International Antimony Association

ANALYSIS: ATO, PSLTs and Lung Tumors

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1. General context and definition of PSLT

The carcinogenic hazard and risk posed by Poorly Soluble Low Toxicity (PSLT) particulate matter has been the subject of recent conjecture and debate.

Humans, and the experimental animals that serve as surrogates for humans in toxicology, have historically been presumed to share basic similarities with respect to the impact that may be associated with the inhalation of particulate materials. Thus, inhalation exposure of rodents to particulate matter has been assumed to yield pulmonary deposition patterns, clearance mechanisms, and effect profiles similar to that which will be observed in humans. This assumption is now recognized as an over-simplification that does not reflect important species-specific factors which modulate the potential impacts of particulate matter (Driscoll and Borm, 2020; Miller et al., 2014; Warheit et al., 2016)[1].

Interest in PSLT toxicology intensified with the observation that the deposition rate of particles in the pulmonary tract can exceed the capacity of mechanisms for particle clearance. As defined by an ECETOC Task Force (ECETOC, 2013) PSLTs are particles that have dissolution half-lives in artificial lung fluids (interstitial, lysosomal and alveolar) that are longer than macrophage-mediated clearance times. As a result, macrophage clearance is the primary mode of particle removal from the lung, imparting sensitivity to impairment of macrophage function, the accumulation of particles within the lung, and the development of pulmonary overload.

Although there has been a tendency to regard PSLTs as a single class of materials, they exhibit significant differences with respect to composition, surface reactivity, mass, volume and surface area.

2. Pulmonary overload and lung tumors

The onset of “pulmonary overload” can initiate a sequence of events that results in cytotoxicity and stimulates inflammatory responses. This includes histopathological alterations that yield adverse impacts upon lung function and structure. In rats, but probably not in mice or humans, the inflammatory response induced by PSLTs is further associated with the development of benign and malignant pulmonary neoplasms (most often in the alveolar or deep recesses of the lung). Pulmonary overload has thus been proposed as a basic mechanism by which lung neoplasms can be induced in the rat, by yet to be defined cellular and molecular processes, which may not be relevant to mice or humans (Warheit et al., 2016).

Although operational definitions have been proposed for the “poorly soluble” aspect of PSLTs, scientific consensus has not been reached for the definition of “low toxicity”. Pulmonary overload was first defined in studies of inert particles, the chemical constituents of which were presumed to have low inherent toxicity

[1] Species-specific differences can be observed in determinants of particle pulmonary deposition patterns (e.g. rats are obligate nose breathers whereas humans exhibit oro-nasal breathing). Other more nuanced species-specific differences reflect the cell population subtypes that mediate particle clearance kinetics, clearance pathways and responses to toxic insults within the lung.

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(Warheit et al., 2016). Impairment of macrophage function resulted from the physical mass and/or volume of phagocytized materials such as carbon black or titanium dioxide. Overload was not applicable to poorly soluble substances such as quartz which were known to produce significant cytotoxicity mediated by reactivity of the particle surface.

Although it can be presumed that there are gradations of toxicity between substances such as quartz and carbon black, and that at some point overload becomes the principle mechanism for particulate induced toxicity, a consensus definition of "low inherent toxicity" has yet to be developed. Lack of a consensus definition for low toxicity lends uncertainty as to which materials should be considered as PSLTs that produce effects via overload-related mechanisms. The following summary statements attempt to capture current scientific consensus regarding the mechanisms for, and toxicological significance of, pulmonary overload in the rat:

- **Pulmonary overload may trigger tumor development only in rats.** Pulmonary overload occurs in rats, mice and humans – but only rats have been observed to exhibit tumor development in response to pulmonary overload.

- **Cellular and molecular processes observed in rat tumors could be relevant for human.** Scientific consensus is lacking on whether or not rat neoplasms induced by pulmonary overload are relevant to humans. Occupational epidemiology studies have failed to observe a relationship between exposure to PSLTs and cancer of the lung in humans. However, the observed cellular and molecular events associated with overload-mediated cancer of the rat lung can be postulated as being relevant to humans. Agencies such as the International Agency for Research on Cancer have judged substances producing lung tumors via pulmonary overload to be possibly carcinogenic for humans (Cat. 2B).

- **Under current ECHA guidelines, extrapolating effects in rats to those that might occur in humans, makes the precautionary default assumption that humans are more sensitive than rats to the toxic and/or carcinogenic properties of substances.** However, rat sensitivity to overload seems to be greater than that of humans. Potential rodent vs human species differences in sensitivity to overload have not be rigorously evaluated, but rats may be more sensitive to overload induction (and the impacts of overload) than humans by a factor of about 40. The potential hypersensitivity of the rat to overload has not been considered in the assignment of assessment factors for the derivation of NOAELs, DNELs or OELs for PSLTs. Instead, default inter-species assessment factors (ranging from 3 to 10) are applied on the precautionary assumption that humans could be more sensitive to PSLT than rats.

- **Onset of overload seems to be driven by interrelated metrics.** Several different metrics have been applied to identify exposure levels associated with the onset of pulmonary overload, and reflect mechanistic uncertainty concerning factors that modulate rates of particle clearance. The onset of overload can be indexed to:
    - Total lung particulate burden (particle mass),
    - The volume of material sequestered within macrophages, and/or
    - The total surface area of phagocytized particulate matter.

  Exposure metrics such as these are not independent of each other, complicating efforts to identify the most critical determinants for the onset of overload.

- **Nature of particles could be an important factor to trigger onset of pulmonary overload.** Initial studies of overload focused upon the impacts of inert particles (e.g. carbon black, titanium dioxide), and assumed that the chemical composition of particulate matter was *not* a critical factor governing the onset of pulmonary overload. More recent study of PSLTs has acknowledged that compound-specific toxicity can occur and may even modulate aspects of overload onset (and potentially overload consequences). However, there is no scientific consensus as to the nature or extent of the "low toxicity" that can be associated with overload induction by a PSLT.

3. Pulmonary overload and lung tumors mediated by ATO

Inhalation studies conducted with ATO observed that ATO: 1) accumulated in the lung of the rat to an extent sufficient to achieve overload; 2) inhibited particle clearance; and 3) could produce lung tumors. These initial observations were consistent with ATO-induced lung tumors being produced as a result of pulmonary overload and the relevance of ATO-induced tumor formation in the rat for humans was uncertain. However, inconsistencies subsequently emerged as further research was conducted.

- Modest inhibition of ATO particle clearance was reported at ATO exposure levels that did not produce overload. This suggested that ATO exerted toxicity towards macrophages and that inhibition of clearance was not a non-specific effect mediated solely by particle overload.

- Although PSLTs are now acknowledged to potentially exert some chemical-specific toxicity, there is no consensus regarding the extent and nature of toxicity that can be exerted while maintaining consistency with the non-specific impacts of particle overload. Transient granulocytic inflammation is to be expected as a prelude to overload, but operational definitions for permitted impacts upon clearance have not been agreed.

- In the absence of agreed upon criteria for the toxicity limits of PSLTs, non-specific induction of pulmonary overload by ATO particles may not be accepted as the mechanism for ATO-induced lung tumor formation in rats.

- The relevance of overload to other Sb compounds is also uncertain. *In vitro* flow-through bioelution studies using fluids present within the lung or within macrophages might be predictive of pulmonary retention and/or Sb release from substances such as antimony metal powder or antimony trisulfide. However, these bioelution test systems are still being validated for the prediction of *in vivo* particle dissolution.

- Solubility in water or cell culture medium may be a surrogate for pulmonary fluids and suggests that ATO may be the least soluble Sb(III) compound under REACH evaluation. Sb substances with faster rates of dissolution may be less likely to accumulate in the lung and produce pulmonary overload and more likely to produce toxicity by Sb release. If particle retention is significantly reduced by dissolution, other Sb substances are unlikely to be classified as PSLTs.

- Tumors have been produced by inhalation exposure of mice to ATO. Overload is not known to produce tumors in mice. The induction of lung tumors in mice suggests that ATO can induce mouse lung tumors via a mechanism that does not entail pulmonary overload.

- Lung tumor induction by ATO in mice does not preclude a role of overload in rat lung tumor formation, but acceptance of overload as the primary mechanism for rat lung impacts is called into question by responses in the mouse lung.

4. Conclusions and next steps

Scientific consensus has not been reached on whether pulmonary overload poses carcinogenic risk solely for the rat – additional research is required to determine if overload can create cancer risk for humans. Given this uncertainty, the following summary conclusions can be drawn with respect to ATO and particle overload:

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- ATO will induce both pulmonary overload and lung tumors in rats. However, ATO particle clearance is modestly inhibited by inhalation exposure levels that do not produce pulmonary overload or tumors. This suggests there is ATO-specific toxicity that impairs particle clearance at ATO exposure levels, that do not produce overload.

- The extent and nature of low toxicity that can be possessed by a PSLT (while still inducing effects via a pulmonary overload mechanism) remain to be defined. Current models for overload induction and carcinogenic effects are not sufficiently well-developed to accommodate compound-specific toxicity within the overall framework of PSLT induced pulmonary overload. Ideally, **as PSLTs are better defined, it will be possible to determine whether the low level of pulmonary toxicity induced by ATO is indicative of a mechanism for carcinogenesis independent of pulmonary overload.**

- Until such time as overload impacts in the rat can be related to the presence or absence of cancer risk for humans, **a portion of the scientific community will assume that lung cancer induction in rodents** after inhalation exposure to ATO is, on a precautionary basis, **indicative of potential cancer risk for humans**.

- ATO induced pulmonary tumors in mice, a species where PSLTs will induce pulmonary overload, but in which overload only is not sufficient for the induction of lung tumors. The induction of mouse lung tumors by ATO inhalation suggest **there are mechanistic pathways for ATO carcinogenesis that do not entail the induction of pulmonary overload**. Unless the induction of mouse lung tumors can be shown to entail mechanisms unique to the mouse, rat lung tumors will be presumed to arise via processes separate from, or in addition to, pulmonary overload.

- Based upon both *in vivo* and *in vitro* studies**, genotoxicity** resulting from ATO exposure (be it from direct or indirect mechanisms) **is likely to be proposed as responsible for lung tumor induction in both rats and mice.** Delineation of the role of genotoxicity in lung tumor induction will be critical for appropriate classification and risk assessment of ATO. In particular, any future in vivo studies should be designed as such that:

    - A sufficiently large range of exposures is covered, including exposures that do not produce overload, and high exposure concentration(s) producing a 2-3-fold prolongation of the retention time associated with overload (i.e. a clearance half-time of approximately 180-240 days).
    - The study includes the assessment of the macrophage function, lung histopathology, and a lung lavage analysis.
    - The possible translocation of ATO to the interstitial space, and the subsequent endocytosis by non-macrophage cells is assessed.
    - Relevant supplemental mechanistic information studies are included in the study design (e.g. micronucleus test, COMET assay).

Based on the above statements, many will judge that ATO will currently not meet the criteria to be considered as a PSLT substance inducing carcinogenic effects via pulmonary overload. The appropriate carcinogenicity classification and risk assessment of ATO will instead rest on clarifications brought through investigations on the compound-specific (geno)toxicity of ATO.

Cited Literature

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