ISSUES RELATED TO THE APPLICATION OF THE GHS STOT CRITERIA TO INHALED POORLY SOLUBLE PARTICULATES OF LOW TOXICITY

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Introduction

The UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS) covers a range of hazard classes related to the physico-chemical, toxicological and environmental properties of chemical substances and mixtures. GHS includes hazard classifications addressing Specific Target Organ Toxicity (STOT) following acute ("STOT-SE") or repeated exposure ("STOT-RE"). With regard to STOT-RE, it describes for different routes of exposure the type of effects and distinguishes between Category 1 and Category 2 STOT-RE hazard classes based on cut-off exposure levels at which effects were observed in animal studies.

Concerns have been raised regarding the suitability of current cut-off exposure levels when applied to the repeated inhalation effects of poorly soluble particles of low toxicity (PSP). Moreover, the lack of detailed guidance for the application of the criteria has the potential for different jurisdictions to interpret and apply the criteria differently.

This investigation illustrates on the basis of two published subchronic particulate inhalation studies the type of effects observed with poorly soluble particles of low toxicity and the issues related to the application of the STOT-RE criteria to these particles. It identifies areas where the existing STOT-RE classification criteria could be further clarified and strengthened within the current framework to ensure harmonized application of the GHS criteria and to avoid inappropriate onerous classifications as STOT-RE of poorly soluble particulates that are considered to be of low toxicity.

GHS criteria for the classification of particles for STOT-RE

GHS distinguishes two hazard classes for Specific Target Organ Toxicity following repeated exposure (STOT-RE):

- **Category 1**: Substances that have produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in humans following repeated exposure;
- **Category 2**: Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure.

Effects of relevance to particle inhalation studies considered to support classification

- Significant organ damage that may be noted at necropsy;
- Multifocal or diffuse necrosis, fibrosis, granuloma formation in vital organs with regenerative capacity;
- Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction;

Table 1: Guidance values to assist in Category 1 or 2 classifications

Study Type	Species	Unit	Category 1		Category 2	
			90d	28d	90d	28d
Inhalation dust/mist/ fume	Rat	mg/l/6h/d	≤0.02	≤0.06	≤0.2	≤0.6
		mg/m³/6h/d	≤ 20	≤ 60	≤ 200	≤ 600

Guiding principles for STOT-RE classifications

- Expert judgment on the basis of the weight of all evidence;
- Use of weight of evidence of **all** data, including human incidents, occupational or environmental epidemiology, and studies conducted in experimental animals (i.e., 28-day, 90-day or lifetime) tapping into the considerable body of industrial toxicology data collected over the years;
- A key consideration for classification of a substance on the basis of animal data is not only the type of effect that has been produced, but also at what concentrations they were produced and how relevant these effects are for humans.

CASE STUDIES

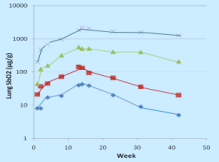
Subchronic inhalation of Diantimony Trioxide particulates in rats

- **Study conducted by Newton *et al.* (1994)**

Male and female rats (50/sex) were exposed to diantimony trioxide (Sb_2O_3) for 6 hrs/day, 5 days/week for 13 weeks via whole body inhalation at target exposure of 0, 0.25, 1, 5 or 25 mg/m³ Sb_2O_3. Satellite groups were satellite groups of 5 animals/sex were examined up to 27 weeks after termination of exposure.

Results

- ***Bodyweight***. Significantly reduced body weight at the two highest exposure groups during exposure and most of the recovery period;
- ***Absolute lung weights***. Mean absolute and relative lung weights were increased in the 5 and 25 mg/m³ Sb_2O_3 exposure groups. While the lung weights in the 5 mg/m³ group appeared to return to normal during 27 week post-exposure period, the 25 mg/m³ group values remained elevated.
- ***Gross and microscopic pathology***. Chronic interstitial inflammation and interstitial fibrosis were seen in the lungs of control and treated animals. Incidences of control and exposure groups II, III, IV were comparable. Granulomatous inflammation in the lungs was most frequent in the 25 mg/m³ group during observation period. Alveolar macrophages were more numerous in the lungs of treated animals than in controls.
- ***Sb_2O_3 tissue levels and pulmonary clearance rates***.



- No indication of lungs reaching a steady state lung burden level of Sb_2O_3;
- Observation of a lung-burden dependent effects on Sb_2O_3 clearance;
- At the highest Sb_2O_3 lung burden levels, pulmonary clearance was significantly decreased suggesting impairment of clearance mechanisms.

Important considerations for a STOT-RE classification review

- High background levels of interstitial inflammation and fibrosis in rats;
- Among the list of possible effects triggering a STOT classification, only granulomatous inflammation appeared to be treatment-specific. It is of note that this effect was not observed in two further investigations of the inhalation toxicity of Sb_2O_3 in rats;
- Calculation of alveolar deposition in rats was estimated to be 2 times that of workers considering particle size distribution of Sb_2O_3 workplace aerosols & differences in breathing rates.

Subchronic inhalation of carbon black in rats, mice and hamsters

- **Study conducted by Elder *et al.* (2005)**

Female rats, mice and hamsters were exposed for 6 hrs/day, 5 days/week for 13 weeks to 0, 1, 7 or 50 mg/m³ carbon black (CB). Additional recovery groups were held for post-exposure periods of 3 and 11 months. Body weight, lung weight, carbon black lung burdens, lung pathology as well as cellular and biochemical parameters (i.e., total BAL cell numbers) in lung lavage fluid were recorded.

Results

- ***Bodyweight***. Significantly reduced body weight at the end of exposure only in hamsters which returned to normal at the end of observation period.
- ***Absolute lung weights***. Lung weights were increased in high-dose carbon black exposure animals, but persist only in rats and mice until the end of study period.
- ***Lung pathology & cellular markers in lavage fluid***. Interstitial fibrosis present in carbon black - exposed rats at the mid and high dose. Responses seen in mice and rats were similar in character to those in rats, but the pulmonary inflammation and histopathological changes were more severe and persistent in rats. No adverse inflammatory changes were observed at the lowest dose in any of the 3 species.

Carbon black particle retention kinetics. Particle retention was achieved in rats and mice for the mid- and high dose CB and in hamsters for high dose; mice cleared particles at highest dose whereas the rats didn't show clearance at the highest dose.

Table 2: Retention half times for following 13weeks of exposure to carbon black

	Rats (days)	Mice (days)	Hamsters (days)
1 mg CB/m³	64	133	42
7 mg CB/m³	115	343	53
50 mg CB/m³	No clearance	322	309

Important considerations for a STOT-RE classification review

- Other existing studies also indicated that CB caused particle overload in the lungs associated with chronic inflammation and epithelial hyperplasia; lung tumors were induced in rats, but not in mice or hamsters;
- Results of the Elder study confirms previous findings that the rat is the most sensitive of the 3 species with respect to PSP-induced adverse effects in the respiratory tract; retention was longest, pulmonary inflammation and histopathological changes were more severe & persistent than in rats and hamsters;
- Epidemiological data on worker exposure to carbon black neither provide adequate evidence that workplace exposure to carbon black is carcinogenic nor that it causes inflammatory (including fibrotic) conditions.

Discussion

- Effects observed in the case studies show similar pulmonary effects as reported in animal inhalation studies of other PSP's of low toxicity like talc, toner or coal particles;
- At exposure levels similar or lower than STOT-RE cut-off levels, PSP caused impaired particle clearance, induced lung inflammation and histopathological changes and increases of alveolar and intra-alveolar particle laden macrophages in rats;
- There is consensus within the scientific community that the rat is more sensitive to exposure to PSP than other rodents (ILSI, 2000);
- A key consideration in this context is the relevance of particle overload-induced effects in the rats to humans: Reviews on specific PSP's (i.e., TiO_2) concluded, that overloaded lungs and reduced clearance rates in humans are not associated with the type of inflammatory responses seen in the rat lung (Hext *et al.*, 2005) ;
- In establishing cut-off levels on the basis of animal studies, it is of critical importance to establish comparable exposure levels considering particle size distribution and species differences in deposition & breathing pattern.

Conclusion

- ❑ Subchronic/chronic exposure to PSP concentrations at or below the STOT-RE cut-off levels can lead to lung overload induced inflammation responses of questionable human relevance;
- ❑ Lack of consideration of the rats unique sensitivity to PSP exposure and dosimetric differences between rat experiment and worker exposure will predominantly lead to STOT-RE classifications of inert particulates without distinguishing between chemical-specific and particle-induced toxicity;
- ❑ STOT-RE classification guidance criteria should be refined to take account of these issues to ensure a harmonized implementation of the STOT-RE hazard to inhaled particles.

References

Elder, A. *et al.*, 2005. Toxicological Sciences 88(2), 614-629
Hext, P.M. et al., 2005. Ann. Occup. Hyg. 49(6), 461-472
ILSI, 2000. Inhalation Toxicology 12, 1-17
Newton, P.E. *et al.*, 1994. Fundamental and Applied Toxicology 22, 561-576

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