California Proposition 65: Proposed Regulatory Amendment – Specific Regulatory Levels Posing No Significant Risk for Antimony Trioxide (ATO)

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1. Introduction

The Office of Environmental Health Hazard Assessment (OEHHA) is proposing to adopt a Proposition 65 (Prop. 65) No Significant Risk Level (NSRL) for antimony trioxide (ATO) by amending Title 27, California Code of Regulations, section 25705(b). The proposed NSRL for ATO is 0.13 mg/day. According to Prop. 65 if this NSRL is accepted a daily exposure to ATO at a level which does not exceed 0.13 mg/day shall be deemed to pose no significant risk.

Proposition 65 requires businesses with ten or more employees to provide warnings prior to exposing people to chemicals that are known to cause cancer. ATO is on this list due to being classified as a category 2A carcinogen by IARC.

This regulatory proposal provides a "safe harbor" value, with the aim of aiding businesses in determining whether a warning is required for a given exposure. The proposed NSRL, whilst aiming to provide compliance assistance to businesses subject to Prop. 65, does not impose any mandatory requirements on those businesses, and if adopted the use of the NSRL is not mandatory and business can calculate their own safe exposure level. However, resulting business-calculated levels may not be defensible in an enforcement action.

The International Antimony Association (i2a) have concerns over the methodology used in the calculation of the NSRL and why a NSRL is needed due to the limited exposure of ATO during normal use which have found to be associated with negligible risks to workers and consumers. These concerns are expanded on in Sections 2 and 3 below.

1.1   Who are i2a?

The International Antimony Association (i2a) is the Brussels-based organization representing the producers, importers, and users of multiple Antimony substances. i2a's aim is the sustainable and responsible production, use and recycling of Antimony.

Our vision is a sustainable and responsible Antimony industry, where Antimony substances continue to be the material of choice for many technology-enabling applications. At i2a we want to ensure the safe use and responsible management of Antimony substances throughout their lifecycle and to inspire positive product stewardship throughout the Antimony value chain, from classification to sustainability profiling.

i2a aims to:

- Address knowledge gaps weakening the safe use and positive image of Antimony substances;
- Make sure that the most up to date evidence is used in any regulatory scrutiny of Antimony substances;
- Determine the most relevant, proportionate, and efficient risk management measures applicable to each use of Antimony substances; and
- Support the Antimony industry in implementing responsible product stewardship practices

2. Calculation of NSRL

The i2a takes note that the proposal for a No Significant Risk Level (NSRL) safe harbor for antimony trioxide (ATO) has been prompted by inhalation studies conducted by the National Toxicology Program (NTP, 2017). Inhalation exposure of rats and mice to ATO aerosol concentrations of 5, 10 or 30 mg/m^{3}resulted in the formation of lung tumors in rats and mice. Other lesions observed include lymphomas in mice and pheochromocytomas in rats. Lung tumor and lymphoma formation in female mice was judged by California's Office of Environmental Health Hazard Assessment (OEHHA) to be the most sensitive cancer endpoints and to provide the most appropriate information for NSRL derivation. Lymphoma incidence in female mice was summed with lung tumor induction even though the lymphomas observed were a seeming reactionary response to lung tumors and hypoxia. The induced lymphomas were of a B cell origin whereas chemically induced lymphomas usually exhibit a T cell origin. (Ward et al.,). Pooling of lung cancer and lymphoma data to generate a dose response function for tumor development is thus of questionable validity but benefits NSRL derivation since dose dependence for lung tumor induction is less than robust and the pooling of lesions at different sites confers dose dependence that facilitates derivation of a NSRL. If there is an etiological linkage assumed between lymphoma and lung tumor formation it should be stated. In the absence of such a linkage, NSRL derivation should be indexed to lung tumor formation only since it is the primary lesion for which a plausible mode of action can be proposed. i2a concurs that the inhalation studies of NTP are the most technically sound cancer bioassays available for definition of ATO's carcinogenic potential. The i2a further acknowledges that the method of NSRL derivation employed is potentially appropriate for a direct-acting genotoxic carcinogen. However, i2a submits that the procedures used to derive the NSRL are predicated upon mode of action assumptions that are not applicable to ATO which has usually been judged to act via indirect mechanisms.

Genotoxicity and carcinogenicity studies of antimony compounds, including ATO, were recently reviewed (Boreiko and Rossman, 2020) and it was noted that antimony compounds are weak *in vitro* genotoxins that do not exert genotoxicity *in vivo* and lack the ability to undergo covalent interactions with DNA. Although NTP asserts they conducted genotoxicity studies that indicated induction of genotoxic impacts *in vivo*. is a probable mode of action for carcinogenesis, the NTP studies were not conducted in compliance with OECD guidelines for genotoxicity testing. For example, The NTP Comet assay studies neglected to include controls for cytotoxicity, apoptosis or terminal differentiation even thought it was plainly evident that ATO inhalation produced pulmonary toxicity sufficient to induce systemic hypoxia. Failure to control for factors known to be the source of false positive test results removes biological significance from the weak positive assay responses observed by NTP. The background document "Initial Statement of Reasons: Antimony Trioxide: Proposition 65 Safe Harbors" proceeds (e.g. p. 4) to cite NTP's study for proof of a of mode of action that entails direct ATO-induced genotoxicity and that such genotoxic effects occur in humans. The i2a respectfully submits that ATO carcinogenicity is most likely mediated by a mode of action that entails effects such as the induction of reactive oxygen species, inhibition of DNA repair and/or alter cell differential. Such modes of action either entail nongenotoxic impacts or lead to genotoxicity via indirect mechanisms that that have a strong non-linear dose response that approximates a threshold. The mechanistic inferences normally made regarding ATO's mode of action are inconsistent with the mechanistic assumptions made by OEHHA in their cancer risk assessment modeling. Finally, the document appears to misunderstand and/or misquote aspects of EPA's Cancer Risk Assessment Guidelines. For example, an electrophilic nature is a characteristic of organic compounds that are

carcinogens since it is can be indicative of an ability to establish covalent interactions with DNA or other critical cell macromolecules. The application of principles of organic chemistry to inorganic substances should be undertaken with caution. In the specific case of metals and metalloid elements, many will be electrophilic (i.e. they will form cations in aqueous environments) but most lack an ability to form stable covalent linkages with organic molecules. Simple methylation is generally the most complex interaction that occurs. While it is true that there may be multiple mechanisms of action for ATO-induced lung tumor formation, the mechanism assumed in the OEHHA risk assessment (direct genotoxicity) is the one for which supportive data is the least compelling.

On page 5 the background documentation goes on to assert that "*there are no specific mechanistic data to suggest any deviation from the standard assumptions, including low dose linearity, . . .*" thus ignoring the large body of data generated over the past several decades indicating indirect mechanisms of action are the likely mediators of ATO carcinogenicity. Indirect modes of action, including ROS induction or impacts upon DNA repair, have been consistently suggested to provide the most probable modes of action (Boreiko and Rossman, 2020; Boreiko et al., 2021) and are acknowledged by the NSRL documentation as playing a potential role in the process of carcinogenesis induced by ATO. If the derivation of an NSRL is to be conducted assuming low dose linearity and other features characteristic of direct-acting genotoxic carcinogen it should at least be acknowledged that the risk assessment is very conservative and assumes a mode of action that yields the lowest levels of permissible Safe Harbor exposure. The process of risk assessment is best served when the critical mechanistic assumptions being made are clearly stated, a rationale given for conservative assumptions made and acknowledgement given to alternative modes of action commonly discussed in the peer-reviewed literature that would lead to different quantitative estimates of risk. Justifying risk assessment modeling decisions by mischaracterization or misunderstanding of the available peer-reviewed scientific literature results in a process lacking in scientific transparency and technical rigor.

Setting aside OEHHA's failure to adopt the mode of action assumptions put forward in the peer-reviewed literature, the NSRL derivation makes multiple assumptions regarding exposure that are overly simplistic to the point of being misleading. ATO is a poorly soluble compound that deposits in the lung in accordance with basic, well-established principles of aerosol physics and inhalation toxicology. Key parameters determining inhaled particle deposition rates and localization include the animal species, particle Mean Mass Aerodynamic Diameter (MMAD), particle size distribution and particle density. Accurate predictors of particle deposition have been developed in accordance with the principles established by the International Council on Radiological Protection (ICRP) and incorporated into computer simulations of inhaled particle deposition such as the Multi-Path Particle Deposition (MPPDep; ARA;) model. Although there have been significant advances in the development of such predictive models, neither the computer models nor the basic aerodynamic factors that govern particle deposition have been used by OEHHA to estimate the ATO lung burdens associated with carcinogenic impacts.

The deposition pattern of ATO in the lung determines the alterations that may occur as a result of the direct "local exposure" of pulmonary tissues as opposed to deep tissue systemic exposures mediated by blood borne Sb. The ATO exposure experienced by lung tissues will vary as a function of aerosol properties that dictate particle deposition patterns and the surface area of the lung tissues that will be impacted by inhaled materials. Expression of an NSRL based upon a dose that is indexed to animal body weight will provide at best be an imprecise surrogate for the intensity of pulmonary exposure that yields carcinogenic effects. Moreover, the inhalation studies of ATO conducted by NTP utilized experimentally generated aerosols with a respirable size distribution (1.1 +/- 1.8 mm) that yields deep lung penetration to the alveolar tissues that give rise to tumors. Clearance mechanisms for such

particles are limited to processes such as macrophage mediated clearance or particle dissolution. As a result, the inhaled dose that gives rise to tumors is not the daily deposition rate but rather the cumulative lung burden that develops over the course of months or years. Upper airway deposition will also occur, particles so deposited being rapidly cleared to the gastrointestinal tract via mucociliary clearance. This translocated material will be available for uptake and systemic distribution with an uptake efficiency of 1% or less (ATSDR, 2019). The methodology used in the NSRL derivation provides no information on the patterns of particle deposition within the lung which are key to predicting effects. Nor is there consideration given to the differential rates of uptake that will result from deposition in different regions of the respiratory tract. As a result, the exposure estimates used in NSRL derivation are unlikely to have any relationship to the exposure pathways and exposure levels that yield effects. As an illustration, the NSRL derivation assumes impacts from 30 mg/m^3 ATO and a daily deposition rate estimated to be 6.21 mg/kg bw/d or about 0.25 mg/day per animal (assuming a body weight of 0.04 kg). The measured values of lung Sb observed by NTP were 2.7 g/kg of lung tissue on day 61 and 8.4 g/kg lung tissue on day 551 for a lung Sb content between 1 and 5 mg per lung. The lung Sb burden associated with tumors by this measure of dosimetry is up to 200-fold higher than the estimated doses that yield effects used by OEHHA for NSRL derivation. Accordingly, dosimetric extrapolation from mice to humans should include comparative estimates of the particle deposition and clearance rates in the lungs of mice and humans. Real world exposures to ATO have further been characterized with respect to the particle size distribution of aerosols in the occupational environment (Vetter, et. al., 2018). The mean MMAD of aerosols in ATO production facilities is well within an inhalable size range and lacking a significant respirable fraction capable of deep lung penetration and deposition. An NSRL that is based upon the effects of experimentally generated respirable aerosols will inflate estimates of carcinogenic effects due to the study of particle aerosols that are not representative of those encountered in real world exposure scenarios. The question asked should be "*what is the concentration of real-world aerosols that will yield a respirable fraction that is quantitatively similar to that which produces effects in mice from experimentally generated respirable aerosols?*" This dose estimate would be the more appropriate starting point for NSRL derivation.

The promulgation of an NSRL limit based upon pulmonary impacts will also be misleading if applied independent of specification the route of exposure for ATO. Whereas the deposition in the deep lung may be followed by high rates of uptake, oral exposures to Sb compounds yields rates of uptake on the order of 1% or less (ATSDR, 2019). Estimates of cancer risk from inhalation, as opposed to oral exposure, will be exaggerated on the simple basis of bioavailability. Moreover, carcinogenic and genotoxic impacts are not seen in most studies of oral ATO administration. Oral cancer bioassays have not been conducted with ATO or other Sb compounds – the conduct of cancer bioassays via oral exposure routes has not been feasible due to the gastrointestinal impacts associated with the emetic and laxative properties of Sb compounds.

3. Potential exposure of ATO

The major uses of ATO are in flame retardant polymers and textiles, where ATO acts as a synergist, or as a catalyst for polyethylene terephthalate (PET) manufacture. When used as a flame retardant synergist ATO is bound and remains in products as part of a polymer matrix, thus precluding any exposure of any toxicological significance.

In industrial settings ATO exposure is controlled. This is either via standard workplace hygiene measures such as closed system bulk transfer, the use of wetter powder suppressing dust generation, engineering controls such as

local exhaust ventilation, or the use of masterbatches. Masterbatches are when the flame retardant components, including ATO, are provided to manufacturers already dispersed in a polymer matrix, allowing addition to be controlled and dust-free, thus removing the inhalation pathway – the only realistic pathway for ATO exposure in an industrial setting.

The principal source of consumer exposure to ATO is from exposure to flame retardant-treated textiles, including upholstery, carpeting, and mattresses, and degradation of polyester textiles (NTP, 2018).

In consumer use settings ATO remains bound in the polymer matrix, however, there is very low exposure potential via the is the addition of antimony, particularly to household dust, that occurs as the polymers, such as those found in polyester textiles and flame-retardant fabrics and upholsteries, break down into particulates.

In 2020 Intertox, on behalf of i2a, produced a human health risk assessment for typical household dust with the focus on ATO. The findings of the modelled risk assessment showed that ATO does not represent a real and attributable risk to consumers by any toxicological endpoints that have been attributed to antimony / ATO exposure.

4   Conclusions

The i2a has reviewed OEHHA's derivation of a proposed safe harbor level for antimony trioxide and has identified multiple areas of concern. The NSRL proposal is indexed to the induction of lung tumors and lymphomas in mice after inhalation exposure to antimony trioxide. The document assumes a mode of action for ATO as a direct-acting genotoxic carcinogen despite the general scientific consensus that Sb genotoxicity and carcinogenicity progress via indirect mechanisms that likely have a threshold.an indirect mode of action should at least be acknowledged. Estimates of risk from inhalation are then generated but no efforts are made to apply widely accepted modeling procedures for estimating patterns of particle deposition and uptake. Instead, worst case general assumption are made to estimate the delivered dose associated with carcinogenic impacts. As a result, the NSRL calculation provided is imprecise and lacking in technical rigor. The document then proceeds to assume equivalency between doses administered by inhalation and by oral exposure even though Sb uptake by different exposure routes varies by several orders of magnitude. Such significant differences in route specific uptake efficiency requires the generation of NSRL estimates specific to the route of exposure of concern.

5   References

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