecular pathways driving the biological processes initiated upon interaction of ATO with the tissue and its solutes within the cellular compartments.

However these studies are at the moment still mainly investigative in nature, and are not ready to take a pivotal role in the assessment of complex in vivo process.

Moreover these in vitro studies are in vitro studies are performed with antimony compounds other than ATO. Knowing that the investigated antimony compounds are barely soluble with an unknown stability of the solutes in the culture medium, making it difficult to define the real concentration effect relationship and its relevance to explain the in vivo processes of ATO.

Unless this chapter is clearly marked as a review exercise rather than a contribution to the cancer assessment, it has no value in the Monograph.

7. Evidence Integration and Preliminary Listing Recommendation

According to our analysis of the presented data in this Peer-Review Draft RoC Monograph on Antimony Trioxide.

1. There is no evidence that ATO is a human carcinogen upon inhalation.

2. Only one high quality study (NTP 2017a), long-term inhalation carcinogenesis study evaluating both sexes of two species, mice and rat.

   In the rat (male and female):

At the two highest exposure concentrations of 10 and 30 mg/m3, lung overload is reached. A condition that is not relevant for human (ECETOC[7]).

At 3mg/m3: there is a low incidence in lung lesions (Alveolar/bronchiolar adenoma or carcinoma), within the historical control range of NTP.

The mice already experience lung overload at the low dose of 3mg/m3, moreover in the mouse pulmonary adenomas and carcinomas do not resemble the common lung tumors in humans (Greaves, 2008) and the mouse malignant lymphomas are spontaneous tumors. Particularly mice are influenced by various factors , which makes human risk assessment very difficult (Greaves, 2008 [8])

Based on this information there is no sufficient evidence to consider ATO inhalation as source of human cancer.

IV. Campine info

1. Workplace and health effects

Campine has monitored and collected its own workers health effects. We can state that no adverse effects by ATO have been logged during all the years of operation (more than 100 years).

We have collected the data, in line with the Belgian legislation and under supervision of IDEWE, an official expert in health and toxicology on the workplace. IDEWE has recently made a thorough screening of the data and drawn conclusions to them. The report is added to this comment.

The following conclusions have been made:

- Relationship between changes in pulmonary function parameters and years of exposure were weak
- There were no clear relationships between mean urinary antimony concentration and
    - changes in pulmonary function parameters
    - liver function
- On the basis of the existing information of chest X-ray's (on a yearly basis for more than 20 years) no pulmonary lesions were detected.

Campine is preparing a publication with these data.

2. Published report on air quality near the Campine production site (Belgium).

Campine is declared a Seveso Company, producing heavy metals as Lead, Zinc, Antimony,… The authorities therefore monitor our direct air quality in order to safeguard residential areas. The

[7] ECETOC. (2013). *Poorly Soluble Particles / Lung Overload, Technical Report No. 122; http://www.ecetoc.org/technical-reports*. https://doi.org/ISSN-2079-1526-122

[8] Greaves P (ed) Histopathology of Preclinical Toxicity Studies, Fourth Edition: Interpretation and Relevance in Drug Safety Evaluation. Elsevier 2008.

report from the VMM (Vlaamse Milieu Maatschappij = the Flemish Environment Authority) shows the following table for 2012:

Tabel 4: Jaargemiddelde zware metalen in PM_{10}-stof in 2012 (uitgedrukt in ng/m³)

ng/m³		arseen As	cadmium Cd	chroom Cr	koper Cu	mangaan Mn	nikkel Ni	lood Pb	antimoon Sb	zink Zn
00BE01	Absheide	6	9	8	143	22	10	383	20	441
00BE02	L. Kwikstraat	0,7	0,7	2	11	7	3	55	80	33
00BE07	Heidestraat	6	6	5	93	16	8	291	20	265
00BE15	Hoevestraat	3	4	5	69	10	4	144	11	197

De gemeten waarden respecteren op alle meetplaatsen:

- de EU-grenswaarde van 500 ng/m³ voor lood;
- de VLAREM II-grenswaarde van 30 ng/m³ voor cadmium;
- de EU-streefwaarde voor nikkel van 20 ng/m³;
- de WGO-advieswaarde voor mangaan van 150 ng/m³.

Translation:

On all monitored locations the measured values were below the regulatory limit

- EU norm 500 ng/m³ for lead
- The Flemish norm of 30 ng/m³ for cadmium
- EU norm for Nikkel of 20 ng/m³
- De WHO advise value of manganese of 150 ng/m³

Point 00BE02 is relevant for Campine emissions, downwind, near a residential area, located at less than 1 km from the emission points. Antimony (Antimoon Sb) equals 80 ng/m³

V. Campine conclusions

Having screened the Monograph with caution and looking to all proof of evidence we conclude that on the basis of the current knowledge and the information put forward in the Monograph and additional literature, there is no reason to conclude that ATO would be a potential human carcinogen in general, and not even by inhalation only.

Even if the experts would conclude otherwise we would urge the committee to wait until Campine can give more leverage to its opinion by the publication of the results of the monitoring of the health effects of its workers over time. Campine also wants to be cooperative for all further investigations and is open to share any information.

In this respect, even if there are deadlines defined in the ROC evaluation program, Campine urges the committee to allow Campine sufficient time to submit the official publication of the monitoring of the health effects of its workers.

We are convinced that Campine data are very valuable for the completeness of the research done by ROC, through the fact that they are the only “recent dataset” for human exposure, generated over a substantial length of time.

For reflection

Statement from *McCallum (2005)*[9]

Antimony has been studied for more than a century, processed by workers in unprotected conditions and been injected in humans at high doses for medical applications. In UK, in the sixties there was the suspicion that antimony might induce lung cancer. Ever since epidemiological surveys to monitor the prevalence were set up, but as of today there is insufficient evidence, either human or animal, to regard antimony as a major hazard. From that time antimony is handled with increasing care and the exposure has been dropped drastically. Recently, old claims for its anticancer action revived and are again under investigation.

Ghent, January 10th, 2018

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[9] McCallum, R. I. (2005). Occupational exposure to antimony compounds. *J Envirmon. Monit.*, *7* (May), 1245–1250.