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Toxicity testing of poorly soluble particles, lung overload and lung cancer

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ABSTRACT

In 2013, an ECETOC Task Force evaluated scientific understanding of the 'lung overload' hypothesis. As there is no evidence that humans develop lung tumours following exposure to poorly soluble particles (PSPs), emphasis was given to the observed higher sensitivity and specificity of rat lung responses and potential impacts of this on human risk assessment. Key arguments and outcomes are summarised here, together with discussion of additional findings published since 2013. Inhalation exposure to PSPs in all species is associated with localised pulmonary toxicity initiated by a persistent pro-inflammatory response to particle deposition. Events in the rat indicate a plausible adverse outcome pathway for lung tumour development following exposure to PSPs under overload conditions. A different particle lung translocation pattern compared to rats make humans less sensitive to developing comparable lung overload conditions and appears to also preclude tumour formation, even under severe and prolonged exposure conditions. Evidence continues to suggest that the rat lung model is unreliable as a predictor for human lung cancer risk. However, it is a sensitive model for detecting various thresholded inflammatory markers, with utility for non-neoplastic risk assessment purposes. It is noteworthy that preventing inflammatory rat lung responses will also inhibit development of neoplastic outcomes.

1. Introduction

Experimental rat study findings in which chronic inhalation of poorly soluble particles (PSPs) give rise to lung neoplasms have been questioned in terms of their relevance and applicability to humans occupationally exposed to high levels of PSPs. This has been the subject of several comprehensive reviews, all resulting from scientific conferences, workshops and/or task forces. An early conference (held at the Massachusetts Institute of Technology) in 1995 concluded there was, 'a consensus of uncertainty that the lung tumour response of rats to chronic high level exposures to particles, taken alone, is an adequate predictor of human lung cancer risk from exposures resulting in much lesser lung loading with particles' (Mauderly and McCunney, 1995). Moreover, at this conference Snipes (1995) concluded that 'the available data from monkeys, dogs and humans suggest that lung overload in rats may not be directly relevant to larger mammals and humans'. The author called for additional research to determine 'the extent to which the various manifestations of lung overload in rats can be applied to other species, including humans'. This Conference provided an excellent foundation for subsequent discussions and was followed by a workshop in 1998 hosted by the International Risk Sciences Institute (ILSI). Although a full consensus opinion was not reached at the ILSI workshop, it was generally acknowledged that lung tumours are specific to the rat following chronic inhalation exposure under conditions of PSP-overload; in addition, it was noted that there was an absence of lung cancers in PSP-exposed production workers (ILSI, 2000). In 2000, the MAK Commission, on behalf of the Deutsche Forschungsgemeinschaft, convened an international workshop, to help define the necessary information to allow a science-based risk assessment of fibres and particles. Participants recognised the sensitivity of the rat to the development of lung tumours with high doses of PSPs, concluding a secondary genotoxic mechanism. Important dose-metrics were concluded to be, surface area, particle number and diameter. Although it was stated that the rat model was the only one available, the relevance to humans was considered unlikely based on the lack of evidence of lung tumours in coal workers chronically exposed to very high levels of dust (Greim et al., 2001). More recently, the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) convened a Task-Force (TF) in 2013 to update findings of the health effects literature on poorly soluble particles/lung overload. The findings of the TF are discussed in detail in this review, however one of the most significant conclusions was that the rat is *unique* amongst all species in

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developing lung tumours under chronic inhalation overload exposures to PSPs (ECETOC, 2013). Additionally, Warheit and colleagues (Warheit et al., 2016) have provided an update and interpretation of data published since the earlier ILSI report (ILSI, 2000).

In the present paper, particular focus is given to discussion of the findings of the ECETOC TF, which was established in 2013 to update the conclusions of the ILSI report (ILSI, 2000) based on examination of current scientific understanding of the 'lung overload' hypothesis. Although the concept of lung overload has been acknowledged for some years, it has become prominent once again due to the requirement under the EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation for derivation of Derived No Effect Levels (DNELs). When deriving DNELs for PSPs, ECHA notes the importance of consideration of the potential for lung overload as a driver of pathologic effects (ECETOC, 2013). One of the main considerations by the ECETOC TF was to assess the sensitivity and specificity of rat lung responses to PSP, particularly with regards to the development of lung tumours, and associated implications of the lung overload hypothesis for hazard identification and human risk assessments, including the setting of regulatory guideline values. In this paper, we supplement the ECETOC discussion with additional findings and developments published after the ECETOC report in 2013. In particular we draw attention to the conclusion that the lung cancer seen in rats under conditions of lung overload is not predictive of the lung cancer risk to humans. An additional question discussed here, that was not considered in the ECETOC report, concerns which models and/or endpoints (if any) may be better reflective for the screening of adverse effects (including putative carcinogenicity) of PSPs in humans.

1.1. What do we mean by 'poorly soluble particles'?

Biosolubility refers to the solubility of particles in biological systems, including *in vitro* cell systems and *in vivo* biological fluids. The biosolubility of a substance may differ substantially from its solubility in water and in different biological fluids. (ECETOC, 2013). Working in parallel with physical translocation, chemical dissolution is a key mechanism of clearance of inhaled particles within the respiratory tract. The biosolubility of particles governs how easily particles are dissolved in intracellular or extracellular fluids and cleared into the blood or lymphatic circulation, and therefore contributes significantly to clearance rates. Thus, differences in biosolubility directly influence the retention rate of particulate matter and can therefore be considered a significant factor in determining the dose at which lung overload is established. According to the ECETOC TF, PSPs are defined as particles that have dissolution half-lives measured in artificial lung fluids (interstitial fluid, pH 7.4; artificial lysosomal fluid, pH 4.5; and artificial alveolar fluid, pH 7.4) longer than macrophage mediated clearance times (e.g., TiO2, carbon black, talc, and toner particles); under this definition, macrophage clearance rather than particle dissolution determines particle residence time in the lung. PSPs are also viewed as particles with no specific inherent toxicity (ECETOC, 2013; Warheit, 2016). These two factors together allow differentiation from other particulates such as quartz (crystalline silica), which exhibit significant surface-related cytotoxicity (ECETOC, 2013). More recently, the German BAuA[1] have produced a report that attempts to define Granular Biopersistent Particles (equivalent to PSPs). In this, the BAuA include various materials such as minerals, metals, metal oxides or polymers that show a negligible solubility in lung fluids (lung lining fluid, lysosomal fluid). This definition is broadly equivalent to the ECETOC definition of PSPs.

The ECETOC TF proposed three clearance classes for PSPs, based on those from the International Commission on Radiological Protection (ICRP) (ICRP, 1994) for radioactive particles. These are detailed in Table 1 below:

1.2. What do we mean by lung overload?

Overload was first described by Bolton and colleagues when investigating pulmonary clearance of inhaled asbestos fibres in rats (Bolton et al., 1983). The phenomenon was later fully demonstrated by Morrow (1988) who confirmed the toxicological implications of impaired pulmonary macrophage clearance of an excessive dust load in the lung. Warheit and colleagues describe overload as 'a condition of impaired macrophage mediated clearance of particles in the lung following prolonged high-dose exposures to PSP of low inherent toxicity' (Warheit et al., 2016). Impaired clearance leads to a number of effects that characterise overload; these include an increased transfer of particles to lymph nodes, accumulation of particles in the lung, increases in lung weight, pulmonary inflammation, epithelial hyperplasia (proliferation), fibrosis and eventually cancer (in the rat) (ECETOC, 2013). Although lung overload as a phenomenon has been extensively described in the rat and other rodents, human biokinetics suggest that great care must be used when using this term to apply to humans (discussed later).

Various physicochemical characteristics may influence the establishment of alveolar macrophage (AM) overload and a number of dose metrics have been proposed in an attempt to describe a relationship between particle dose and the toxicological effects of lung overload. Whilst, for inhalation studies in general, the most practical and widely used dose metric is particle mass, this is not considered the most suitable metric when describing lung overload effects. Particle volume and particle surface area are considered to be of greater use. However, it is unlikely that a universal dose metric will be identified as the most appropriate metric that may be specific to a particular substance and the type of effect investigated (ECETOC, 2013). Dose metrics are discussed more fully in Section 2.

2. Particle characteristics associated with lung overload

The discussion of which is the most appropriate particle dose metric for toxicological investigations and risk assessment purposes has been ongoing for a number of decades, and there are differing opinions on this. However, it is not the intention of this review to add to this discussion but to outline the current arguments and views relating to the relevance of rat lung tumours for humans. The three dose metrics of particle mass, particle volume and particle surface area have most often been used to identify a relationship between particle exposure and the occurrence of lung overload. Particle mass is probably the most accessible of these descriptors and Morrow identified a mass-based lung burden of 1–2 mg dust/g (presumed dry weight) lung as a threshold for the development of a significant prolongation (> doubling) of the pulmonary retention half-time in Fischer F344 rats (Morrow, 1988). Pauluhn has proposed that particle overload is governed by particle retention kinetics and pulmonary inflammation, driven primarily by the volumetric particle dose and the macrophage pool. These factors determine the particle displacement volume and the author has concluded that this is the most prominent contributor linking retained particulate dose with toxicity in the lung (Pauluhn, 2011, 2014).

Morrow also considered particle volume *per* AM as a quantitative descriptor of overload and identified two important issues: 1) particles with higher density would achieve the volume load at respectively higher mass load; 2) the void spaces would increase the volume of phagocytised agglomerates by more than 30%. The volumetric hypothesis has been strongly supported in later studies (e.g. Snipes and Clem, 1981; Oberdörster, 1995) and recently modified for nanoparticles to include agglomerate density (Pauluhn, 2011). However, the two requirements are difficult to define in a quantitative way and reliable

[1] Methodology for the identification of Granular Biopersistent Particles (GBP) at workplaces. Available at: http://file:///C:/Users/Admin/Downloads/BAuA%20GBS%202017%20(1).pdf.

Table 1
Proposed clearance classes for particulate matter.

Clearance class	Pulmonary clearance in humans (t½ days)	Proposed solubility class	Example
D (days)	< 10	Soluble	ZnO, CuO
W (weeks)	10–100	Partly soluble	SiO_2
Y (years)	> 100	Poorly soluble	Co3O4, TiO_2, CeO_2

values of density are critical (ECETOC, 2013).

Surface area-based dose and biological activity *in vivo* have been shown to correlate well, leading to the hypothesis that decreasing particle size, at equivalent mass concentrations, is associated with greater toxicity. This hypothesis has gained prominence over the last 15 years with regard to the toxicity of nanoparticles, because at the same mass load, surface area increases as particle size decreases (e.g. Oberdörster et al., 1994; Tran et al., 2000; Cullen et al., 2000; Sager et al., 2008, 2009). Donaldson et al. proposed a threshold value for onset of inflammation of 1 cm^2 particle surface area burden per 1 cm^2 of proximal alveolar region (PAR), (Donaldson et al., 2008).

Other particle characteristics have also been shown to play a significant role in the way the respiratory system responds to particle exposure and the importance of determining physicochemical characteristics of the material being assessed was emphasised by the ECETOC TF (ECETOC, 2013). Several physicochemical properties were considered of importance:

- particle density - directly linked to volume by division of particle mass by particle volume. For any given particle, ECETOC states that the density of the envelope (determined as mass divided by the displacement volume in a non-wetting liquid) is smaller than that of the apparent density, which, in turn, is smaller than the true density (ECETOC, 2013). Which of these is used depends on the 'wettability' of the particle when engulfed by macrophage fluid, however only the apparent density can be considered in the volumetric hypothesis. Determination of the density of powdered material is usually carried out using pycnometric methods. For airborne materials, measurement of the envelope density has recently become possible through determination of mass and envelope volume (Park et al., 2004; Liu et al., 2012).
- particle size refers to the linear extension of the particles (e.g. fines, ultrafines and nanoscale). Size is directly related to the surface-based dose metric and is expressed as an 'equivalent diameter' to account for density and shape; this includes the aerodynamic diameter for particles > 0.5 μm and the diffusion equivalent diameter for the smaller size fractions. According to the EC,[2] nanoscale materials are defined when '*50% or more of the particles in the number size distribution have an external dimension in the size range* 1 nm – 100 nm' however, when released into the air, most nanoparticles exist as agglomerates. ECETOC stated that importantly, de-agglomerisation of agglomerates is not considered to occur *in vivo* (Cruetzenberg et al., 2013; Morfeld et al., 2012; Cruetzenberg et al., 2013; Morfeld et al., 2013). Although increasing pulmonary toxicity with decreasing particle size has been shown to occur for some PSPs, this is not universally applicable (Donaldson and Poland, 2013; ECETOC, 2013).
- particle shape can modulate a particle's aerodynamic behaviour, directly impacting deposition sites in the lung. Size also impacts the ability of macrophages to internalise particles through the actin-driven movement of macrophage membrane (Champion and Mitragotri, 2006, 2009).
- surface reactivity – different biological effects have been reported for chemically similar particles with differences in surface reactivity (Warheit et al., 2007; Duffin et al., 2007). Using TiO_2 as an example, Warheit concludes that the toxicity of a particle is not dependent on particle size but on surface characteristics, however, where there is comparable surface chemistry *per* unit area, then the number of reactive groups increase with decreasing particle size, but equal particle mass (Warheit et al., 2007).

The dosimetry of inhaled particles is determined by the transport of material into the respiratory tract, with subsequent transfer to the surfaces/lining fluids of the different compartments, followed by a re-distribution of the deposited matter and removal from the lung by physiological processes. These parameters have been well defined in both laboratory animals and humans, and models allowing the estimation of the deposited dose have been developed and validated. It should be noted however, that in using these models, it is critical that the methodologies used to obtain the input parameters are well developed and understood (ECETOC, 2013). Importantly however, as noted elsewhere (section 3), it is not just deposited dose that is a driver of any pathological consequences in the lung but, the proportional location of the particles within lung compartments; e.g. alveolar in rodents versus interstitialisation in primates (including humans). Thus, comparisons of equivalent lung burdens and doses between species alone may be somewhat misleading. This is explored further in the next section.

3. Pathology of lung overload

3.1. Which parts of respiratory system are involved/impacted?

Nikula et al. (1997) carried out morphometric studies to directly compare anatomical patterns of particle deposition following chronic inhalation (24 months) of the PSPs diesel exhaust, coal dust, and diesel exhaust combined with coal dust in rats and cynomolgus monkeys. Exposure levels were 2 mg respirable particulate/m^3 (7 h/day, 5 days/week), equivalent to the permissible airborne concentration in underground coalmines in the US at the time of the study. *Within* each species the authors reported that particle retention and lung tissue responses were similar for each PSP. Differences were noted *between* species in patterns of retention. In the rat, a greater proportion of particulates (approximately 73%) were retained in the lumens of alveolar ducts and alveoli when compared with the monkey (approximately 43%). In the latter species a significantly greater portion of the particulate material (approximately 52%) was retained in the interstitium than was seen in the rat (approximately 27%). Differences were also noted in lung tissue responses between species. Rats, but not monkeys, showed significant alveolar epithelial hyperplastic, inflammatory, and septal fibrotic responses to the retained particles. The authors concluded that retention patterns and tissue responses to inhaled PSPs in rats at occupationally relevant levels may thus not be predictive of those in humans and non-human primates.

Importantly, anatomical and histological differences between species most probably affects particle deposition, retention, and clearance. Rats and mice have simple acini due to the lack of respiratory bronchioles. However, monkeys and humans have more complex and larger acini, accommodating respiratory bronchioles and larger alveoli and alveolar ducts than in the rat (Mercer and Crapo, 1988). In addition, the amount of septal tissue is greater in primates than in rodents (Pinkerton et al., 1982; Kapanci et al., 1969, 1972) and the thickness of the pleural tissues also differs; in the rat the pleura is thin with relatively few pleural lymphatics, in nonhuman primates the pleura is thicker with a greater number of lymphatics than rat pleura, and in humans the pleura is thick with relatively abundant pleural lymphatics (McLaughlin et al., 1961, 1966; Tyler and Julian, 1992; Leak and Jamuar, 1983).

[2] http://ec.europa.eu/environment/chemicals/nanotech/faq/definition_en.htm.

Morphometric analysis of particle disposition patterns in deceased coal miners showed similar patterns to those seen in cynomolgus monkeys, with most of the coal particles having translocated from respiratory bronchioles/alveolar epithelium to interstitial sites. These results strongly suggest that anatomic patterns of particle retention and lung tissue reactions in rats may not be predictive of retention patterns and tissue responses in primates that inhale PSPs at concentrations representing high occupational exposures (Nikula et al., 2001). This is supported by the findings of Gregoratto et al. (2010) who used an updated and revised version of the ICRP Human Respiratory Tract Model to demonstrate that a large proportion of inhaled PSPs translocate from initial deposition sites within alveolar/respiratory bronchiolar regions, to the interstitium.

3.2. Alveolar macrophage accumulation

AMs represent primary phagocytes of the innate immune system, providing a non-specific pulmonary defence to inhaled particles through phagocytic and cytotoxic activities. A rapid increase in phagocytic active cells, including polymorphonuclear leucocytes (PMNs) and AMs, is observed in the lung following particulate exposure and may be considered an early indicator of pulmonary particle deposition (ECETOC, 2013). Different pulmonary macrophage phenotypes can be distinguished, depending on their localisation, e.g. alveolar, interstitial, pleural and surface associated, with the latter considered to be potentially responsible for differences in clearance rates of particulates. Bronchoalveolar lavage (BAL) samples from rats and humans show differences in composition with respect to surface macrophage content, with rodent BAL comprised of ≥95% macrophages, few lymphocytes and neutrophils and human BAL consisting of ≥80% macrophages, around 18% lymphocytes and few neutrophils.

Generic elimination half-times for PSPs of 60 days for rats and approximately 1 year for humans have been proposed (Oberdörster, 1995) which may result from the greater AM pool of humans relative to rats (approximately 7 times greater) (Pauluhn, 2011). The alveolar clearance rate in humans seems to be independent of the particle load, whereas the clearance rate in rats depends on the amount of particles in the alveolar region. This may contribute to a more pronounced impairment of macrophage mediated alveolar clearance and thus, the higher susceptibility of rats to lung overload (Brown et al., 2005). Krombach and colleagues also reported that AM in humans are significantly larger than in the rat, hamster or monkey (Krombach et al., 1997) suggesting that differences in the number and size range of phagocytosed particles will also exist. Difference in translocation processes between species (human, monkeys, rats and mice) will also play a crucial role in clearance rates (Nikula et al., 2001; Gregoratto et al., 2010). This latter difference is becoming increasingly recognised as critical in the potential cascade of events in any interspecies AOP considerations (Warheit et al., 2016).

In addition to their phagocytic function, alveolar macrophages exert their regulatory function within lung tissue via the secretion of antimicrobials and nitric oxide as well as the release of numerous mediators like tumour necrosis factor (TNF)-α and interferon (IFN)-γ) (Murray and Wynn, 2011; Laskin et al., 2011; Byrne et al., 2015). Originally, classification of macrophages has broadly been defined as classically activated pro-inflammatory "M1" and "alternatively" activated anti-inflammatory "M2" macrophages respectively (Fig. 1). Classically activated "M1 macrophages" are produced during cell-mediated immune responses and release reactive oxygen/nitrogen species (ROS/RNS) and pro-inflammatory cytokines (e.g., IL-1 IL-6, IL-12, IL-23, TNF-α) so exerting strong anti-proliferative activities – but are also known to generate microbicidal and tumoricidal activity (Gao et al., 2018). Macrophages and their activation states are characterised by plasticity and flexibility. In this respect, macrophages respond to exposure against foreign bodies apparently through a carefully balanced system consisting of M1 macrophages releasing pro-inflammatory and cytotoxic mediators important in host-defence and M2 macrophages involved in down-regulation of inflammatory processes and the initiation of cell proliferation and wound-repair. However, rather than existing at the extreme points of the M1/M2 spectrum, macrophage phenotype is tailored to meet the immediate immunological need. Due to their plasticity, macrophages are capable of fine-tuning their activity by developing mixed phenotypes in response to the local microenvironment (Bazzan et al., 2017).

If this system becomes dysregulated, for example through prolonged exposure to high levels of particles, macrophages may become hyperresponsive leading to excessive release of mediators and growth factors. These, in turn, promote acute tissue injury and progression of chronic conditions such as fibrosis, and in the case of the rat, cancer (Mosser and Edwards, 2008). Species differences in the pulmonary micro-environmental conditions responsible for the activation of "M1" and "M2" macrophages, and/or differences in generating mediators and ROS/RNS, may play an important role in the observed species differences in lung injury following chronic exposure to PSPs of low toxicity.

3.3. Inflammatory and associated processes

Evidence showing the absence of rat lung tumours when pulmonary inflammation is not induced (Levy, 1995; Oberdörster, 1997; ILSI, 2000; Greim et al., 2001; Kolling et al., 2011) confirms that chronic pulmonary inflammation is a key driver for carcinogenesis in rats (Oberdörster, 1997; Kolling et al., 2011). However, preliminary findings from a recent study (reported as a conference abstract[3]) have shown that chronic inhalation exposure to cerium oxide (CeO_2) in the rat at exposure levels sufficient to cause inflammation of the lung, did not result in an increase in lung tumour incidence. This may imply that inflammation of the lung can be considered basically to have a threshold in terms of tumour induction in the rat and that additional rat specific factors may account for the known higher sensitivity regarding neoplastic lung responses of rats. Schwotzer et al. (2017) confirmed that following a 90-day inhalation exposure to CeO_2(NM-212), nanoparticles reached the alveolar space and induced persistent inflammatory reactions, considered to be overload-related; the authors proposed a NOAEL below 1.0 mg/m^3.

It has been concluded that the tumourogenesis of PSPs in the rat involves a mechanism of secondary genotoxicity at doses that induce inflammation (Greim et al., 2001), with genetic damage caused by oxidative DNA damage mediated through ROS/RNS generated during inflammation (Schins and Knaapen, 2007). This is of significance to hazard and risk assessments and derivation of DNELs in risk characterisation and risk management decisions (discussed later). There seem to be no new findings that contradict this generally-held paradigm.

- **Cell proliferation**

The induction of persistent lung inflammation initiates cell proliferation and tissue remodelling which are necessary changes for non-neoplastic lung lesions (e.g. fibrosis) as well as the fixation of secondary caused mutations in affected target cells and, in the rat, the progression of these cells also to neoplastic lesions. Hence it follows, that the induction of cell proliferation and all subsequent events resulting hereof, are threshold related (ECETOC, 2013).

- **Oxidative stress**

During lung overload conditions, an inflammation-dependent increase of oxidative stress becomes dominant resulting in secondary

[3] http://www.jeangilder.it/icoam2017/wp-content/uploads/2017/09/ISBM_AbstractBook.pdf.

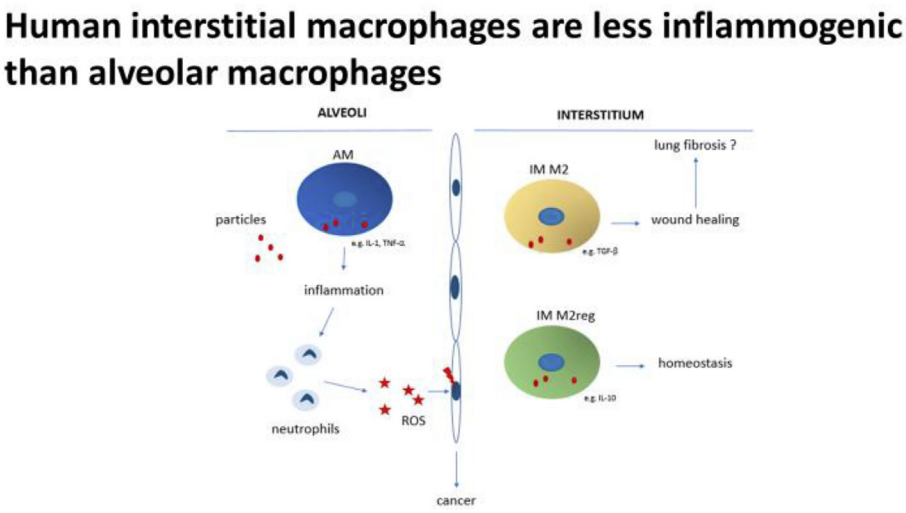

Fig. 1. Inflammogenic properties of human interstitial and alveolar macrophages.

genotoxic events which are therefore also thresholded.

3.4. Translocation

Compared to rats, in which between 15 and 18% of particles are translocated to the interstitium (Nikula et al., 2001), in humans > 70% (average) of particles are translocated to the interstitium (Nikula et al., 1997, 2001; Warheit et al., 2016). Uptake into the interstitium is inversely related to particle-size and is also species dependent. Whilst rats generally accumulate more particles in the lumen of alveolar ducts and alveoli, humans retain much more material in the interstitium, from where fractions of particles can translocate to extra pulmonary organs, in particular to the liver. Translocation to lymph nodes can also occur in humans and is a process of clearance related to inflammation rather than particle size. Chemical composition and physical structure of particles are considered to be important in determining systemic translocation of particles (ECETOC, 2013).

4. Mechanism of lung tumour formation - adverse outcome pathways

4.1. Inter-species differences in lung tumour formation following chronic exposure to PSPs

Inter-species differences in response to particle overload clearly show that the rat has *a particularly sensitive, and most probably unique*, response in the development of lung neoplasms under such conditions. An extensive toxicological database for the PSPs TiO_2 and carbon black exists in several experimental species, and in humans through occupational exposure. The following findings from the experimental studies were discussed by the ECETOC Working Group (ECETOC, 2013):

- chronic exposures to PSPs at high concentrations leads to development of lung tumours in rats, but not mice;
- rats, mice and hamsters have been exposed to identical test substances at identical concentrations of pigment-grade TiO_2, ultrafine TiO_2 as well as carbon black particles (at particle overload concentrations). Rats, but not mice, developed a pathological sequelae of sustained lung inflammation and cytotoxicity, followed by increased cell turnover and fibroproliferative effects (e.g., hyperplasia, septal fibrosis, etc.) ultimately leading to metaplasia and secondary genotoxicity.

This is illustrated in Table 2 which shows that lung tumours have been reported exclusively in rats, but not in mice, hamster, non-human primates or humans following chronic exposure to PSP. Importantly, epidemiological studies to date have not found comparable `lung overload' conditions, or an increased risk for cancer in workers exposed chronically to PSPs; this is a particularly notable finding for former coal miners, a group that have experienced worst-case exposure conditions (ECETOC, 2013).

Fundamental differences in the histological characteristics of tumour cell types within rat and human lung tumours have also been highlighted (Green et al., 2000; Warheit et al., 2016). Green and co-workers report that in humans, lung tumours are typically bronchiolar in origin, i.e. the initial site of deposition, and are comprised of four major cell types, namely, adenocarcinomas, squamous cell carcinomas, small cell and large-cell anaplastic carcinoma; in contrast, the majority of rat lung cancers are adenocarcinomas and squamous cell carcinomas (Green, 2000). In rats exposed chronically to PSPs, non-tumorigenic keratinising cysts and adenomas are also common. In humans, lung tumours do not develop following chronic exposure to PSPs and non-tumorigenic keratinising cysts and adenomas are rare or absent (Green,

Table 2
Interspecies lung responses following long-term or chronic inhalation exposure to GBS (equivalent to PSPs).
Source: ECETOC, 2013; Severity low +, moderate + +, high + + +, or questionable (+); ADs – alveolar ducts; ISs – interstitial sites

Species			
Rat	Mouse	Hamster	Primate/Human
Likelihood for developing particle overload (slow lung clearance)			
+ + +	+ + +	+	Not determined
Alveolar macrophage participation			
Active (accumulation in ADs)	Active (accumulation in Ads)	Extensive (rapid clearance)	Not as extensive (translocation to ISs)
Pulmonary (neutrophilic) inflammation			
+ + +	+ + +	+	+
Epithelial and interstitial cell proliferation			
+ + +	+	(+)	(+)
Septal fibrosis			
+ + +	+	(+)	(+)
Anatomical location of retained particulates			
Primarily alveolar[a]	Primarily alveolar[a]	Rapid clearance	Primarily interstitial
Lung tumours following chronic exposure			
Yes	No	No	No

[a] Some increased translocation at overload.

2000).

Warheit and colleagues also report that from a histopathology perspective, occupational exposures to TiO_2, carbon black, or coal dust particles do not produce epithelial hyperplasia, lung inflammation or lung cancer in humans. In the rat, under PSP overload conditions, histopathological changes are characterised initially by macrophage accumulation, followed by necrosis of alveolar epithelial cells, granuloma formation, lung fibrosis, and bronchiolisation and squamous metaplasia of alveoli. Further, in the rat, bronchiolar-alveolar adenomas and squamous proliferative cysts occur as well as keratinising and squamous cell carcinomas and adenocarcinomas (Warheit et al., 2016). Development of squamous lesions appear to be unique in rats and have not been observed or reported in any other species following chronic exposures. Schultz (1996) emphasises the importance of distinguishing between the nonspecific, primarily alveolar tissue impacts of particle overloading in the rat, from the more specific and relevant non-tumorigenic, interstitial loading effects that are common to occupationally-exposed humans (Schultz, 1996). Warheit and colleagues comment that the characterisation and histopathological identification of lung lesions and tumour types found in rats under conditions of PSP overload has been controversial. This was due to the uncommon nature of the pulmonary lesions seen, which were dissimilar from human pulmonary tumour-types and later judged to be proliferative keratin cysts (PKC) (Carleton, 1994; Levy, 1994) which are not seen in humans (Warheit et al., 2016).

4.2. Adverse outcome pathways

The OECD describe an adverse outcome pathway (AOP) as the '*sequential progression of events evolving in an organism from the first contact of a toxicant at the molecular level, via a subset of following key effects or biological responses to a final adverse outcome at the individual or population level*' (OECD, 2013). Although often presented as a linear cascade, one molecular initiating event in an AOP may lead to variable adverse outcomes, just as different molecular initiating events can cause the same adverse outcome. It should also be noted that although the adverse outcome is the result of a sequential cascade of biological events, each step in this pathway may itself be influenced by other pathways ongoing and/or dominating within the biological system of interest. It is therefore the intrinsic chain of causally linked biological events that determines the AOP, and in cases where species specific toxicodynamics can influence various possible MoAs, different phenotypic outcomes may be triggered (ECETOC, 2013).

With regard to lung overload effects following chronic inhalation exposure to PSPs, obvious species-specific differences exist in the adverse outcome. Although accumulation of particles in the deep lung is a common finding in all investigated species, there are significant differences in the adverse outcome between rats and all other mammalian species; that is, lung tumours are reported exclusively in rats, but not in mice, hamsters, non-human primates or humans. A conceptual AOP was proposed by the ECETOC TF (ECETOC, 2013), as detailed in Fig. 2. The impairment of pulmonary particle clearance is viewed as an initiating event, triggering induction of persistent neutrophilic pulmonary inflammation, apoptosis, generation of ROS/RNS and increased cell proliferation, which are viewed as intermediate events. The final adverse outcome *in rats* is considered to be lung tumour formation, however in other species non-neoplastic changes, including fibrosis, are the adverse outcome. Translocation processes may significantly influence the AOP in that different compartments of the biological system become affected (alveolar spaces versus interstitum).

4.2.1. AOP in the rat

An IARC Monograph meeting considered the pathways of biochemical and patho-physiological events in the rat under overload conditions of TiO_2 and carbon black and proposed a mode of action as outlined in Fig. 3 (reproduced from ECETOC, 2013).

The IARC Monograph meeting described a generic mode of action (MoA) in rats beginning with the deposition of particles within critical target cells or tissues within the lung leading to "sustained inflammation, production of reactive oxygen species, depletion of antioxidants and/or impairment of other defence mechanisms, cell proliferation and gene mutations." Further, whilst they acknowledged that some of the steps in Fig. 3 have been shown to occur in humans exposed to PSPs, the group concluded that "it is not known to what extent humans are susceptible to particle-induced lung cancers associated with titanium dioxide, carbon black or talc".

The National Institute for Occupational Safety and Health (NIOSH) also consider the key steps leading to lung tumour development in the rat as; particle-induced lung inflammation; oxidative stress; lung tissue damage; and epithelial cell proliferation, i.e. tumour formation is through a secondary genotoxic mechanism (NIOSH, 2011).

The ECETOC TF proposed that in particle-overload exposed rats, continuous exposures promote a scenario including enhanced transfer of particles to lymph nodes, accumulation of particles in the lung, increases in lung weight, alveolar macrophage accumulation, pulmonary inflammation, alveolar epithelial hyperplasia (proliferation) and metaplasia, fibrosis and eventually cancer. Importantly, they considered the tumourigenic response to particle overload conditions to be unique to the rat as this was not observed in mice, hamster, non-human primates and humans.

4.2.2. AOP in the mouse and hamster

Experimental studies that compare findings from inhalation studies with particles in the rat, mouse and hamster provide evidence that the rat model is the most sensitive to development of lung inflammation, cytotoxicity, fibro-proliferative effects, and septal fibrotic response under conditions of lung overload. In the mouse and hamster, although lung inflammatory responses can be detected under conditions of overload, the pulmonary tissue responses to this are substantially different than in the rat. The proliferative cell changes and fibrotic responses that are most likely to lead to secondary genotoxic changes and tumour formation are not present in the mouse and hamster. In addition, the hamster has a faster particle clearance mechanism which also contributes to the low inflammatory response to exposure to high doses of particulates. It should be noted that inflammation between species is not always the same and so much care should be taken in the interpretation of inflammatory markers and subsequent events. As an example, and most importantly, Carter et al. (2006) demonstrated that following inhalation exposure to carbon black particles, genes for anti-inflammatory mediators were expressed in the mouse and hamster, whereas genes for pro-inflammatory mediators were expressed in the rat. They concluded that these differences in pro- and anti-inflammatory responses may contribute to the apparent species differences in inflammation and tumourigenesis.

4.2.3. AOP in the primate

Although AOPs in primates exposed to poorly soluble particulates has not been clearly defined, a number of 2-year inhalation studies have been reported comparing rat and nonhuman primate pulmonary toxicity responses. Klonne et al. (1987) exposed monkeys and rats to petroleum coke at exposure concentrations of 0, 10 or 31 mg/m^3 for 24 months. At the end of the exposure period, the investigators reported that rats developed chronic inflammation, focal fibrosis, alveolar metaplasia and keratin cysts. In contrast, the response in monkeys was characterised as black discoloration concomitant with normal particle deposition and phagocytosis of the inhaled test material. Thus, the differences in rats and monkeys inhaling the same test materials at equivalent exposure concentrations, was a hyper-reactive and inflammatory response (rats) vs. normal physiological clearance responses (monkeys). MacFarland et al. (1982) exposed monkeys and rats to raw or processed shale dust at levels of 30 and 10 mg/m^3 (respirable dusts) respectively for 6 h per day, 5 days per week for 24 months.

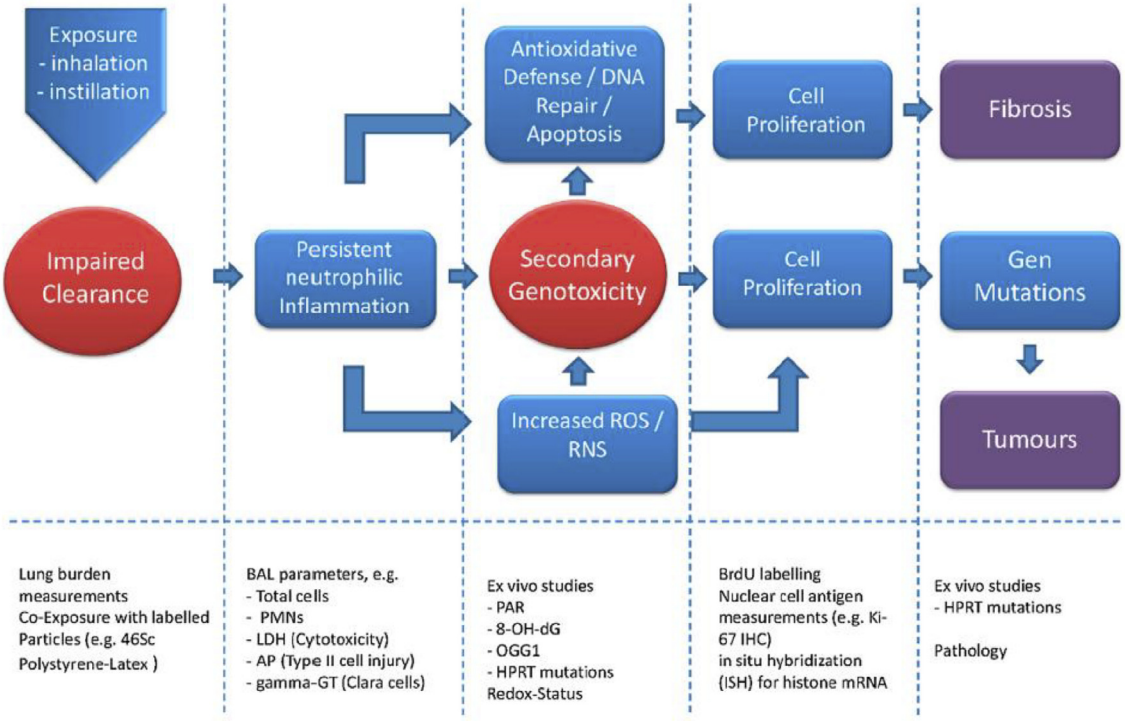

Fig. 2. Conceptual AOP model of lung overload in rats exposed to aerosols of PSPs.
Source – ECETOC (2013).

Following the end of exposure, the rats developed proliferative bronchiolitis/alveolitis, fibrosis and microgranulomas. In contrast, in monkeys little or no adverse pulmonary reactions were observed; normal pigment-laden macrophages were reported present as a consequence of pulmonary clearance. Finally, Lewis et al. (1986) and Nikula et al. (1997) exposed monkeys and rats to aerosols of diesel exhaust 2 mg/m^3 or coal dust 2 mg/m^3 for 24 months and analysed histopathological responses of lung tissues as well as morphometric analysis of particle distribution patterns in various anatomic compartments of the respiratory tract. The results indicated that in rats, the majority of particle retention was located in alveoli/alveolar dusts. In addition, significant alveolar epithelial hyperplasia and pulmonary inflammation was observed. In monkeys, the particle distribution was observed in the pulmonary interstitium, together with very limited pulmonary responses consisting primarily of normal clearance patterns. Overall, one can conclude that, using these comparative studies, the

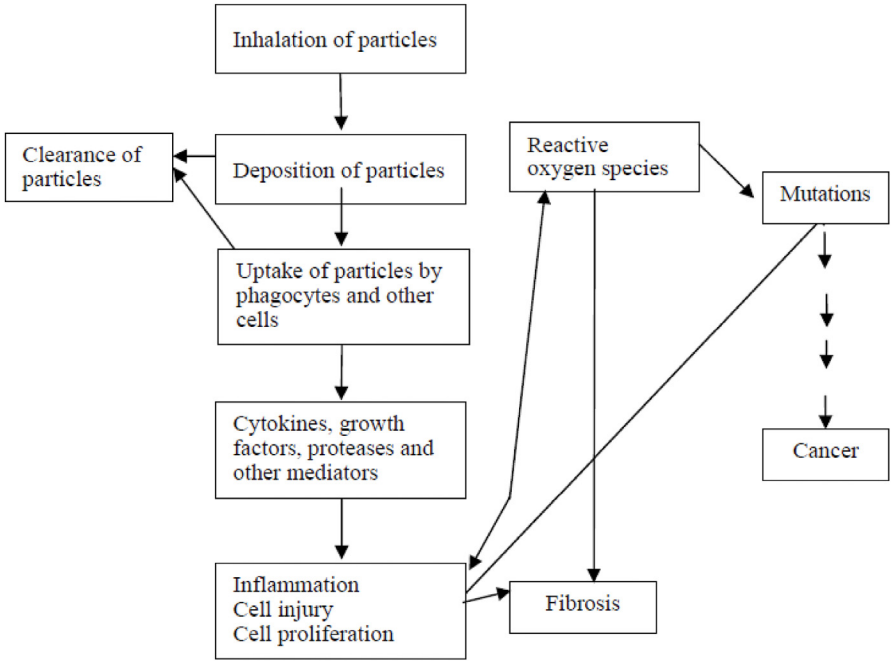

Fig. 3. Conceptual framework of carcinogenesis induced by poorly soluble particles in rats.
Source IARC, 2010.

AOP for monkeys represents a normal physiological clearance response to inhaled particulates; whereas rats exposed to the same concentrations of the same dusts developed hyper-inflammatory and hyper-reactive responses following chronic exposures to inhaled poorly soluble particulates.

4.2.4. AOP in the human

Chronic occupational exposures to high doses of PSPs such as TiO_2, carbon black particles, toner particles or coal dust do not result in lung cancers in humans (Green, 2000). The absence of tumourigenic responses in humans has been confirmed by data reported from a variety of epidemiological studies of > 50,000 occupationally exposed TiO_2, CB, and talc production workers (Warheit et al., 2016). The ECETOC TF considered that the studies by Nikula et al. (1997) provided essential information to help elucidate differential mechanisms between the pulmonary responses of rats and humans under conditions of high exposure to dusts. As detailed previously in Table 2, the authors reported lung morphological and morphometric responses under similar conditions of exposure, showing fundamental differences in particle retention patterns and lung tissue and cellular responses. Inhaled diesel soot was shown to be retained in the alveolar region in the rat, however in humans the main area of retention was the interstitial compartment, indicating a faster transfer from deposition sites through pulmonary epithelium, enabled by anatomical differences. These differences are also represented in the schematic below (Fig. 4).

In humans, the majority of particles which deposit on the respiratory bronchiole or alveolar surfaces are translocated to the interstitium, making them unavailable for sustained pulmonary inflammation or cell proliferation, or indeed other pathologic events that can lead to the formation of lung tumours (Warheit et al., 2016).

4.3. Biomodelling of respirable dust in humans

The International Commission on Radiological Protection (ICRP) – Respiratory Tract Model has been regarded as the standard dosimetric model of the human respiratory tract since it was adopted in 1994 (ICRP, 1994). It was recently updated to account for the transit of particles into the interstitial compartment, as around 40% of inhaled/deposited particles have been estimated to be sequestered there (Gregoratto et al., 2010, 2011) which had caused underestimation of lung retention burden of PSPs. Warheit and colleagues (Warheit et al., 2016) highlight the excellent degree of correlation between the Gregoratto modified model findings and those in rats and non-human primates/humans reported by Nikula et al. (2001). The authors also comment that the retention pattern of retained particles in interstitial sites of coal workers results in pulmonary reactions initially occurring within the interstitial compartments, which has been shown in coal workers with pneumoconiosis and pulmonary anthracosis. The site of reaction is therefore away from the alveolar regions, unlike in the lungs of PSP exposed rats, which may explain the negative findings of lung tumours in epidemiological studies (Warheit et al., 2016).

4.4. Consideration of implications for regulatory guidelines

Although the 'lung overload' phenomenon has been recognised for decades, it has recently become more prominent due to its impact on regulatory decision making. For the calculation of DNELs under REACH registrations, ECHA cautions the interpretation of data for PSPs, particularly with regards to the mechanism behind observed pathogenic effects (ECHA, 2012a). In addition, ECHA also notes, that "*effects occurring secondary to a threshold stimulus such as inflammation … could also be considered threshold in nature and as such a DNEL can be derived" and that* " a possible example of such a driver is the induction of lung overload in experimental animals exposed to poorly soluble low toxicity (nano)particles leading to inflammation, oxidative stress and culminating in lung tumour formation" (ECHA, 2012b).

Hazard classification systems such as the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS) and the EU implementation law CLP have direct regulatory consequences, whilst others such as the IARC and the German MAK cancer

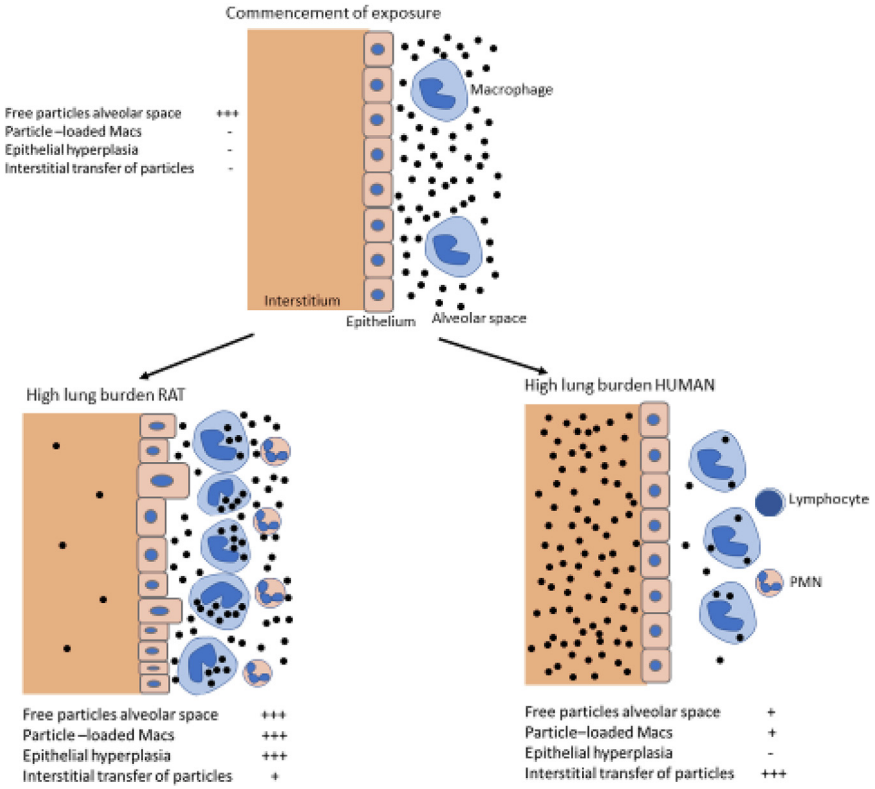

Fig. 4. Schematic of rat and primate/human particle overload.

classifications inform regulators for classification and labelling decisions or the setting of occupational exposure limits. However, all of these systems are hazard-based and utilise findings from animal studies, most often carried out in the rat. Whilst human data can also be used to establish hazard, substance/chemical-specific good quality human studies are not always readily available. Due to the unique sensitivity of the rat to lung overload related effects, both the ILSI and ECETOC Working Groups questioned whether the rat is an appropriate model for the extrapolation of `lung overload' related pulmonary effects to humans. Further, ECETOC highlighted the limited consideration given under existing regulatory frameworks to rat-specific overload responses, with only the EU CLP Regulations identifying the hazard of 'specific target organ toxicity following repeated exposure' (STOT-RE) the condition of rat lung overload as a mechanism which may not be relevant to humans. For carcinogenicity classifications, neither the UN GHS nor the EU CLP Regulation recognise the lung overload condition as a rat-specific mechanism of toxicity (ECETOC, 2013).

For carcinogenicity classification, the UN GHS and EU CLP give only limited guidance as to the consideration of the tumourigenic effects of PSP in experimental animals, following inhalation under the conditions of lung overload. Strength of evidence should be determined according to IPCS guidelines and of particular note are: (1) progression of lesion to malignancy; (2) possibility of a confounding effect of excessive toxicity at test doses; (3) mode of action and its relevance for humans (e.g. secondary mutagenicity, cytotoxicity with growth stimulation, mitogenesis, immunosuppression). UN GHS notes that 'if a mode of action of tumour development is conclusively determined not to operate in humans, the carcinogenic evidence for that tumour may be discounted following expert review and weight of evidence analysis' – i.e. lung tumours in rats with PSPs (ECETOC, 2013).

Although IARC does not go so far as to specifically consider the mechanism of action of lung overload in rats as a species-specific effect, and therefore not relevant to humans, IARC has recognised this issue in the assessments of carbon black, titanium dioxide and talc. IARC has proposed a conceptual framework of carcinogenesis induced in the lungs of rats (Fig. 3) after high exposures to PSPs (IARC, 2010).

The German MAK-Commission has defined five groups of carcinogens based on mode of action and has also identified carcinogenic substances for which a threshold can be established. Granular persistent dusts (equivalent to PSPs) have been classified as Category 4 carcinogens – 'substances with carcinogenic potential for which a non-genotoxic mode of action is of prime importance; no significant contribution to human cancer risk is expected at MAK and BAT values'. Justification is the evidence that inhalation exposure to high levels of GBS leads to inflammation in bronchial and alveolar region with associated release of free radical species leading to tumour formation in the rat. Importantly the MAK-Commission concludes that the effects seen in the rat are also applicable to humans (Hartwig, 2014) – however, this has been disputed (Morfeld et al., 2016).

Morfeld et al. (2013) contend that the MAK classification depends highly on debatable assumptions and calculations on lung particle overload. They argue that the total surface area of particles retained in the lung is the more appropriate metric for the inflammation and impaired clearance function (Borm et al., 2015). In contrast, Pauluhn (2014) argues that the volumetric particle dose and the AM pool has driven existing mechanistic information because this determines the percentage of macrophage volume that is displaced by particles, leading to overload and associated impaired clearance function. However, Pauluhn also acknowledges the importance of surface area and, particularly, particle surface chemistry and associated potential for particle dissolution, in the development of host responses. Volume alone may therefore not be the only critical parameter that determines the likelihood of a carcinogenic potential of PSPs (Borm et al., 2015).

Borm et al. (2015) also comment that Pauluhn (2014) and Morfeld et al. (2012) cite the earlier papers by Cullen et al. (2000) and Tran et al. (2000) which detail effects, deposition and retention kinetics and lung lavage neutrophil data of inhaled $BaSO_4$ and TiO_2 particles in rats. These studies are considered to provide an important contribution to the overload discussion by viewing accumulation and retention kinetics of the inhaled particles and also through determining *in vivo* particle dissolution rates. However, in their evaluations of these studies, Pauluhn (2014) and Morfeld et al. (2012) come to different conclusions about the data. Whilst Morfeld et al. (2013) supported the conclusions of Cullen et al. (2000) and Tran et al. (2000) that particle surface area rather than volume or mass was the appropriate metric to confirm that both particle types are PSP particles, Pauluhn et al. (2014) highlighted the importance of consideration of an enhanced dissolution of $BaSO_4$ when fitting the data of the accumulation phase. Borm et al. (2015) conclude that studies aimed at generating new insights regarding the fate of phagocytised particles in AM of rodents and primates are required to advance the thinking about lung particle overload. In addition, Morfeld et al. (2016) strongly emphasise the abundant negative findings of a lung cancer excess in studies in coal miner, carbon black and TiO_2-production workers which support the uniqueness on the rat lung tumour findings and links back the very different fate of inhaled particles between rats and humans.

More recently, Pauluhn (2018) and Li and Pauluhn (2018) have reported the use of prospective dosimetry kinetic modelling to predict the no observed adverse effect level (NOAEL) and maximum tolerable dose (MTD) for chronic repeated inhalation exposure of rats to the PSPs, nano-CeO_2 and multi-walled carbon nanotubes (MWCNT) and black iron oxide (Fe_3O_4, magnetite) respectively. The model is based on the hypothesis that increasing volume density prompts kinetic lung overload and is proposed by the authors as a useful tool to predict shortcomings in experimental design and/or AOPs. However, it still needs to be verified whether this model is appropriate to humans.

5. Conclusions and recommendations

Following evaluation of scientific evidence available at that time (2013), the ECETOC TF concluded the following with regards to lung overload:

- The rat represents a particularly sensitive model concerning the development of pulmonary non-neoplastic lesions and, moreover, a unique model with regards to lung neoplastic responses under conditions of lung overload;
- Lung tumours have to be regarded as the final phenotypic adverse outcome only in rats, whereas in other species – non-neoplastic lesions seem to be the respective adverse outcome;
- Humans are less sensitive to lung overload as epidemiological studies thus far have not been able to detect an association between occupational exposures to poorly soluble particles of low toxicity and an increased risk for lung cancer.
- The divergence in the largely common mechanistic sequence of the adverse outcome pathway may be related to a biological diversity of detoxification systems, especially in species specific anti-oxidant defences resulting in a more pro-inflammatory environment in rats compared to a more anti-inflammatory environ in other rodent species.
- The measured differences of particle retention, distribution and clearance patterns in the lungs of exposed rats vs. primates or humans, may account for both the greater sensitivity in rats and corresponding differences in pulmonary pathological responses to long-term particle exposures.
- Slight differences in the bio solubility of deposited poorly soluble particles in biological fluids may influence chemical dissolution and based hereupon accelerate or slow down the process of overload development;
- Independent of particle size, inhalation exposure to high concentrations of low soluble particles of low toxicity are eliciting comparable localised pulmonary toxicity via process that are pro-

inflammatory in nature, causing oxidative stress and a persistent pulmonary inflammatory response.
- The mechanisms leading to an oxidative and inflammatory pulmonary status are clearly threshold related;
- There is no nanoparticle-specific lung overload toxicity and mechanistic findings for conventional micro-particles apply also for nanostructured particles.

A crucial additional point that could be considered to be added to this list concerns interstitialisation as a key driver for both clearance and retention in humans rather than alveolar macrophage clearance as in the rat.

In addition to the key findings of the ECETOC Task-Force, the relevance of particle overload related lung tumours for human risk assessment following chronic exposures of PSPs has been explored further here. We would suggest that rat lung tumour responses are not relevant for human risk assessment based on the following criteria: 1) inhaled particulates in humans translocate to interstitial sites and clearly have a different mechanism of action when compared to particle-overloaded rats. In rats, the AOP is well defined with the common features including: macrophage particle overload, chronic lung inflammation, cell proliferation, septal fibrosis, secondary genotoxicity, metaplasia, bronchoplasia, keratin cysts, and subsequently the development of lung tumours. In humans, particle overload in coal miners leads to coal workers pneumoconiosis – but not lung tumours; 2) Differential functions of macrophages and alveolar epithelial cells in rats (stimulating inflammogenic and cell proliferative responses) vs. M1 (alveolar/respiratory bronchioles) and M2 (interstitial macrophages) which are wound healing but not tumour promoting macrophages.

There are other examples of species-specific tumours that are not viewed as applicable for humans. Moreover, it is important to note that these tumours that are known to occur in rats are not accepted at the regulatory level for humans – meaning that they are known to occur in experimental hazard-type studies using rats – but are not viewed as pertinent for humans. Some examples include rodent tumours induced by peroxisome proliferators and renal tubule tumours in male mice mediated through α-2 μ globulin.

A key question that may arise from our discussions is how to assess potential cancer risk in humans from PSPs if lung cancer seen in the rat inhalation model findings cannot be utilised. Unfortunately, the science/technology does not permit the implementation of any *in vitro* studies, other than acute studies; even 1-week or 4-week toxicity studies are difficult using *in vitro* methodologies as the cell-types cannot be grown and maintained for extended periods. Moreover, the formulation of particles for delivery to cells is also problematic. Developments to allow implementation of longer-term studies are being made but we are many years away from developing true longer-term *in vitro* studies to model *in vivo* inhalation studies (Warheit, 2018). The application of a battery of *in vitro* investigations could provide some useful mechanistic insights into toxicity pathways, although several shortcomings to current *in vitro* methodologies need to be addressed first. Optimising the relevance of *in vitro* studies to real-world toxicity considerations would require the utilisation of experimental designs that require dose-response behaviours over a full range of doses and should also require the duration of exposure using relevant cell types (focussing on route of exposure regimens), including time-course evaluation concomitant with comparisons and validation with corresponding *in vivo* systems (Warheit, 2018).

It could be viewed that as the ECETOC TF concluded that the rat is a unique model with regards to lung cancer, as no other species was seen to develop lung tumours at comparable inhalation exposure, the key question of alternatives to the rat model may be unanswerable. There may not be a need to identify or develop a better model for the human situation, as the end point of exposure in humans may not be tumour formation. In addition, it is the view of the authors of this paper that the rat model could be better used to predict risk of exposure in humans if the primary event in the AOP of inflammation is used as a benchmark. By preventing the development of inflammatory responses in the lung, all subsequent key events in the AOP including AM and neutrophil accumulation, hyperplasia, metaplasia, secondary mutations and, in rats, lung tumours will also be prevented. By focussing on inflammation as a primary key event, well designed targeted studies using bronchoalveolar lavage fluid (BALF) (as described in ECETOC, 2013) could provide information concerning thresholds of response. The applicability of using the shorter-term endpoint of inflammation in rats is also supported in a recent discussion paper in which a group of eminent scientists discussed the utility of animal models in general, and particularly those of rats and mouse origins, for reflecting human hazards (Aschner et al., 2016). The authors of that paper are broadly supportive of findings from short-term animal studies of < 90 days, as they consider that adverse effects occurring within that time frame '*concern basic and stable physiologic mechanisms that are conserved in many species including humans, and are not significantly overcome by transient random disturbances*'. However, for chronic exposure studies that assess effects occurring over the long-term, including cancer and neurological deficits, Aschner et al., do not consider animals to be appropriate surrogates due to the impact of species-specific causal opportunities. In our opinion, the views of Aschner and his colleagues are consistent with those discussed in this paper. However with the current absence of an alternative model, should or where regulatory requirements necessitate the assessment of chronic exposure scenarios for PSPs, we feel that the rat inhalation model conducted at reasonable, non-overload particle concentrations is a pragmatic solution. In such cases, it is our belief that the alternative end-point of inflammation could be assessed in studies of 90 days duration, or even less, and would be sufficient for risk assessment and risk management considerations.

Our findings both support and update those reported by the ECETOC TF (ECETOC, 2013). Here, we have had highlighted that the predominant interstitialisation of inhaled respirable PSPs in humans is the probable key initial driver which precludes the marked alveolar responses seen in rat lung. The rat-specific cascade of inflammatory events can lead to lung cancer, however this does not appear to happen in humans as evidenced by the extensive epidemiological data in coal miners and other occupational groups chronically exposed to PSPs.

Conflicts of interest

One of the authors (DBW) is employed by a company that manufactures and sells titanium dioxide particles. LSL is a member of the Scientific Advisory Group (SAG) that provides scientific opinion to the International Carbon Black Association (ICBA). RK is scientific advisor to VCI Steering Committee TiO2.

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Transparency document

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