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REVIEW ARTICLE

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Expert workshop on the hazards and risks of poorly soluble low toxicity particles

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ABSTRACT

'Lung particle overload' refers to the impaired lung particle clearance and increased particle retention occurring with high lung doses of poorly soluble low toxicity (PSLT) particles. In rats, lung particle overload is associated with inflammation, epithelial hyperplasia, and, in extreme cases, lung cancer. While the human relevance of rat lung tumors occurring under overload has been questioned, recent regulatory decisions have considered these outcomes evidence of possible human hazard. To better understand the state-of-the-science on PSLT toxicology, an Expert Workshop was held to document agreements and differences amongst a panel of highly experienced scientists and regulators. Key outcomes included: a functional definition of PSLTs; agreement the rat is a sensitive model for PSLT inhalation toxicology; identifying lung inflammation as a critical endpoint for PSLT risk assessment; and, agreement rat lung cancer occurring only under conditions of lung particle overload does not imply a cancer hazard for humans under non-overloading exposures. Moreover, when asked – should PSLTs be considered as human lung carcinogens based on rat data alone (and no supporting data from other species), the expert consensus was: 'No. However, the experts noted the current default regulatory position on rat lung overload data alone would be the suspicion of human carcinogen hazard.' The many areas of the expert agreement provide guidance for design, interpretation, and extrapolating PSLT inhalation toxicology studies. Considering the workshop outcomes, the authors recommend guidelines for evaluation and classification of PSLT be reassessed; and, prior decisions on PSLT hazard classification be revisited to determine if they remain appropriate.

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KEYWORDS
PSLT; particles; lung particle overload; hazard; risk; lung cancer; inhalation

Introduction

It has been over 30 years since Lee et al. (1985) described the development of lung tumors in rats exposed chronically to 250 mg/m^3 titanium dioxide (TiO_2) and Morrow (1988) proposed a general hypothesis for the increased particle retention occurring in rats after inhalation of insoluble particles now denominated poorly soluble low toxicity (PSLT) particles. Subsequent to the Lee et al. (1985) study, several chronic inhalation studies exposing rats to various concentrations of PSLT (e.g. carbon black, TiO_2, and talc) also demonstrated lung cancer only at exposure concentrations which increased particle retention (Mauderly et al. 1994; Heinrich et al. 1995; NTP 1995). The process that Morrow described has become known as lung particle overload and is now well documented in the rat to be associated with a nonspecific lung response including: the accumulation of particle-laden macrophages, persistent neutrophilic inflammation, epithelial hyperplasia, and metaplasia; and, in extreme cases, lung tumors.

While, the human relevance of lung particle overload associated rat lung cancer has been questioned (Warheit et al. 2016), several regulatory agencies and health advisory organizations have interpreted these outcomes as evidence of human hazard (IARC 2010; ECHA 2017). In 2006, TiO_2 and carbon black were classified by IARC as 'possibly carcinogenic to humans' based on inhalation studies in rats and the absence of increased lung cancer in occupational epidemiological investigations (IARC 2010). In 2017, the European Chemicals Agency's (ECHA) Committee for Risk Assessment assessed the carcinogenic potential of TiO_2 against the criteria in the Classification, Labeling, and Packaging (CLP) Regulation and concluded it meets the criteria to be classified as suspected of causing cancer (category 2, through the inhalation route) (ECHA 2017). The classification of these materials as possible or suspected human carcinogens was based on the occurrence of lung cancer in only rats, as mice or hamsters did not develop lung cancer after chronic inhalation exposure to similarly high concentrations of carbon black and TiO_2 and increased lung cancer has not been observed in epideliology studies. Consideration is now being given to classify for carcinogenic hazard inhaled PSLT as a category, of which carbon black and TiO_2 are considered representative. Collectively, these actions have sparked debate on how decades of inhalation toxicology and epidemiology research is being applied to evaluate, classify and assess the risk of inhaled particulate materials.

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Recently we surveyed 23 international experts extensively involved in PSLT inhalation toxicology research, risk assessment, and/or regulation of inhaled particulate materials (Borm and Driscoll 2019). As reported, there appeared to be agreement on several topics including: (1) the need for a clear definition of the term PSLT; (2) concern about grouping PSLTs for hazard classification; (3) incorporating the concept of lung particle overload in the design and interpretation of inhalation toxicology studies; and (4) the relevance of rat lung cancer responses to PSLT for hazard identification and risk characterization. As follow-on to that survey, a workshop was organized to facilitate an open, face-to-face discussion and debate amongst highly experienced scientists and regulators well-informed on the subject matter and to document the experts' opinions on the state-of-the-science for PSLT inhalation toxicology. This manuscript presents a summary of that workshop describing the approach taken, the participants, and the areas of expert agreement and disagreement.

Workshop charges, participants, format, and sponsorship

Charges: The workshop covered four topic areas on PSLT toxicology and risk assessment:

1. Definition and grouping of PSLT
2. Lung particle overload: definition and implications for study design
3. Relevance of the rat as a model for PSLT inhalation toxicology
4. Human health hazard and risk due to PSLT

The workshop focused on current scientific understanding and did not debate hazard classifications previously made on PSLT materials. The charges addressed by the experts are presented in Table 1. The workshop charges, the format of the workshop, and the plan for publishing the outcomes were reviewed with all experts and observers prior to the workshop.

Participants: Workshop experts were identified based on their substantial knowledge and experience with PSLT toxicology and/or related regulatory and scientific matters (see Table 2 for a list of experts). The role of the experts included: participate in plenary and breakout discussions, provide points of view on the charges, and contribute to the preparation of summaries during the workshop. The experts acted in an individual capacity and not as representatives of any organization or committee to which they are currently or were previously associated. In addition to invited experts, observers attended the workshop to hear first-hand the debate and discussion (see Table 3 for a list of observers). Observers were invited based on the following considerations: individuals playing a critical role in regulatory and/or health-related matters on PSLTs, individuals with involvement in product stewardship and/or decision-making regarding occupational exposure, and ensuring balanced stakeholder representation (academia, regulatory, and industry). The workshop was moderated by Drs. Kevin Driscoll and Paul Borm (Table 4).

Workshop format and approach: During the workshop, mixed groups of experts and observers met in breakout groups to discuss charges, formulate responses and document agreements, differences, and the associated rationale. The outputs from breakout groups were subsequently discussed in plenary sessions with all experts and observers present. The breakout group outputs were revised as required during plenary sessions to reflect the collective views of all the experts. Consensus statements or statements on differences were developed and circulated to the expert panel for review during and after the meeting.

Sponsorship: The workshop was held in Edinburgh, Scotland, and sponsored by the Institute of Occupational

Table 1. Breakout topics and charges to the expert panel.

1. Definition and grouping of PSLT
   - What is the definition of Poorly Soluble Low Toxicity Particles?
   - What criteria should be used to group PSLTs – solubility; toxicity; particle size; surface area; others?
   - What data are needed to support defining a material as a PSLT?
2. Lung Particle Overload: definition and implications for study design
   - What is the definition of 'lung particle overload' as it relates to the clearance of particles? What criteria should be used to determine lung particle overload?
   - Is the volumetric hypothesis of lung particle overload still valid? Are other mechanisms involved and/or more critical to the process?
   - Does lung particle overload occur in all species including humans? Is it manifested in the same way?
   - How should lung overload in rats be considered in the design of inhalation toxicology studies with PSLTs e.g. setting the maximum functional tolerated dose?
3. Relevance of the rat as a model for PSLT inhalation toxicology
   - Is the rat lung biological response to PSLTs (e.g. inflammation, hyperplasia, fibrosis, and tumors) unique from other rodent species or humans?
   - Is the rat a relevant model of human lung cancer hazard and risk for inhaled PSLTs under conditions of lung particle overload?
   - If adverse lung effects (e.g. fibrosis and tumors) are observed in a PSLT study with rats only under conditions of lung particle overload how should the results be extrapolated to lower, non-overloading conditions?
4. Human health hazard and risk due to PSLT
   - Should PSLTs be considered as human lung carcinogens based on rat data (and no other supporting species) alone?
   - What are the implications of the coal mine epidemiological studies (in combination with titanium dioxide and carbon black) to PSLT hazard classification and risk assessment?
   - What is the most appropriate animal species for assessing human health hazards and risks from the inhalation of PSLTs?

Table 2. List of workshop experts, their current and/or affiliations and areas of expertise.

Expert	Affiliation(s)	Areas of expertise
Armelle Baeza-Squiban, Ph.D.	• Professor, Functional & Adaptive Biology, Paris Diderot University	Nanotechnology, Cell Biology, and Biochemistry
Flemming Cassee, PhD	• RIVM • Professor, Utrecht University, IRAS	Exposure, Deposition Modeling, and Inhalation Toxicology
Rodger Duffin, PhD, MRC Path, FRSB	• Reader in Respiratory Medicine, University of Edinburgh	Thoracic and Particle Toxicology; Nanotechnology; Inhalation Toxicology, and Inflammation
Tom Gebel, Prof. Dr.	• Unit Toxicology Head, German Federal Institute for Occupational Safety and Health	Toxicology
Helmut Greim, M.D.	• Professor Emeritus, Toxicology, Technical University Munich	General and Inhalation Toxicology and Regulatory Toxicology
Uwe Heinrich, Dr. rer. nat., Dr. rer biol. hum. Habil	• Professor Emeritus, Toxicology and Aerosol Research, Medizinische Hochschule, Hannover • Former Director, Fraunhofer Inst. Toxicology and Exp Med • Fellow Academy of Toxicological Sciences	Inhalation Toxicology, Regulatory Toxicology, Toxicology of Particles and Fibers and Metals
Wolfgang G. Kreyling, Dr. rer. Nat.	• Retired Researcher & External Scientific Advisor of Institute of Epidemiology, Helmholtz Center Munich – German Research Center for Environmental Health	Inhalation + Lung Deposition of Aerosol Particles, Biokinetics of Inhaled Particles, and Biophysics of Lung-Particle Interactions
Robert Landsiedel, Dr. rer. nat. habil.	• BASF SE. Experimental Toxicology and Ecology, Vice President • Free University of Berlin, Privatdozent • German Society of Toxicology, Vice President • SCOEL, full member	Biokinetics, Inhalation Toxicology, and *In Vitro* Toxicology
Len Levy, Ph.D.	• Emeritus Professor, Cranfield University • SCOEL	Occupational Toxicology, Metal Toxicology, Occupational Cancer, and Chemical Risk Assessment
Dominique Lison, M.D. Ph.D.	• Professor Toxicology, Louvain Center for Toxicology and Applied Pharmacology (LTAP)	General Toxicology, Particle Toxicology, and Metals Toxicology
Fred J. Miller., Ph.D. Fellow, Acad. Toxicol. Sciences	• Fred J. Miller and Associates LLC • Duke University Medical Center, Pulmonary Division • Inhalation Toxicology Division Director, EPA • Vice President for Research, CIIT	Inhalation toxicology, Dosimetry, Extrapolation Modeling, and Risk Assessment
Günter Oberdörster, Prof	• Professor Emeritus, University of Rochester School of Medicine and Dentistry, Departments of Environmental Medicine	Inhalation Toxicology of Fibrous and Nonfibrous Particles, Dosimetry, Biokinetics, and Extrapolation Modeling
Lang Tran, Ph.D.	• Professor Institute of Occupational Medicine, Edinburgh, UK	Inhalation Toxicology and Mathematical Modeling
David B Warheit, Ph.D.	• Warheit Scientific LLC	Particle Toxicology, Nanotoxicology, and Pulmonary Toxicology
Mei Yong, Dr rer. Medic.	• Head, Institute for Occupational Epidemiology and Risk Assessment, Evonik Technology & Infrastructure	Epidemiology and Occupational Medicine

Medicine (IOM), the University of Edinburgh Lung and the Environment Group Initiative (ELEGI), and Heriot-Watt University. In addition to IOM, ELEGI, and Heriot-Watt University, financial support was provided by the International Carbon Black Association (ICBA), the TiO_2 Manufacturers Association (TDMA), Eurometaux, International Antimony Association, Industrial Mineral Association (IMA), Vliegasunie, and the Iron Platform. Comments on this workshop summary were neither solicited nor provided by any Sponsors.

Workshop outcomes: expert panel responses to charges

In the following sections, the text in italics represents the expert opinions as documented and agreed at the workshop.

Topic area 1. Definition and grouping of PSLT

Despite the frequent use of the term PSLT in scientific and regulatory literature, a clear consensus definition has not been published. A review of the literature finds many other terms which appear to be used synonymously with PSLT and also without a precise definition, a few examples: Poorly Soluble Particles (PSPs; Pauluhn 2014); Granular Biopersistent Particles without known Specific toxicity (GBS) (Morfeld et al. 2015); and, biopersistent granular dusts (MAK 2014). A definition of PSLT can be inferred from the publication of Morrow (1988) referencing data from Xerox toner, TiO_2, carbon black, diesel soot, PVC, and talc inhalation studies in describing lung particle overload, however, this does not reflect an expert consensus. Subsequently, other scientific publications have defined PSLT by analogy to other materials, typically without details as to chemical, physical and toxicology attributes of a PSLT (ILSI 2000; Greim et al. 2001; NIOSH 2011; MAK 2014; ECHA 2017). Some specifics for what constitutes low solubility for PSLT can be found in OECD No. 39 (2018) and ECETOC Technical Report 122 (2013), although these still do not provide the robust technical definition of both solubility (and other important physical and chemical properties, e.g. size, surface reactivity, and crystallinity) and 'low' toxicity. A first topic taken up at the workshop was aligning on the characteristics of a PSLT and providing a framework by which the PSLT nature of a material can be determined. The outcomes of this discussion served to ensure subsequent workshop deliberations were based on a common understanding of the materials in question.

Table 3. List of workshop observers, their current and/or recent affiliations and areas of expertise.

Observer	Affiliation(s)	Areas of expertise
Damjana Drobne	• University of Ljubljana, Biotechnical Faculty (SL) • National representative/ member of *ad hoc* CARACAL sub-group on ATPs to CLP classification of TiO_2 and mixtures.	Experimental Toxicology, Nanotoxicology, Standardization, and Nanomaterials
Craig Boreiko	• Consultant Toxicologist, CJD Risk Analysis LLC • Consultant to Antimony Association	Genetic Toxicology, Experimental Oncology, and Metal/ Metalloid Human Health Effects
Fiona Murphy	• Herriot Watt University- Edinburgh (UK) • Member of the EU GRACIOUS Consortium	Nanomaterials, Classification, and Grouping
Annie Jarabek	• Senior Research Advisor, Research and Development, U.S. EPA • National Center for Environmental Assessment (NCEA)	Risk Assessment, Extrapolation Modeling, and Toxicology
Terry Gordon	• Professor, New York University School of Medicine • ACGIH TLV Committee	Inhalation Toxicology, Risk Assessment, and Ambient PM
Klaus Kamps	• Unifrax (Director – Risk Management & Regulatory Affairs) • President of ECFIA • Chair of Eurometaux REACH working group	EU Regulation, Occupational Hygiene & Risk Management, and Product Stewardship
Roger Battersby	• Scientific Director at EBRC Consulting	Regulatory Toxicology, Toxicokinetics, and Nanomaterials
Frank Luetzenkirchen	• Quarzwerke GmbH, Frechen, Germany (DE) • IMA-Europe: Chairman IMA Technical Board	REACH-Workers Protection, Product Classification, Exposure and Risk Assessment, and Epidemiology
Robert McCunney	• Harvard Medical School, MD, PhD • Consultant to International Carbon Black Association	Occupational Toxicology, Epidemiology, and Risk Assessment
David Lockley	• Product Defense and Toxicology Manager, Venator Corp • Chair of Scientific Committee and CLH TF, TDMA	Toxicology, Classification, and Risk Assessment
Sue Hubbard	• Consultant Regulatory Toxicologist SahCo Ltd (UK) • Member of Iron Platform	Toxicology, Classification, and Risk Assessment
Andrew Smith	• Health & Safety Executive (UK), • Chemicals Regulation Division, Team leader: REACH-CLP-PIC • Member of ECHA's Risk Assessment Committee	Toxicology, Regulation, and Classification
Tim Bowmer	• European Chemicals Agency (ECHA), Helsinki (FI) • Chairman of the Committee for Risk Assessment	Regulatory Processes: Harmonized Classification, Restriction, Authorization, and Occupational Exposure Limits, Environmental Science
Ari Karjalainen	• European Chemicals Agency (ECHA), Unit C1 – Hazard I	Toxicology, Regulation, and Classification
Yufanyi Ngiewih	• Orion Engineered Carbons GmbH • ICBA- SAB member	Toxicology, Regulatory Processes and Classification, and Product Stewardship

Table 4. Workshop moderators, their current and/or recent affiliation, and areas of expertise.

Moderator	Affiliation(s)	Areas of expertise
Kevin E. Driscoll	• Adj. Professor, Rutgers University, Ernest Mario School of Pharmacy, New Jersey, USA • President, Healthcare Innovation Partners, LLC, New Jersey, USA	Inhalation Toxicology; Risk Assessment; Consumer Product Safety; Drug Discovery and Development; and Regulatory Affairs
Paul J.A. Borm	• Clinical director, Nano4Imaging GmbH (Dusseldorf, Germany) • Managing director- Consultant Toxicologist, Nanoconsult BV (Meerssen, NL) • Professor Toxicology, University of Dusseldorf (Germany)	Inhalation and Particle Toxicology; Nanotechnology; Risk Assessment; Medical Imaging; Medical Device Regulation; and Clinical Evaluation

What is the definition of poorly soluble low toxicity particles? What criteria should be used to group PSLTs – solubility; toxicity; particle size; surface area; others?

The expert panel agreed that a definition is needed for solubility and toxicity of particles and proposed the most practical way to accomplish this was through a tiered approach, first defining PSPs and then Low Toxicity (LT) as a subgroup of PSP. Experts considered biopersistence to be a critical component of the definition.

The expert consensus on PSP:

To define PSP, biopersistence needs to be further elaborated. There was consensus in the group that biopersistence can be split into release of components (relates to toxicity) and persistence in the biological environment. PSPs can be defined as particles for which their alveolar macrophage-mediated clearance rate is not shortened by their dissolution rate in the lung. This is made more specific by the following observations and conditions:

- *The processes concern respirable particles with aspect ratio <3.*
- *In the rat, we consider an alveolar macrophage-mediated clearance rate to be equivalent to a pulmonary retention half-time of 60–80 d.*
- *Shortened retention half-times can be assessed in vivo; dissolution rates can be assessed in vitro at both pH 7.4 and 4.5.*
- *Thresholds and benchmark materials to classify a particle as PSP need to be established. Clearly, PSPs include TiO_2 and carbon black, clearly, readily soluble particles include ZnO and CuO.*

Expert consensus on LT particles:

To define the LT component of PSLT, the endpoint should be chronic inflammation since this underlies other responses such as fibrosis and hyperplasia. The time, nature and extent of inflammation are crucial as to the resulting final endpoint. The following factors need to be considered:

- *An LT particle should not cause more than minimal and transient granulocytic inflammation up to a lung burden causing overload in the rat*
- *Granulocytic inflammation is a crucial characteristic of rat inflammation*
- *Inflammation is relevant to several endpoints across different species*
- *Dose-response benchmarking is important for inflammation and can be established by conventional methods*
- *The most adequate dose metric still needs to be established*

To make the definition more pragmatic the expert panel elaborated the following guidance for a Tiered Approach toward a PSP/PSLT definition:

- *Define the material and respirable fraction*
- *Particle dissolution in vitro should be assessed under biologically relevant conditions (pH and flow-through). The dissolution rate constant, k_{diss}, as well as dissolution half-time (or '% dissolution per day') can be used to classify particles as PSP. Recently a method and thresholds have been proposed for grouping nanoparticles based on k_{diss}* (Koltermann-July et al. 2018).
- *In vivo biopersistence can be determined in a rat inhalation study with an appropriate post-exposure monitoring period. If the retention half-time under non-overload is at or above 60 d particles are considered PSP.*
- *If upon dissolution in vivo or in vitro, quantities of components are released that exhibit toxic effects the material cannot be considered* LT.

Regarding the grouping of PSLT particles, the Experts agree that PSLT should NOT be considered as a group into which particles with unknown toxicological profiles can be placed – there should be evidence on their toxicological properties demonstrating a materials' PSLT nature.

Topic area 2. Lung particle overload: definition and implications for study design

The first description of lung particle overload was based on observations in rats exposed chronically to high concentrations of dust (Morrow 1988). It was hypothesized that increased retention of PSLT occurs when the lung particle burden exceeds a threshold related to the volume of particles phagocytized by alveolar macrophages and causing impaired macrophage function (Oberdörster et al. 1992). The lung responses associated with lung particle overload were described as 'artefactual' involving the accumulation of macrophages; persistent inflammation; and increased interstitial translocation of particles (Morrow 1988). Despite the considerable research undertaken on the lung particle overload phenomenon debate continues on the underlying mechanism(s); species similarities and differences; and the implications for toxicology study design and interpretation (ILSI 2000; ECETOC 2013; Borm et al. 2015; Warheit et al. 2016; Bos et al. 2019).

What is the definition of 'Lung Particle Overload' as it relates to the clearance of particles?

Expert consensus definition:

Lung Particle Overload is a phenomenon of impaired clearance in which the deposited dose of inhaled PSLT in the lung overwhelms clearance from the alveolar region leading to a reduction in the ability of the lung to remove particles. Lung Particle Overload results in an accumulation of particles greater than that expected under normal physiological clearance. This definition is relevant for all species (not just rat). This definition is independent of the underlying mechanism(s) (e.g. macrophage mobility impairment).

A key issue is that increased particle retention due to large lung burdens needs to be differentiated from that due to high cytotoxicity particles (e.g. quartz).

What criteria should be used for determining lung particle overload?

Expert consensus opinion:

Lung particle overload has occurred when a statistically significant retardation in lung particle clearance is demonstrated in well-designed studies using valid approaches to measure particle retention. Valid approaches include administration and measurement of low doses of tracer particles; time-course measurement of actual lung particle burdens; and, comparing lung burdens in long-term studies to those predicted based modeling of short-term clearance data

The expert panel also agreed:

Other measures useful to interpret lung particle overload include assessment of macrophage function and lung histopathology.

The use of intratracheal instillation as an exposure method for lung toxicology studies was discussed by the panel. The experts agreed:

Intratracheal instillation studies can be problematic as it results in the focal deposition of material.

Does Morrow's original hypothesis on particle volume loading of alveolar macrophages and impaired macrophage function (i.e. volumetric hypothesis) remain valid?

Expert consensus opinion:

The volumetric hypothesis has validity for PSLTs under certain circumstances.

Are other mechanisms involved and/or more critical to the process?

Expert consensus opinion:

Mechanisms other than exceeding macrophage volume can contribute to increased particle retention in lung particle overload with PSLTs.

Studies with nanosized particles indicate that particle surface physiochemical properties (e.g. surface area) involve mechanisms other than exceeding macrophage volume. Translocation of particles to the interstitium and endocytosis

by non-macrophage cells are important additional mechanisms.

Two studies experts cited in support of the importance of particle surface area contributing to increased retention included Oberdörster et al. (1994) and Tran et al. (2000).

Does lung particle overload occur in all species including humans?

Expert consensus opinion:

Lung particle overload has been demonstrated in all laboratory animal species tested.

Regarding the occurrence of lung particle overload in humans, there were differing points of view expressed by the experts:

- *A majority of the experts (10 of 15) agreed that lung particle overload could occur in humans.*
- *Some experts (5 of 15) were of the opinion that, while particle clearance overload could potentially occur in humans, there was insufficient information to draw a clear conclusion that it does occur in humans.*
- *One expert asserted lung burdens in coal miners after very high exposures are indicative of impaired particle clearance supporting overload occurs in humans.*

Studies by Kuempel et al. (2000, 2001) were cited by some experts as supporting the occurrence of lung overload in highly exposed coal workers. Other experts were of the opinion the coal miner data, while suggestive, were not definitive.

Is lung particle overload manifested in the same way across species?

There were different opinions regarding species differences in the lung response to PSLT.

A majority of experts (12/15) asserted lung particle overload is not manifested the same way across species, in particular, regarding the distribution of particles in the lung and tissue responses. Evidence cited supporting this opinion included multiple studies on the nature of particle distribution in the lungs of rats, non-human primates, and humans as well as studies of lung tissue responses in rats, mice, hamsters, nonhuman primates, and humans.

A minority of experts (3/15) considered the question based solely on retention and were of the opinion similarities exist with increased retention times seen in multiple species including humans (e.g. coal miners). Evidence cited in support was coal miner data indicating impaired clearance (Kuempel et al. 2001).

Several published reports were referenced by the experts in support of species differences in response to lung particle overload including Green et al. (2007), Bermudez et al. (2002, 2004); ECETOC (2013) Nikula et al. (1997, 2001), Elder et al. (2005), and Carter et al. (2006). These studies demonstrated that rats exposed at lung particle overloading doses of PSLT exhibit neutrophilic inflammatory and epithelial proliferative responses which are more pronounced and persistent than observed in the other tested species. Most of these differences are quantitative in nature and not qualitative.

Regarding interspecies extrapolation, several experts noted the importance of considering species differences in the numbers of macrophages per alveolus and alveolar surface area when determining the potential for overload. The publication by Stone et al. (1992) was cited as providing such comparative data for rats and humans.

How should lung overload in rats be considered in the design of inhalation toxicology studies with PSLTs e.g. setting the maximum functional tolerated dose?

Expert consensus opinion:

Chronic inhalation studies with PSLT should include a high concentration that produces an overload of particle clearance. A high exposure concentration producing a 2–3-fold prolongation of retention time was considered sufficient. The 2–3 factor was based on the need to ensure both a statistically significant increase in retention is seen and the study includes exposures in the non-overload range.

The experts agreed on the following guidance for the design of PSLT inhalation toxicology studies:

- *Measurements of lung burden and retention times should be performed to validate that overload was reached.*
- *The study should include exposures not causing lung particle overload.*
- *An exposure level producing lung inflammation should be included.*
- *Studies should have a valid statistical design to control the probability of type 1 and type 2 errors.*
- *Study doses should be equally spaced on a log scale.*
- *Histopathology should be conducted, accompanied by measures of lung inflammation (e.g. bronchoalveolar lavage fluid analysis).*
- *The high exposure level can be estimated/predicted by subacute and/or subchronic studies. It may also be possible to estimate the high exposure level based on occupational exposure levels of other PSLTs.*
- *These design criteria are scientifically based and do not consider specific regulatory requirements.*

Topic area 3. Relevance of the rat as a model for PSLT inhalation toxicology

Results from several PSLT inhalation studies indicate the rat lung responds differently from other small animal species, non-human primates and humans (Nikula et al. 2001; Boffetta et al. 2004; Carter et al. 2006; Baan 2007; IARC 2010; Morfeld et al. 2015; Warheit et al. 2016; Bos et al. 2019). Differences include: the clearance and translocation of PSLT in the lung, inflammatory and epithelial hyperplastic and metaplastic responses, and development of lung cancer. Considered along with the epidemiology data on PSLT there is debate on the relevance of the rat as a model to assess the hazards of PSLT inhalation and, in particular, lung cancer. In addressing these questions, the expert panel considered separately non-neoplastic and neoplastic responses as well as responses occurring with lung particle overload and under non-overload conditions.

Is the rat lung biological response to PSLTs (e.g. inflammation, hyperplasia, fibrosis, and tumors) unique from other rodent species or humans?

There was consensus among the experts on the following points:

- *Under non-overload conditions, responses in the rat should be considered relevant to other species, although the rat is more sensitive than other species.*
- *Under overload conditions, the rat was considered not to be unique in its inflammatory, hyperplastic, and fibrotic responses to PSLT.*
- *There was consensus that the rat is more sensitive than other species and humans in the lung response to PSLT for inflammation, epithelial hyperplasia, and fibrosis.*
- *Support cited for these positions were the many acute, subchronic, and chronic inhalation studies in rodent and non-human primates and the pathology from coal miners' lungs.*

Is the rat lung cancer response to PSLTs unique from other rodent species or humans?

On the question of neoplastic responses to lung overload there were two points of view among the experts:

A majority of the experts (10 of 15) asserted the rat is more sensitive than other species but there was not sufficient information as to the uniqueness between rats and humans.

A minority (5 of 15) of experts asserted the rat lung cancer response to PSLT was unique from other species including humans.

Points raised and considered by the experts in the discussion of lung cancer and lung particle overload were:

- *Other species tested (mice, hamster) have not developed lung cancer in response to PSLT*
- *It is important to consider the adverse outcome pathway (AOP) and whether it can occur in rats and humans. On this point, one expert cited the relationship between crystalline silica and lung cancer in rats and humans as supporting the potential of an inflammation-based AOP in both rats and humans.*
- *Epidemiology Data and the unique nature of rat lung tumor response*
  - *Several Experts asserted the epidemiology data for carbon black, TiO_2 and coal workers clearly indicate lung cancer does not occur in humans even with very high historical exposures. This was interpreted as evidence the rat is different.*
  - *Some Experts asserted the epidemiology data strongly supported the rat was unique but, as yet, was not conclusive.*
  - *One Expert cited a recent study on coal dust exposed workers as supporting lung cancer occurrence in coal workers exposed to high dust levels* (Taeger et al. 2015). *Several Experts strongly disagreed with the quality and interpretation of this study, citing several recent studies that found no association between coal dust exposure and lung cancer* (Attfield and Kuempel 2008; Miller and MacCalman 2010; Stayner and Graber 2011; Morfeld 2013; Morfeld et al. 2015).

A majority of experts were of the opinion the challenge in much of the debate is hazard classification and its implications versus risk assessment.

All experts agreed to the following statement:

While it may not be possible to conclude the rat is unique in its lung cancer response to lung particle overload and there is no hazard, considering the sensitivity of the rat and expected human exposure, PSLT do not pose a risk of lung cancer under non-overload exposure conditions.

Is the rat tested under overload conditions a relevant model of human lung cancer hazard and risk under non-overload conditions?

Expert consensus opinion:

Rat lung tumors occurring only under lung particle overload are not relevant to humans under non-overloading exposure conditions.

If adverse lung effects (e.g. fibrosis and tumors) are observed in rats ONLY under conditions of lung particle overload how the results should be extrapolated to lower, non-overloading conditions?

The extrapolation of adverse lung effects from PSLT inhalation studies was discussed in the context of the setting of Occupational Exposure Limits (OELs).

Expert consensus opinion:

Rat lung tumors occurring with PSLT only under lung particle overload are not relevant to humans under non-overload exposure conditions.

Setting OELs should focus on inflammation as this would precede other adverse responses (e.g. fibrosis) and can be seen at lower exposures.

The guiding principle is protecting for inflammation protects for fibrosis risk.

Other principles that should be considered in setting OELS for PSLTs:

- *Use inflammation as the key endpoint in applying Benchmark Dose (BMD); NOAEL or LOAEL approaches with adjustments for lung dose based on physiologic differences in species (e.g. lung deposition, ventilation).*
- *The rat is a sensitive animal species for PSLT associated inflammation.*
- *Incorporate uncertainty factors as can be justified.*
- *Use all relevant data (e.g. acute, subchronic, studies in other animal species, and data from humans).*

Topic area 4. Human health hazard and risk due to PSLT

An important driver for the workshop was to evaluate the meaning of animal and human data on PSLT for assessing human health hazards and risks. This has been a topic of debate exemplified in how various authoritative groups have approached hazard classification and published reviews on the topic (IARC 2010; NIOSH 2011; ECETOC 2013; ECHA 2017; Borm and Driscoll 2019; Bos et al. 2019). For example, working within their established hazard classification schemes, IARC and ECHA classified carbon black

(IARC) and TiO_2 (IARC, ECHA) as possible or suspected human carcinogens based solely on rat lung cancer responses occurring under lung particle overload (IARC 2010; ECHA 2017). In contrast, NIOSH considering likely mechanisms and exposure-dose-response relationships, differentiated between fine and ultrafine (nanosized) TiO_2 concluding for micro-sized particles 'there is insufficient evidence to classify fine TiO_2 as a potential occupational carcinogen'; and, conversely for nanosized particles 'inhaled ultrafine TiO_2 is a potential occupational carcinogen' (NIOSH 2011). Subsequently, an ECETOC Task Force, asserted 'there is no nanoparticle-specific lung overload toxicity and mechanistic findings for conventional "micro" particles apply also for nanostructured particles' (ECETOC 2013). The MAK Commission, taking into consideration mechanism and the conditions under which rats developed lung cancer, classified biopersistent granular dusts (exemplified as TiO_2, toner and carbon black) as Category 4, which presumes a threshold for cancer and that there will be no significant human risk at OELs (i.e. MAK values) (MAK 2014). For the workshop discussions, experts were asked to consider the state-of-the-science in offering their opinions on PSLT hazard and risk. The experts were not constrained by established regulatory classification schemes (e.g. IARC, ECHA).

Should PSLTs be considered as human lung carcinogens based on rat data (and no other supporting data from other species) alone?

Expert consensus opinion:

No. However, the expert panel noted that currently, the default regulatory position on rat lung particle overload data alone would be the suspicion of a human carcinogen hazard

The expert panel agreed on the following statement:

At the moment, from a theoretical standpoint overload could occur in humans, but we do not have sufficient information regarding the qualitative and quantitative differences (e.g. toxicodynamic, kinetics) between rats and humans that impact pathogenic endpoints. The experts recommend a Weight-of-Evidence (WoE) approach including other species (i.e. mice, hamsters, and non-human primates) and human epidemiological data. The WoE approach needs to incorporate effects in other species and within long-term cohort epidemiological studies in humans.

What are the implications of the coal mine epidemiological studies (in combination with titanium dioxide and carbon black) to PSLT hazard classification and risk assessment?

The expert consensus opinion:

Currently, Coal Mine Dust (CMD) represents the best source of high dose particulate exposure in humans showing inflammation that conceptually may be associated with lung particle overload. We can use CMD to look at PSLT effects in humans. However, the data are insufficient on the qualitative and quantitative differences (e.g. toxicodynamic, kinetics) between rats and humans that impact on pathogenic endpoints.

There were different opinions on the relevance of the coal miner's cancer risk for PSLT. A majority of workshop experts (9 of 15) asserted that studies in coal miners do not provide proof for elevated lung cancer risks. However, one expert was of the opinion, based on the study of Taeger et al. (2015), that elevated lung cancer risk in coal miners cannot be ruled out. As stated previously, several experts strongly disagreed with the quality and interpretation of the Taeger et al. (2015) study and cited several studies in support of their position including: IARC (1997), Attfield and Kuempel (2008), Miller and MacCalman (2010), Stayner and Graber (2011), Morfeld (2013), and Morfeld et al. (2015).

To provide additional perspective on the possibility of overload in coal workers, an analysis was conducted to estimate the coal dust burden in coal workers lungs and this was compared to dust burdens known to cause lung particle overload in rats. The following expert consensus was reached:

Historical coal dust lung burden values in humans have been shown to achieve levels of 12–15 mg/g wet lung (Kuempel et al. 2001). Considering the dose and tissue distribution of CMD in miners' lungs, one would expect lung particle overload when extrapolating from rat studies. Lung particle overload in rats, in terms of impaired particle clearance, is considered to occur at lung burdens above 1 mg/g of lung tissue. Given that 68–91% of the coal miners lung burden is in the interstitium at the end of life, and only 8–30% is in the alveolar lumen (Nikula et al. 2001) to load up the macrophages, a lung burden of 1–4.5 mg/g lung wet weight is estimated. This value is equal to or exceeds the lung burden resulting in ling particle overload in rats but not necessarily induction of lung tumors in rats.

What is the most appropriate animal species for assessing human health hazards and risks from the inhalation of PSLTs?

Expert consensus opinion:

The most sensitive species for inhalation testing of PSLT is the rat. The rat is also the species for which most of the data on PSLT has been generated. There are differences in the degree and nature of lung tissue response in the rat versus other species. It is recommended studies be performed in the rat along with mechanistic studies to better understand the differences between rats and humans and enable improved extrapolations.

Summary and authors' commentary

The PSLT workshop provided a forum to debate the state-of-the-science on PSLT toxicology amongst a panel of scientists and regulators who have contributed significantly to research on PSLTs with many extensively engaged in science advisory and/or government oversight roles on risk assessment of inhaled materials (e.g. IARC, SCOEL, MAK, US EPA, RIVM, and BAU). The approach taken for the workshop was similar to IARC Monograph meetings in which participants openly express their agreements or differences on specific charges and written summaries are prepared and aligned with all participants. To our knowledge, this workshop was the first time an expert panel was assembled along with stakeholders to provide their collective opinions on

several important and controversial topics regarding PSLT inhalation toxicology and risk assessment.

This article provides a brief summary of workshop outcomes, along with our perspective on implications for how the toxicology of PSLT is evaluated and risks assessed. Briefly, the experts reached agreement on a number of important workshop charges including: (1) defining a process for characterizing a material as poorly soluble and LT thus providing a framework for developing the data needed to support read-across approaches for assessing the safety of new PSLT-like materials. This data-based definition can help ensure any grouping of PSLT-like materials and extrapolation across studies is done appropriately. (2) The experts supported the use of the rat as a sensitive species for PSLT inhalation toxicology and provided specific guidance for inhalation study design. The latter builds on the concept of maximum functionally tolerated dose (Oberdorster 1997) and provides a sound technical rationale for the selection of maximal exposure concentrations. This study design guidance can serve as a basis to reevaluate the adequacy of prior PSLT inhalation studies. (3) Regarding PSLT risk assessment and OEL setting, the Expert Panel agreed the prevention of inflammation should be a driving principle in setting exposure limits for PSLTs as inflammation was considered to precede and contribute to other adverse lung responses e.g. fibrosis.

Possibly one of the most significant outcomes of the workshop was the agreement reached on the human relevance of rat lung cancer observed under conditions of lung particle overload. Regarding hazard, the expert panel agreed that, in the absence of supporting data from other species, overload-associated lung tumors in rats do not imply a human hazard. From a risk perspective, there was consensus that rat lung tumors occurring only under conditions of lung particle overload are not relevant to non-overloading PSLT exposure conditions in humans. As noted by the experts, their position on the relevance of rat lung cancer differs from the interpretations underlying current regulatory classifications of PSLT (i.e. carbon black and TiO_2) for which lung cancer seen only in rats and only under conditions of lung particle overload was interpreted as evidence of a possible human hazard.

There were some questions on which the experts did not reach consensus. For example, whether the presumed inflammation-dependent mechanism for cancer in overloaded rat lungs was unique to this species; and, whether lung particle overload can occur in humans (e.g. coal miners). Notably, for these topics, several experts felt there was insufficient data to draw a firm conclusion. The lack of consensus and suggested data gaps support the need for additional research on mechanisms underlying the unique inflammatory, epithelial hyperplastic and metaplastic and lung cancer responses of the rat along with reference data on these pathways in humans.

In summary, the understanding of PSLT toxicology has grown substantially since the first observations on TiO_2 and rat lung cancer (Lee et al. 1985) and the first description of lung particle overload (Morrow 1988). The advances in the state-of-the-science are reflected in the many significant areas of agreement reached by an expert panel having in-depth knowledge and experience in PSLT toxicology and regulatory matters. Based on the outcomes of the workshop the authors recommend: (1) guidelines for evaluation and classification of inhaled particulate materials be reassessed, taking account of the state-of-the-science on lung particle overload and the definitions, guidance and consensus opinions of the expert panel; and, (2) accordingly, PSLT hazard classifications be revisited to determine if they remain appropriate.

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Disclosure statement

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