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PII: S0300-483X(16)30292-X
DOI: http://dx.doi.org/doi:10.1016/j.tox.2016.11.013
Reference: TOX 51788

To appear in: *Toxicology*

Received date: 1-8-2016
Revised date: 16-11-2016
Accepted date: 17-11-2016

Please cite this article as: Warheit, D.B., Kreiling, R., Levy, L.S., Relevance of the Rat Lung Tumor Response to Particle Overload for Human Risk Assessment—Update and Interpretation of new data since ILSI 2000.Toxicology http://dx.doi.org/10.1016/j.tox.2016.11.013

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Toxicology, Volume 374, 30 December 2016, pp. 42–59
DOI:10.1016/j.tox.2016.11.013

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Revised 11-16

Relevance of the Rat Lung Tumor Response to Particle Overload for Human Risk Assessment – Update and Interpretation of new data since ILSI 2000

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ABSTRACT

The relevance of particle-overload related lung tumors in rats for human risk assessment following chronic inhalation exposures to poorly soluble particulates (PSP) has been a controversial issue for more than three decades. In 1998, an ILSI (International Life Sciences) Working Group of health scientists was convened to address this issue of applicability of experimental study findings of lung neoplasms in rats for lifetime-exposed production workers to PSPs. A full consensus view was not reached by the Workshop participants, although it was generally acknowledged that the findings of lung tumors in rats following chronic inhalation, particle-overload PSP exposures occurred only in rats and no other tested species; and that there was an absence of lung cancers in PSP-exposed production workers. Since the publication of the ILSI Workshop report in 2000, there have been important new data published on the human relevance issue. A thorough and comprehensive review of the health effects literature on poorly soluble particles/lung overload was undertaken and published by an ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) Task Force in 2013. One of the significant conclusions derived from that technical report was that the rat is unique amongst all species in developing lung tumors under chronic inhalation overload exposures to PSPs.

Accordingly, the objective of this review is to provide important insights on the fundamental differences in pulmonary responses between experimentally-exposed rats, other experimental species and occupationally-exposed humans. Briefly, five central factors are described by the following issues.

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1) Interspecies differences in lung responses of rats vs. other rodents, triggering different adverse outcome pathways (AOPs);
2) Interspecies differences in inhaled particle kinetics in rats vs nonhuman primates and humans triggering differential particle-related pulmonary responses.
3) Advanced and updated human respiratory tract deposition and retention models allowing more realistic particle translocation/retention estimates.
4) Differences in morphologies and characterizations of rat vs. human pulmonary tumor types and locations within the respiratory tract.
5) Comprehensive in-depth analysis of available epidemiological data from PSP production workers that demonstrate no correlation between particle exposures and lung cancers or other non-malignant respiratory diseases.

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Focusing on these five interrelated/convergent factors clearly demonstrate an inappropriateness in concluding that the findings of lung tumors in rats exposed chronically to high concentrations of PSPs are accurate representations of the risks of lung cancer in PSP-exposed production workers. The most plausible conclusion that can be reached is that results from chronic particle-overload inhalation studies with PSPs in rats have no relevance for determining lung cancer risks in production workers exposed for a working lifetime to these poorly soluble particulate-types.

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Keywords: Particle overload;
Species differences; Adverse Outcome Pathway;
Poorly Soluble Particles;
Nonhuman primates
Rats

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Introduction

Particle overload describes a condition of impaired macrophage mediated clearance of particles in the lung following prolonged high-dose exposures to poorly soluble particles (PSP) of low inherent toxicity. The term “overload” was first described by Bolton and colleagues in studies on pulmonary clearance of inhaled asbestos fibers in rats (Bolton *et al.,* 1983). However, the pioneering studies of Morrow (1988) clearly have demonstrated that particle overload and toxicological implications occur when the ability of pulmonary macrophages to clear particles are impaired due to an excessive dust load in the lung. Morrow suggested that this particle overload and reduced/lack of pulmonary clearance could be quantified by assessing volumetric kinetics. Wolff *et al* (1987) reported on alterations in particle accumulation and clearance in rats chronically exposed to diesel engine exhaust particles (DEEP). In this study, groups of rats were chronically exposed to 0, 0.35, 3.5 or 7.0 mg/m^3 concentrations of DEEP. Parallel measurements of particle deposition and clearance were conducted to assess mechanisms of particle accumulation in the lungs. Following 24-month exposures, it was reported that long-term clearance half-times were 81 days (0.35 mg/m^3), 264 days (3.5 mg/m^3) and 240 days (7.0 mg/m^3) for the respective exposure groups. The investigators concluded that lung burdens of diesel soot at the 3.5 and 7.0 mg/m^3 exposure levels were associated with impaired clearance. Oberdorster (1995) demonstrated pulmonary overload in rats exposed subchronically to TiO_2 particles; and Warheit *et al. (1997),* exposed groups of rats to 5, 50 or 250 mg/m^3 pigment grade TiO_2 particles for 4 weeks and evaluated pulmonary clearance

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patterns for 6 months postexposure (Figure 1). Lung burden and biokinetic studies demonstrated that clearance of TiO_2 particles was significantly retarded following exposures to 250 mg/m^3 TiO_2. The calculated $T_{1/2}$ clearance rates for the various exposure concentrations were the following: TiO_2 – 5 mg/m^3 = 68 days; TiO_2 - 50 mg/m^3 = 110 days; TiO_2 -250 mg/m^3 = 330 days.

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. Based upon mechanistic information obtained both in chronic and subchronic inhalation studies, a sequence of cellular and pathobiological effects can be measured at various time points postexposure or at various times of continued repeated exposure and are evidenced by progressive cellular processes and pathological sequelae commencing with macrophage overload, concomitant with sustained pulmonary inflammation and cytotoxicity; and ultimately progressing to unique tumorigenic responses to particle overload exposures not observed in any other species (e.g. mice, hamster, non-human primates, humans). A total mechanistic understanding of cellular events in terms of species differences has not been fully established. However, factors such as persistent inflammation and epithelial cell proliferation appear to be the dominant mechanistic drivers in all species under lung overload conditions, whereas additional rat-specific factors lead to tumor development only in this species (Warheit et al., 1997; ECETOC, 2013).

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According to NIOSH (2011), chronic pulmonary inflammation is characterized by increased and persistent numbers of inflammatory cells, specifically neutrophils. Neutrophils are recruited from the pulmonary vasculature in response to chemotactic stimuli emanating from pulmonary macrophages and alveolar epithelial cells and this process becomes sustained or persistent following prolonged particle exposures. Particle-induced lung inflammation, oxidative stress, lung tissue damage and epithelial cell proliferation are considered to be key steps leading to lung tumor development in the rat acting through a secondary genotoxic mechanism (NIOSH 2011; Knaapen *et al.*, 2004; Baan, 2007, Wagner et al., 1969).

The term poorly soluble particulates (PSPs) is defined herein as particles that have dissolution half-lives measured in artificial lung fluids longer than macrophage mediated clearance times (e.g., TiO_2, carbon black, talc, and toner particles). As a consequence, macrophage clearance and not particle dissolution would determine particle residence time in the lung. PSPs are also viewed as particles with low toxicity. This definition of PSPs differentiates these particle-types from other particulates that are cytotoxic, such as quartz (crystalline silica), which exhibit significant surface-related cytotoxicity (ECETOC, 2013). The ILSI Working Group (ILSI, 2000) identified PSP materials as talc, titanium dioxide, carbon black, coal, and diesel soot. In this workshop document it was noted that inhalation exposures to these specific particle-types share several common features of the rat lung response to chemically distinct, poorly soluble, nonfibrous particles of low acute toxicity. Moreover, chronic inhalation studies of PSP can result in pulmonary inflammation, fibrosis, epithelial hyperplasia, and in most

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instances, adenomas and carcinomas in the distal regions of the respiratory tract, but only in rats.

In 2006, the International Agency for Research on Cancer (IARC) Working Group re-evaluated the carcinogenic hazards of three different, low toxicity, poorly soluble particulates (PSP), namely titanium dioxide, carbon black and talc particles (IARC, 2010). In its preamble, IARC maintains that its Monographs represent the first step in "carcinogen risk assessment". According to IARC, a carcinogenic "hazard", is defined as an agent that, upon exposure, may cause cancer under some circumstances. In contrast, IARC defines a cancer "risk" as an estimate of the carcinogenic effects expected from exposure to a carcinogenic hazard. IARC maintains that the Monographs identify cancer hazards even when risks are very low at current exposures (IARC, 2010).

IARC defines in its preamble a cancer "hazard" as an agent that is capable of causing cancer under some circumstances, while a cancer "risk" is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. Cancer hazards are identified even when risks are very low at current exposure levels, because new uses or unforeseen exposure could engender risks that are significantly higher. IARC also states that "The classification indicates the weight of evidence as to whether an agent is capable of causing cancer (technically called hazard), but it does not measure the likelihood that cancer will occur (technically called "risk") as a result of exposure to the agent (IARC, 1996).

The general format for the IARC classifications is designed to combine assessments of epidemiological data along with the results of experimental

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cancer studies in animals and any supporting information such as mechanistic data and genotoxicity (see IARC preamble). The general IARC classifications on cancer hazards for titanium dioxide and carbon black particles were similar in scope (IARC 2010) – i.e., possibly Carcinogenic – 2B. Numerous published epidemiological studies investigating TiO_2 exposed production workers demonstrated no correlation between TiO_2 lifetime work exposures and lung cancer risk. Accordingly, the IARC Working Group concluded that there was an *inadequate evidence* of carcinogenicity in exposed humans. In contrast, the results from two chronic rat inhalation cancer studies with titanium dioxide produced lung tumors and were considered to provide *sufficient evidence* of carcinogenicity. Given the conclusion of "*inadequate*" for humans and "*sufficient*" for rats, the combined, final "hazard" classification was - possibly carcinogenic to humans, Group 2B. Indeed one might conclude that in developing their final classification scheme, IARC provides equivalent weighting to particle overload studies in rats with working lifetime epidemiological studies in PSP production workers (IARC, 2010). However, this approach of overall possibly carcinogenic to humans, Group 2B, seems to be the default classification wherein there are such evaluations for epidemiological and animal evidence.

A variety of epidemiological studies published in both carbon black (CB) production workers as well as in the rubber industry, were judged to be negative for lung cancer risks; and similarly were concluded by the IARC Working Group to constitute *inadequate evidence of carcinogenicity*. In contrast, two-year, chronic inhalation studies in rats exposed to CB particles produced lung tumors and met the criteria for experimental studies to provide *sufficient evidence* of carcinogenicity. Accordingly, when combining the

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human epidemiological data with experimental carcinogenicity data according to the IARC formula, the Working Group classified inhaled carbon black particles as *possibly carcinogenic to humans*, Group 2B.

The use of the reliance on positive rat particle overload inhalation studies when considering poorly soluble particulates in the face of an abundance of negative findings from well-conducted epidemiological investigations in workers has important ramifications for carcinogenic hazard classifications As noted above and described in detail below, the overwhelming evidence is that the rat is the only species that develops lung tumors following long-term inhalation particle overload studies (Mauderly, 1997; Heinrich et al., 1995; Wehner et al., 1979; ILSI, 2000). As will be discussed below, the chronic inhalation toxicity/cancer findings with PSPs are negative for other rodent species (i.e., mice and hamsters). Moreover, comparisons of pulmonary responses in rats vs. nonhuman primates exposed for 2 years to aerosols of low solubility particles such as shale dusts, petroleum coke and diesel exhaust particles demonstrated significant disparities between the pulmonary responses of the two species responses to inhaled dusts– i.e., biokinetics/particle distribution and pulmonary inflammatory responses to the inhaled dusts – with the nonhuman primates being very low responders in comparison to the hyper-responsiveness of rats. It should also be noted, however, that the two year duration represents a much smaller fraction of the lifespan for non-human primates than for rats.

In our opinion, the IARC classification scheme and mechanism for identifying cancer hazard agents, has limited utility for identifying PSP carcinogenic risks in humans, particularly when there exists a clearly identifiable discrepancy between experimental carcinogenicity results in a

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distinctly sensitive species (i.e., the rat) – contrasted both with the results of experimental inhalation studies in monkeys, and numerous epidemiology findings in long-term exposed production workers. In addition, the validity of the epidemiology findings in PSP-exposed humans are substantiated when considering other approaches to particle inhalation kinetics and responses in nonhumans primates and humans. These include 1) histopathological/morphometric findings in the lungs of nonhuman primates and coal workers demonstrating a significantly different biokinetic/particle distribution pattern of inhaled dusts compared to exposed rats (i.e., greater translocation of particles to interstitial sites in monkeys/humans vs. rats – whereas inhaled/deposited particles remain within lung macrophages on alveolar surfaces (see Figures 2-6); and 2) confirmation of significantly enhanced particle transmigration to interstitial sites in humans, (due in part to anatomical differences) using newly updated ICRP modeling. Accordingly, these findings; employing totally diverse approaches provide convergent results that help explain, in part, the epidemiological conclusions which demonstrate an absence of neoplastic effects in particle-exposed humans.

The conclusion of a questionable relevance and inappropriateness of using rat data as a model for the estimation of human neoplastic pulmonary response was also recognized previously by other scientific committees which had taken the view that only the rat lung tumors were reported in that species.. Indeed, the US Presidential and Congressional Commission on Risk Assessment and Risk Management (CRARM) considered that it was wasteful to expend limited risk assessment resources, risk management time and public and legal involvement revisiting the issue of human relevance of

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the specific response chemical by chemical. The CRARM specifically identified TiO_2 particles as one such chemical because observed rodent tumor responses associated with exposure to TiO_2 particles are not relevant to human risk. (Presidential/Congressional Commission on Risk Assessment, 1997). The Health Effects Institute, also has concluded that the rat data should not be used for assessing human lung cancer risk from diesel-exhaust exposure (Health Effects Institute, 1995). Finally, based on the clear pulmonary differences in outcomes documented when comparing rats and humans exposed to PSPs, it is clear that mechanistic data should be used for risk assessment purposes only when they are more likely to represent a mechanism operative to humans at plausible levels of exposures (McClellan, 1997).

In 1998, the International Life Sciences Institute (ILSI)- Risk Science Institute convened a workshop designed to gauge the relevance for human risk assessment of the aforementioned rat lung tumor response to particle overload. It was well known at that time that chronic exposures of rats to poorly soluble particles (PSPs) such as TiO_2 and CB produced tumors under conditions occurring when particle deposition overwhelmed lung clearance mechanisms, resulting in a condition termed “particle overload”. It was also known that PSPs did not produce tumors following chronic exposures in mice or hamsters. In addition, the available epidemiological data in the highest exposed PSP production workers were negative for lung cancer. The two main objectives of the Workshop were 1) to provide guidance for human risk assessment with respect to lung cancer and other non-neoplastic pulmonary changes when considering exposures to PSPs; and 2) to identify important data gaps in our understanding of pulmonary responses of rats and other

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species to PSPs. The Workshop participants concluded that there was uncertainty regarding whether high lung burdens of PSPs could lead to lung cancer in humans via mechanisms similar to those of the rat. Therefore, taking a conservative and precautionary view, it was argued that, in the absence of mechanistic data to the contrary, it should be assumed that the rat model may identify potential carcinogenic hazard to humans. Finally it was concluded that the apparent responsiveness of the rat model at overload conditions is dependent on persistent/coexistent chronic active inflammation and cell proliferation. As a consequence, at lower doses where chronic active inflammation and cell proliferation are not present, no lung cancer hazard for human workers was anticipated. These Workshop conclusions were published in 2000 (ILSI, 2000).

In 2013, an ECETOC (European Center for Ecotoxicology and Toxicology of Chemicals) (ECETOC, 2013) Task Force issued a Technical report (No. 122) entitled “Poorly soluble Particles/Lung Overload”. The ECETOC Task Force was convened to examine the current scientific standing of the “lung overload” hypothesis – including identifying and reporting new findings on lung toxicity of PSPs and corresponding mechanistic interpretation; concomitant with analyzing the progress made (since 2000) on elucidating the mode(s) of action for the lung responses reported in rats, and its relevance for human risk assessment. Clearly this is a critical question when evaluating the health risks related to chronic human exposures to PSPs.

A number of conclusions were derived from this thorough investigation of the literature by this ECETOC Task Force, which form the basis for this review paper, and are briefly detailed below. The major findings of the Task Force were:

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- The rat represents a unique and particularly sensitive model with regard to lung tumor responses following chronic particle overload exposures;

- Lung tumor responses are regarded as the final phenotypic adverse outcome pathway only in rats but not in other similarly exposed species;

- Numerous human epidemiology studies conducted in occupationally exposed workers to PSPs have demonstrated no association between exposures and increased risk for lung cancer;

- Particle disposition, retention and clearance patterns in the lungs of primates or humans are fundamentally different from rats and could account for differences in lung pathological responses following chronic exposures to PSPs.

The ECETOC Task Force concluded that there have been no compelling weight-of-evidence studies published since the conclusions of the ILSI Workshop conclusions that would demonstrate that the rat lung overload results are a reliable predictive model for human risk assessment for lung cancer (ECETOC, 2013).

When considering what has changed in our scientific knowledge-base since the ILSI 2000 publication, it would be prudent to focus on relevant data published since the ILSI publication as well as to re-emphasize important issues (e.g., differences in particle disposition/retention patterns and pulmonary response findings in comparative studies of similarly exposed rats vs. nonhuman primates) that were not available at the time of the meeting.

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Below are “5 factors” or features highlighting differential response data between rats and other species from comparative studies focusing particularly on those those that have been published since the 2000 ILSI Workshop, which provide, in our opinion, compelling evidence that the pulmonary tumors reported in particle-overload studies with PSPs in rats are not relevant for humans:

1) Data and findings from three subchronic, 90-day interspecies rodent inhalation studies provide convincing mechanistic justifications to better understand the differences in cellular responses to particle overload exposures when comparing rats vs. mice or hamsters. In addition, a conceptual AOP (Adverse Outcome Pathway) scenario has been developed (ECETOC, 2013) for the rat pulmonary response to particle-overload leading to lung tumors that is substantively different from pulmonary responses demonstrated in particle-exposed mice or hamsters and/or in either nonhuman primates or coal workers. See Table 1 for a summary of rodent species differences.

2) Several 2-year inhalation studies conducted primarily in the 1980’s and 1990s compared the effects of similar or identically exposed rats and monkeys to a variety of low solubility dusts, such as shale dust, petroleum coke dust and diesel exhaust particles (Wagner *et al.,* 1969; MacFarland *et al.,* 1982; Klonne *et al.,* 1987; Lewis *et al.,* 1989; Nikula *et al.,* 1997). In every case, the lung cellular responses of rats exposed chronically to particles were considered hyperinflammatory and hyperplastic; while the pulmonary responses in monkeys were limited to general, normal physiological effects (particle accumulation, macrophage responses) to inhaled particles. In addition, morphometric studies reported by Nikula *et al*., 1997 were developed to investigate the distribution patterns of inhaled particles in both chronically-exposed rats and cynomolgus monkeys. The results demonstrated that the majority of inhaled particles that deposited in the distal regions of the lung had transmigrated to interstitial anatomical compartments of the lung of nonhuman primates. In contrast to the pulmonary responses and particle distribution patterns measured in monkeys; in diesel and coal dust exposed rats, inhaled particles that had deposited were retained primarily on alveolar surfaces and

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subsequently stimulated active inflammatory responses**.** In another set of morphometric studies assessing the particle disposition pattern in deceased coal miners, particle distribution patterns similar to cynomolgus monkeys were measured. In this regard, most of the coal particles had translocated to interstitial sites (Nikula *et al.,* 2001).
See Tables 2 -3 for a summary of species differences in comparing rat lung responses to those of nonhuman primates and/or coal workers.

3) The ICRP – Human Respiratory Tract Model has been an internationally-recognized standard model to estimate the deposition, clearance and retention patterns for workers in the nuclear and coal dust industry. The model has been updated and revised by Gregoratto and coworkers (2010) to demonstrate that a greater proportion of inhaled low solubility dusts translocate from alveolar/respiratory bronchiolar sites of initial particle deposition to interstitial sites. This updated revision has important implications for lung clearance and retention estimates of inhaled particles and supports species differences in particle distribution patterns; including the finding of enhanced translocation to the interstitium. The impact of the model supports increases in the retention time of particles in the human lung. It is also noteworthy that the finding of enhanced transmigration rates in these models also correlates well with the morphometric findings reported by Nikula *et al*. (2001) in particle-exposed nonhuman primates and coal workers.

4) Fundamental differences have been recognized by human and veterinary pathologists when considering the characterization and location of tumor types in rats chronically exposed to PSPs vs. humans exposed to cigarette smoke or asbestos fibers. First, many PSP-induced rat neoplasms are species-specific entities that are consistently observed only in particle overload instances. Furthermore, there is no known documentation of human production workers developing lung cancers following exposure to PSPs. Moreover, the types of lung tumors characterized in humans exposed to cigarette smoke or asbestos fibers – occur primarily in the bronchiolar regions of the respiratory tract and do not have the “squamous or keratinizing” features of rat lung tumors which are more prominent in these regions of the lung following chronic exposures to PSPs. It generally is acknowledged that comparing asbestos and cigarette smoke-induced tumors in humans to PSP-induced neoplastic entities in the rat

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probably does not contribute meaningfully to comparisons of cancer risk of PSPs, as the lungs differ in morphological aspects such as the presence (humans) and absence (rodents) of a respiratory bronchiole (Schultz, 1996). Nonetheless, it should be recognized that cystic keratinizing tumors of rats arise very differently than squamous lesions in humans and appear to be adaptive versus true neoplastic changes (Schultz, 1996; Green, 2000).

5) Updated epidemiology studies on TiO_2, CB, and toner production workers demonstrate no association between working life-time exposures to PSPs and lung cancer and/or non-cancer respiratory disease.

1. Rodent Interspecies Comparisons of Lung Responses to Particle Overload of Poorly Soluble Particles

Mauderly and colleagues (1987; 1996) reported on the results of chronic inhalation studies in rats and mice with diesel engine exhaust particles (DEEP). These investigators initially reported that diesel exhaust is a pulmonary carcinogen in rats by inhalation exposures (Mauderly *et al.,* 1987). Male and female F344 rats were exposed 7 hr/d, 5 d/week for up to 30 months to soot concentrations of 0, 0.35, 3.5 or 7.0 mg/m^3. It was reported that focal fibrotic and proliferative lung disease accompanied a progressive accumulation of soot in the lung and the prevalence of lung tumors was significantly increased at the high (13%) and medium (4 %) dose levels vs. controls (1%). Mauderly *et al. (1987)* concluded that diesel exhaust, inhaled chronically at a high concentration, is a pulmonary carcinogen in the rat. In parallel with the rat inhalation study, CD-1 mice were also exposed to identical aerosol concentrations of DEEP for 24 months. In contrast to the dose-related neoplastic response of rats, however,

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the exposures of mice did not increase the incidence of lung neoplasms. The authors concluded that their findings are consistent with other data showing that mice, as well as Syrian hamsters, differ from rats in their lung neoplastic and non-neoplastic responses to heavy, chronic inhalation exposure to diesel exhaust soot as well as several other particles (Mauderly et al., 1996). The investigators concluded that this finding is consistent with other data showing that mice, as well as Syrian hamsters, differ from rats in their lung neoplastic and non-neoplastic response to heavy, chronic inhalation exposure to diesel exhaust soot and several other particles. (Mauderly *et al.*, 1996).

Both Mauderly (1997) and Oberdorster (1995) have commented on the relevance of particle-induced rat lung tumors for assessing lung carcinogenic hazard and human lung cancer risk. Mauderly has noted that “present evidence warrants caution in extrapolation from the lung tumor response of rats to inhaled particles to human lung cancer hazard, and there is considerable uncertainty in estimating unit risk for humans from rat data”. Mauderly (1997) has suggested that a positive finding in both rats and mice would provide greater confidence that a particle test material presents a carcinogenic hazard to humans.

Oberdorster (1995) has concluded that significant species differences exist among the pulmonary responses of rodents to chronic high concentrations of inhaled low solubility dusts. Rats are highly responsive, but mice and hamsters appear to be less prone to developing pulmonary effects such as chronic inflammation and pulmonary fibrosis. Furthermore, lung tumors have been reported only in rats under conditions wherein impaired lung clearance occurs. As a consequence, lung tumors observed

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at very high particulate exposure concentrations may not be relevant for extrapolating to low exposure concentrations in humans (Oberdorster, 1995).

By focusing on these five factors, which are comprised of the data generated by various researchers, convincing scientific conclusions can be derived which clearly demonstrate fundamental differences that occur between rat lung tumors and the human responses to inhaled PSPs. The first of the five factors involves rodent interspecies comparisons of lung responses to inhaled dusts. In the ILSI 2000 Workshop report, a number of chronic studies had demonstrated the particular species sensitivity of the rat lung tumor response (e.g., Lee *et al*. 1985, Mauderly *et al.,* 1987; Heinrich *et al.,* 1995; Levy, 1995) to particle overload inhalation studies, which has not been reported in other rodent species following chronic exposures. Subsequently, hypothesis-driven, mechanistic-based subchronic inhalation studies were conducted with rats, mice and hamsters exposed to identical concentrations of pigment-grade TiO_2 (Bermudez *et al*., 2002), ultrafine TiO_2 particles (Bermudez *et al*., 2004) and nanoscale CB particles (Elder *et al*., 2005; Carter *et al*., 2006). All of these studies evaluated lung responses at several time periods post 90-day exposure; and measured a variety of pulmonary indices using bronchoalveolar lavage assessments of cells and mediators, as well as cell proliferation evaluations and histopathological analyses. These are discussed in greater detail in the following sections.

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Chronic Inhalation studies with Titanium Dioxide and Carbon Black Particles

Two-year study in rats with pigment-grade TiO_2 particles

In a two-year inhalation study on pigment-grade TiO_2 (Lee *et al*. 1985), groups of 100 male and 100 female Sprague Dawley rats were exposed whole-body, 6 hours a day, 5 days a week to 10, 50 or 250 mg/m^3 of rutile-type, pigment-grade TiO_2 particles. It was ironic that the aerosol exposure concentrations were set very high, due to the low toxicity of pigment-grade TiO_2 particles and the corresponding attempt to design a maximum tolerated dose at 250 mg/m^3 (MTD). Survival of the TiO_2-exposed animals was comparable to that of the control group. Exposures to 250 mg/m^3 TiO_2 produced particle overload and lung tumors and with one exception, the tumors produced were ultimately characterized as primarily pulmonary keratin cysts (Warheit *et al*., 2006) The incidence of benign lung tumors and what were originally classified as cystic keratinizing squamous cell carcinomas also were significantly increased in the group of rats exposed to 250 mg/m^3 of TiO_2. Because similar lesions were reported after chronic exposure to high concentrations of other particulate-types, such as chromium dioxide, titanium tetrachloride, and p-aramid respirable fibers, it was suggested that this keratinizing pulmonary response may be indicative of a normal, biological response to particle overload exposures in the lungs of rats. The benign lung tumors that developed in rats exposed to 250 mg/m^3 TiO_2 particles were characterized as bronchoalveolar adenomas. Also observed were dose-related increases in pleurisy, slight collagenized fibrosis

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associated with cholesterol granulomas, alveolar bronchiolarization, pneumonia, and alveolar cell hyperplasia. The degree of pulmonary fibrosis seen at the two higher levels was slight. In subsequent experimentation (Warheit *et al.* 1997, summarized below) using these same dosing rates for four weeks, it was demonstrated that high-dose exposed animals had substantially increased lung clearance times.

To further characterize the bronchoalveolar lesions, in 1992, a group of pathologists from North America and Europe examined lung lesions produced by para-aramid RFP and TiO_2 (Carlton 1994; Levy 1994). This panel diagnosed the lesion as a "proliferative keratin cyst" (PKC). Additionally, the pathologists agreed that the lesion was not a malignant neoplasm and is most likely not even neoplastic. A minority opinion was that the lesion is probably a benign tumor.

Another subsequent international pathology workshop was convened to develop standardized histological criteria for classifying pulmonary keratin lesions (Boorman *et al*., 1996). As a consequence, most of the lesions that had originally been diagnosed as “cystic keratinizing squamous cell carcinomas” were re-classified by the consensus panels as non-tumorous “proliferative keratin cysts” (Warheit and Frame, 2006).

In the aftermath of these two international pathology workshops designed, in part, to establish histological criteria for classifying pulmonary keratin lesions, these lesions were evaluated by four pathologists using current diagnostic criteria. Microscopic review of 16 proliferative squamous lesions, previously diagnosed as cystic keratinizing squamous cell carcinoma in the lungs of rats from the two-year inhalation study (Lee *et al.,* 1985) was performed. Unanimous agreement was reached as to the

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diagnosis of each of the lesions. Two of the lesions were diagnosed as squamous metaplasia and one as poorly-keratinizing squamous cell carcinoma. Most of the remaining 13 lesions were diagnosed as non-neoplastic pulmonary keratin cysts (Warheit and Frame, 2006).

It is also noteworthy that The National Institute for Occupational Safety and Health (NIOSH) considered the the exposure of 250 mg/m^3 in the Lee *et al.* 1985 study to represent an aerosol concentration that exceeded the maximum tolerated dose (MTD), and therefore the finding of lung tumors would be inappropriate to be considered as a positive tumor response. According to the NIOSH Executive Summary of the Current Intelligence Bulletin for TiO_2 (NIOSH 2011 - pages VI – VII), the 250 mg/m^3 concentration in the Lee *et al.,* 1985 study was an excessive dose and is not relevant for human risk assessment. Accordingly, it was written in the Executive Summary "However, exposure concentrations greater than 100 mg/m^3 are generally not considered acceptable inhalation toxicology practice today. Consequently, in a weight-of-evidence analysis, NIOSH questions the relevance of the 250 mg/m^3 dose for classifying exposure to TiO_2 as a carcinogenic hazard to workers and therefore concludes that there are insufficient data at this time to classify fine TiO_2 as a potential occupational carcinogen" (NIOSH, 2011).

In their 1985 chronic inhalation study with TiO_2 particles, Lee *et al*. noted that, due to excessive loading in the lungs of rats exposed chronically to 250 mg/m^3, the observed lung tumors were different from common human lung cancers in terms of tumor type, anatomic location, tumorigenesis and were devoid of tumor metastasis. As a consequence, Lee and coworkers considered the biological relevance of the observed lung tumors as

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negligible (Lee *et al.,* 1985). Moreover, it should be noted that lung burdens of 118 and 130 mg/lung of TiO_2 in male and female rats exposed to aerosol concentrations of 50 mg/m^3 TiO_2 for 2 years did not result in the development of lung tumors (Lee *et al*., 1986). The average measured lung burdens for the male and female rats exposed for 2 years to to 250 mg/m^3 were 785 and 546 mg/lung respectively (Lee *et al*., 1986).

Chronic study in rats and mice with ultrafine TiO_2 particles

Heinrich *et al.* (1995) reported on a chronic inhalation toxicity study wherein – as part of a satellite group – female Wistar rats and NMRI mice were exposed to ultrafine P25 TiO_2 particles (80% anatase: 20% rutile), 5 days/week for 17 hours/day at an average concentration of 10 mg/m^3 for 24 months – followed by a six month postexposure period. The results of chronic exposures were examined at 30 months in rats and 19+8 months in exposed mice. The particle size of the ultrafine TiO_2 exposures ranged from 15 – 40 nm, with a MMAD of 0.8 μm (agglomerates of ultrafine particles). The surface area of the TiO_2 particles was 53 m^2/g. Statistically significant increases in tumors vs. controls were observed in rats and were characterized as benign cystic squamous-cell tumors, adenocarcinomas, squamous cell carcinomas and adenomas. Exposures of female mice to ultrafine TiO_2 particles under the same conditions as rats described above did not produce an increase in lung tumors vs. control mice.

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Chronic studies in rats and mice with carbon black particles

A group of 80 female mice was exposed to high-purity furnace black particles (MMAD = 0.64 μm) at 7.4 mg/m^3 for 18 hours a day, 5 days a week, for 4 months, then to 12.2 mg/m^3 for 9.5 months, followed by a 9.5-month recovery period. There was no statistical difference in lung tumor incidence between exposed and control mice (Heinrich *et al.*, 1995).

Two groups of 72 female Wistar rats were exposed for 17 hours a day, 5 days a week to 6 mg/m^3 of a high-purity furnace black. One group was exposed for 43 weeks and kept for an additional 86 weeks in clean air; another group was exposed for 86 weeks and kept for 43 weeks in clean air. The respiratory tract was examined histopathologically. The 43-week exposure group had a lung tumor rate of 18%, while the 86-week exposure group had a lung tumor rate of 8%. The tumor rate in concurrent controls was less than 1% (Heinrich *et al.*, 1994).

Heinrich and coworkers (Heinrich *et al.,* 1995) exposed a group of 100 female Wistar rats 17 hours a day, 5 days a week, to 7.4 mg/m^3 of high-purity furnace black for 4 months, followed by exposure to 12.2 mg/m^3 for 20 months, and then 6 months exposure to clean air. At 30 months, only 1 of 217 controls had a lung tumor compared to 28% of exposed rats with lung tumors and 11% with keratin cysts (Heinrich *et al.*, 1995).

In a fourth study, groups of male and female Fischer rats were exposed 16 hours/day, 5 days a week for 24 months to 2.5 or 6.5 mg/m^3 furnace black (67% MMAD of 2.0 μm and 33% MMAD of 0.1 μm). The incidences of adenomas and adenocarcinomas were significantly increased in females

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(0% in controls, 7.5% in low-dose, and 26.7% in the high-dose group). The lung tumor incidence in males was similar between exposed and control rats (Nikula *et al.*, 1995).

Conclusion: The database of chronic inhalation studies in rats and mice exposed to either TiO_2 or CB particles demonstrate a clear species difference in pulmonary responses. Lung tumors develop in rats but not in mice exposed to PSP particles conditions of particle overload exposures. In subsequent years, three subchronic rodent interspecies studies were reported wherein female rats, mice and hamsters were exposed to identical particle-types, concentrations of pigment-grade TiO_2 particles (Bermudez *et al.*, 2002); ultrafine TiO_2 particles (Bermudez *et al.*, 2004); or nanoscale CB particles (Elder *et al.*, 2005). The results of all three of these 90-day inhalation studies provide important comparative information on the sensitivity of the various rodent species; but perhaps more importantly inform the likely mechanisms of action for the pathogenesis of lung tumor responses of the rat to particle overload as compared with similarly exposed mice and hamsters.

Subchronic, 90-day study in female rats, mice and hamsters with pigment-grade TiO_2 particles

Female rats (F344), mice (B3C3F1/BrlBR) and hamsters (Lak:LVG (SYR) BR) were exposed whole-body to identical concentrations of pigment-grade, rutile-type titanium dioxide particles (0, 19, 50 or 250 mg/m^3) 6 hr/day, 5 d/wk for 13 weeks followed by several postexposure pulmonary assessments up to one year (Bermudez *et al.,* 2002). Particle size analysis of the pigmentary TiO_2 aerosols were the following: hamster – MMAD = 1.36

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μm; GSD = 1.50; mouse – MMAD = 1.39 μm; GSD = 1.72; rat – MMAD = 1.44 μm; GSD 1.71. It was reported that retained lung burdens were greatest in mice following the end of exposure; although particle retention data demonstrated that particle overload was reached in both rats and mice at the 50 and 250 mg/m^3 exposure concentration groups. In this regard, there were dose-related changes in the pigment-grade TiO_2 lung burdens for all three species. Following 13 weeks of exposure, mice exhibited the greatest (170 mg/g dry lung) pigment-grade TiO_2 lung burdens followed by rats (120 mg/g dry lung) then hamsters (114 mg/g dry lung). Pulmonary inflammation was recorded in all species at the two highest exposure concentrations as measured by bronchoalveolar lavage methods. However, lung inflammation persisted in rats and mice at the highest exposure concentrations throughout the one-year post exposure recovery period. In hamsters, pulmonary inflammation was finally resolved due to the more rapid lung clearance kinetics. In rats exposed to 250 mg/m^3, pulmonary lesions were characterized by epithelial proliferative changes evidenced by enhanced alveolar epithelial cell proliferation. Concomitant with the enhanced cell proliferative changes was the development of alveolar septal fibrosis. Although rats exposed to 50 mg/m^3 exhibited minimal alveolar cellular hypertrophy, macrophage accumulation and lung inflammation, no alveolar septal fibrosis or significant cellular turnover was measured at this exposure concentration. Similar alterations to those described above for pigment-grade TiO_2-exposed rats were not observed histopathologically or pulmonary indices in the lungs of exposed mice or hamsters. The results from this study demonstrated clear differential adverse pulmonary effects in the responses of rats at particle overload concentrations when compared to similarly exposed mice or hamsters. To be specific, exposures to high dose TiO_2

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produced both common as well as different particle-overload related effects in exposed female rats and mice. Particle-exposed mice demonstrated characteristics of particle overload - alveolar macrophage accumulation, reduced particle clearance and sustained inflammation; however and importantly, similarly-exposed mice did not develop the pathological sequelae consisting of significant cell proliferation, septal fibrosis, alveolar epithelial cell hyperplasia and metaplasia– leading to tumor formation, as measured in the Lee et al. (1985) study.

Subchronic, 90-day study in female rats, mice and hamsters with ultrafine TiO_2 particles

Using a similar experimental protocol design, a subchronic 90-day study rodent interspecies study was conducted with ultrafine or nanoscale TiO_2 particles (Bermudez *et al.,* 2004). The ultrafine TiO_2 sample consisted of a crystal structure containing 80% anatase: 20% rutile; along with a particle surface area = 55 m^2/g; and an average primary particle size = 21 nm. Female rats (F344), mice (B3C3F1/BrlBR) and hamsters (Lak:LVG (SYR) BR) were exposed whole-body to aerosol concentrations of 0, 0.5, 2.0 or 10 mg/m^3 ultrafine TiO_2, 6 hrs/d, 5 d/wk for 13 weeks followed by a variety of assessments at postexposure time periods ranging from 4 weeks to 1 year. Particle size analysis of the ultrafine TiO_2 aerosols were the following: hamster – MMAD = 1.29 μm; GSD = 3.65; mouse – MMAD = 1.45 μm; GSD = 2.46; rat – MMAD = 1.44 μm; GSD 2.60. Bronchoalveolar lavage parameters and lung tissues were evaluated for cell proliferation indices, lung retention burdens, and histopathological endpoints. Retention of ultrafine TiO_2 particles was increased in a concentration-related manner for

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all three species. Mice and rats demonstrated similar particle lung burdens at the end of exposures, but as demonstrated in the pigment-grade study, hamsters cleared dusts at a faster rate and accordingly, lung burdens were significantly reduced compared to the other species. Retardation of particle clearance occurred in exposed rats and mice at the 10 mg/m^3 (highest) level, and was a strong indication that particle overload had been achieved. Lesions in the respiratory tracts of rats exposed to 10 mg/m^3 included increased alveolar epithelial cell proliferation, along with metaplastic epithelial cells (alveolar bronchiolarization); focal areas of (particle-containing) macrophage accumulation; interstitial particle accumulation and alveolar septa fibrosis. These lesions were progressive and were more prominent at the later post-exposure time points. Results measured with uf-TiO_2-exposed mice demonstrated a reduced lung inflammatory response and an absence of fibroproliferative changes – unlike those measured in the lungs of rats. Lung clearance in particle-exposed hamsters occurred at a rapid pace – relative to the other two species. The characteristic data patterns generated from this ultrafine TiO_2 study are rather similar in scope to the results of the interspecies comparisons study measured in the pigment-grade TiO_2 study. Although the pulmonary effects were more potent at lower particle concentrations in the ultrafine TiO_2 study, the interspecies relationship of rats vs. mice vs. hamsters clearly demonstrate that rats develop the most pronounced pulmonary effects – particularly at particle overload concentrations. At higher particle concentrations, exposed mice demonstrate particle overload kinetics and corresponding lung inflammatory responses but do not develop the progressive pathological sequence that occurs in rats: namely, increased cell proliferation, septal fibrosis, and fibroproliferative changes – which can lead to lung tumors following chronic

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exposures. Particle–exposed hamsters exhibited a more efficient pulmonary clearance pattern and did not demonstrate progressive effects as measured in rats. These differential effects between rats, mice and hamsters can best be explained by the diversity in both pulmonary responses (rat vs. mouse) and/or by particle dosimetry disparities (rat=mouse vs. hamster) between these rodent species exposed to identical concentrations of aerosolized particles.

Subchronic, 90-day study in female rats, mice and hamsters with high surface area and low surface area CB particles

In a third interspecies comparison subchronic inhalation study (Elder *et al.,* 2005), female rats (F344), mice (B6C3F1) and hamsters (276 F1B) were exposed whole-body by inhalation to high surface area CB particles (HSCb) at aerosol concentrations 0, 1, 7, and 50 mg/m^3 selected to span a no-observable adverse effect level (NOAEL). Additional rats were exposed to low surface CB (LSCb) at a single concentration of 50 mg/m^3 for 13 weeks. Particle retention and pulmonary effects were measured at 0, 3 and 11 months postexposure. Equivalent mass burdens were achieved to compare high-dose HSCb and LSCb, whereas surface area burdens were equivalent for mid-dose HSCb and LSCb. Particle size analysis of the high concentration HSCb aerosols were the following: hamster – MMAD = 1.4 μm GSD = 2.4; mouse – MMAD = 2.0 μm- GSD = 2.5; rat – MMAD = 1.5 μm - GSD 2.5. LSCb = 0.8 μm ; GSD = 3.2. The investigators reported that prolonged particle retention was determined in rats exposed to mid-and high-dose HSCb and to LSCb, but LSCb was cleared faster than HSCb. Retention of particles was also slowed in mice exposed to mid-and high-dose

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HSCb. The findings indicated that lung inflammatory and morphological effects were more severe and prolonged in rats when compared to mice or hamsters. In addition, measured adverse pulmonary effects were similar in rats exposed to either mid-dose exposures of the HSCb or high-dose exposure of LSCb. Similar to the effects measured in both the pigment-grade and ultrafine TiO_2 studies, particle clearance in hamsters was most efficient compared to the other two rodent species. Consistent with the findings in both subchronic interspecies studies with titanium dioxide particles, the authors concluded that the rat was particularly sensitive to the development of pulmonary lesions following subchronic exposures to CB particles.

As a component of this interspecies study to inhaled CB particles, Carter *et al*. (2006) implemented additional sequential bronchoalveolar lavage and lung tissue studies post 90-day exposure to assess indices that might explain the sensitivity of particle-exposed rats when compared with similarly-exposed mice and hamsters in the subchronic CB inhalation study. The investigators reported that unlike mice and hamsters, rats generated pro-inflammatory mediators in response to CB particle exposures, while the pulmonary responses in mice and hamsters were generally anti-inflammatory in scope (Carter *et al.,* 2006).

In comparing the pulmonary responses of particle overload in rats and mice it is clear that following high-dose inhalation exposures, particle retention and reduction in clearance were similar in both rats and mice. However, the inflammatory, cell proliferative and histopathologic responses in rats were significantly greater when compared to mice in all three subchronic inhalation studies with pigment-grade and ultrafine TiO_2 particles;

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as well as with carbon black exposures. For example, in the interspecies subchronic study with ultrafine titanium dioxide, in rats inhaling 10 mg/m^3 uf TiO_2, the histopathological responses included epithelial and fibroproliferative changes, and alveolar septal fibrosis. At 52 weeks postexposure, minimal to mild metaplastic changes and minimal to mild particle-induced alveolar septal fibroplasia were observed only in rats. In mice inhaling 10 mg/m^3 uf TiO_2, lesions were described as aggregations of heavily particle laden macrophages in the centriacinar sites. No epithelial, metaplastic and fibroproliferative changes were observed by histopathology in the mice or hamsters.

Understanding the mechanisms of the rat lung response to particle overload

Warheit *et al.* (1997) performed a 28-day inhalation toxicity study that was designed to provide a mechanistic explanation for the responses observed in the pivotal rat oncogenicity TiO_2 study (Lee *et al.,* 1985). Male Sprague-Dawley rats were exposed nose-only to 5, 50 and 250 mg/m^3 pigment-grade TiO_2, 5 d/wk for 4 weeks and the lungs of animals were evaluated at several postexposure time periods through six months. The study demonstrated that the lungs of particle-overload exposed rats are characterized by impaired pulmonary clearance, sustained pulmonary inflammation, cellular hypertrophy and hyperplasia; and that these effects, following continuous exposure at 250 mg/m^3 (for two years), likely could result in the development of overload-related pulmonary tumors. The study with pigment-grade TiO_2 in rats used exposure concentrations similar to those in the Lee *et al.* study (1985) to detail the characteristics of “lung overload” in this species, along with an assessment of the rat’s ability to

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recover from this challenge. Exposure to high dust concentrations produced pulmonary inflammation, proliferation of pulmonary cells, impairment of particle clearance, deficits in macrophage function, and the appearance of macrophage aggregates at sites of particle deposition (see Figures 2-5). Rats exposed to 250 mg/m^3 TiO_2 had lung burdens of 1600 μg/g of fixed lung tissue or 12 mg/lung. TiO_2 particle exposures produced sustained pulmonary, neutrophilic, inflammatory responses in animals exposed to 250 mg/m^3. Rats exposed to 250 mg/m^3 also demonstrated diminished lung clearance after 1 week through 1 month post-exposure. Mono-exponential clearance modeling indicated that TiO_2 particles were cleared with half-times of approximately 68, 110, and 330 days for the 5, 50, and 250 mg/m^3 test groups, respectively (Fig. 1). Lymph node burdens of rats exposed to 250 mg/m^3 TiO_2 demonstrated TiO_2 particles had translocated to tracheobronchial lymph nodes. Rats exposed to 50 mg/m^3 TiO_2 had small, but sustained lung inflammatory responses. *In vitro* phagocytosis studies demonstrated that alveolar macrophages exposed to 250 mg/m^3 TiO_2 were impaired in their phagocytic responses. At high concentrations (50 to 250 mg/m^3) of TiO_2, cellular hypertrophy and hyperplasia were evident at alveolar wall and duct bifurcations that were adjacent to the macrophage. The findings of this study clearly demonstrated that 4-week exposures in rats to high dust concentrations of TiO_2 resulted in persistent neutrophilic inflammation, enhanced cellular proliferation, impairment of particle clearance, deficits in macrophage function as significant biomarkers of lung toxicity and likely serves as a roadmap for the lung tumors measured at 250 mg/m3 following 2 year inhalation exposures.

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2. Differences between the pulmonary responses of rats to chronic exposures to PSPs vs. Nonhuman primates and humans

A variety of studies have been reported that measured the pulmonary responses of nonhuman primates (such as squirrel and cynomolgus monkeys) and rats to identical particle types and aerosol exposure concentrations of low solubility dusts. These comparative studies included exposures to diesel exhaust, coat dust, petroleum coke, beryl ore dusts, and shale dusts. Most of the studies measured conventional histopathological pulmonary effects following two year exposures (see Tables 2-3). The two-year duration represents a near lifetime exposure to the rat but not for the squirrel and cynomolgus monkeys – yet still provides important insights into the fundamental differences in disposition of inhaled particles within the anatomical regions of the respiratory tract - as well as pulmonary responses of the two species. However, in addition, a few of the studies also investigated the particle distribution/translocation patterns of dusts in exposed rats and monkeys following particle deposition in the distal lung. Apart from the comparative rat vs. monkey studies, one study compared the disposition/biokinetic patterns in the lungs of rats exposed to diesel exhaust to nonsmoking coal miners (postmortem) over a working life of 40 years. The study descriptions are detailed below:

Wagner *et al.*, (1969) exposed SD rats, Syrian golden hamsters, and squirrel monkeys to betrandite or beryl ore dusts at 0 or 15 mg/m^3 (6 hr/d; 5 d/wk) for either 17 months (rats, hamsters) or 23 months (monkeys). During the exposure period and following completion of exposures, the lungs were evaluated. Chronic inflammation associated with granulomas and alveolar

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epithelial hyperplasia were common features of the rat pulmonary responses to both dusts. Following 17-month exposures, adenomas and adenocarcinomas were observed in the lungs of 7 and 9 of 19 rats, respectively, while 4 of 19 rats contained pulmonary epidermoid tumors. The pulmonary responses of ore-exposed hamsters were limited to alveolar epithelial hyperplasia, bronchiolar-alveolar metaplasia, and a few granulomatous lesions. For the squirrel monkeys, morphological investigations did not identify any lesions, other than macrophage accumulations (containing particles) located at sites of respiratory bronchioles and blood vessels.

In a 2-year study with raw or processed shale particles, MacFarland *et al.* (1982) exposed F344 rats and cynomolgus monkeys to 0, 10, or 30 mg/m^3 for 6 hr/d, 5 d/wk and evaluated the pulmonary effects post-exposure. Morphological assessments revealed that all of the rats developed proliferative alveolitis and bronchiolitis; and most developed, what was described as chronic inflammation with nonprogressive fibrosis, cholesterol clefts, and microgranulomas. In contrast to the response of rats, for monkeys, accumulations of pigment-laden macrophages were observed in alveolar and bronchiolar walls, concomitant with occasional foci of subacute inflammation in a few of the lungs.

Klonne and coworkers (1987) reported on a study wherein SD rats and cynomolgus monkeys were exposed to aerosols of micronized petroleum coke particles 6 hr/day, 5 days/wk for 24 months (0, 10, or 31 mg/m^3). Morphological assessments demonstrated that rats retained particles primarily in alveolar regions within macrophages and the major effects observed were characterized by chronic inflammation, squamous metaplasia

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of alveolar epithelium, formation of keratin cysts, focal fibrosis, along with alveolar/bronchiolar metaplasia and sclerosis. In contrast to the rat responses, in exposed monkeys, the pulmonary histopathological effects were limited to on accumulation of macrophages containing petroleum coke particulate material.

In another set of comparative studies, male and female F-344 rats and male cynomolgus monkeys were exposed to filtered air, diesel exhaust or coat dust 2 mg/m^3 for 7 hr/d, 5 days/wk for 24 months and the respiratory tract was assessed for biokinetic factors and histopathological responses. The anatomic distribution patterns of both diesel exhaust and coal dust particles were different when comparing effects in rats and monkeys. In rats, the vast majority of inhaled particles did not translocate from alveolar sites, but instead were retained within alveolar macrophages located within the alveoli and alveolar ducts compartments – at sites of particles deposition. In contrast to the patterns observed in rats, to a greater extent, inhaled particles translocated from original deposition sites in the alveoli to the interstitium and pleura in the lungs of monkeys. In addition, significant alveolar epithelial hyperplastic and inflammatory responses were observed in rats but not in monkeys exposed to both diesel and coal dust particles (Lewis *et a*l., 1989). The lungs from these studies were further evaluated by Nikula and coworkers (1997).

In subsequent morphometric studies, Nikula *et al*., (1997, 2001) compared the distribution patterns of particle retention and lung tissue responses between rats and cynomolgus monkeys following two-year exposures to diesel exhaust and coal dust particles (diesel -2 mg soot/m^3; coal dust – 2 mg respirable dust/m^3, and DEEP (diesel engine exhaust particles) and coal

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dust combined (1 mg soot and 1 mg/ coal dust/m^3). Morphometric and morphological/histopathology studies were implemented to gauge pulmonary particle retention patterns and lung tissue reactions in both species. The relative volume density and volume percentages of retained particulate material within defined anatomic compartments of the respiratory tract were assessed using morphometric techniques. With the exception of DEEP (for which clearance was determined to be equivalent between the two species), greater amounts of particulate material were retained in the lungs of monkeys vs. rats**,** demonstrating that the clearance of inhaled particles was faster in rats when compared to cynomolgus monkeys. Moreover, particle distribution and translocation patterns were very different in the lungs of rats vs. the monkeys. In rats, most of the inhaled particles remained on alveolar surfaces – located either in alveolar epithelial cells or within alveolar ducts. In contrast for cynomolgus monkeys, inhaled particles had translocated from sites of initial particle deposition, across epithelial cells, and retained/sequestered within interstitial sites to a significantly enhanced degree; concomitant with slower particle clearance thereafter. For comparisons of pulmonary histopathological responses, rats but not monkeys demonstrated significant alveolar epithelial hyperplastic and lung inflammatory (neutrophilic) responses**,** along with evidence of septal fibrosis. To summarize their results, it was concluded that the intrapulmonary particle retention and tissue reaction study results identified fundamentally different responses when comparing rats with nonhuman primates following identical particle-type exposures. These findings of interspecies differences (i.e., rat vs. cynomolgus and squirrel monkeys) in particle kinetic patterns as well as the intensity of pulmonary responses following exposures to identical inhaled particles have important ramifications for evaluations of human occupational

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exposures. Accordingly, the lung responses in rats to the various inhaled particle exposures were severe when compared to the nonhuman primate responses – wherein the exposures were handled without adverse consequences (Nikula *et al.*, 1997).

In another study, the pulmonary impacts of exposure concentration/dose on the particle distribution of patterns following pulmonary exposures to rats and humans were compared (Nikula *et al.*, 2001). Morphometric techniques of lung sections processed from rats and humans were implemented to assess particle retention patterns within the respiratory tract. Rats had been exposed for two years to diesel soot exhaust particle at aerosol concentrations of 0.35, 3.5 or 7 mg/m^3 (see Mauderly et al., 1987). In addition, lung disposition patterns in three human subject groups were studied. The subjects were characterized as 1) nonsmokers who did not work as coal miners; 2) nonsmoking coal miners who worked for 10-20 years under the presumed occupational standard of 2 mg/m^3; and 3) nonsmoking coal miners who had worked for 33 – 50 years under the previous occupational standard of < 10 mg/m^3. The comparative results demonstrated that the distribution of retained particles within the compartments of the respiratory tract were substantially different when comparing rats to humans. In the diesel exhaust exposure groups of rats, > 82 % of the retained particulate material was identified within pulmonary macrophages observed in alveoli or alveolar ducts. In contrast, in the human groups 57, 68 and 91% of the retained particles were identified within the interstitial regions. Similar to the results obtained from the cynomolgus monkey morphometric studies, the authors concluded that there was a differential pattern particle distribution/retention when comparing exposed

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rats to humans. Chronically inhaled diesel soot particles were retained predominantly in alveolar regions of rats; whereas in humans, most of the inhaled coal dust particles had translocated to the interstitial compartments. Differences in particle distribution and retention patterns likely to account for differential pulmonary patterns /responses of rats when compared to humans and nonhuman primates.

The studies by Nikula and colleagues comparing lung responses to inhaled particles in rats vs. larger mammals (i.e., monkeys/humans) are important because they demonstrate differential particles distribution patterns following chronic inhalation exposures to PSPs: 1) the disposition and dosimetry differences between rats and monkeys/humans are very different. Following particle deposition, Inhaled PSPs are retained within alveolar ducts. In contrast, in monkeys and humans, inhaled particles preferentially translocate across epithelial cells to be retained/sequestered at interstitial sites; and 2) rats produced significantly augmented and sustained pulmonary inflammogenic, epithelial and fibroproliferative responses when compared to either monkeys or humans. The available data also suggest that rats are significantly more sensitive in the development of adverse lung responses to inhaled PSP exposures when compared to 1) other rodent species**,** and 2) larger mammals such as monkeys and humans. These findings are confirmed by the study results of numerous investigators cited above (Wagner *et al.*, 1969; MacFarland *et al.*, 1982; Klonne *et al.*, 1987; and Lewis *et al.*, 1989).

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3. Updated Modeling Studies Demonstrate the Differences in Particle Disposition Kinetics Between Rats and Occupationally Exposed Workers

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). The model is useful because it provides important information for calculations on the potential doses retained in the respiratory tracts of human subjects following exposures to radioactive particles. The human respiratory tract long-term particle retention model from 1994 (ICRP, 1994) has recently been revised, in part, because the model underestimated the lung retention burden of inhaled insoluble materials (i.e., particles) (Gregoratto *et al.*, 2010). This was necessary because the earlier model did not adequately account for the transit of particles into the interstitial compartment - wherein about 40% of deposited inhaled/deposited particles were ultimately sequestered. Gregoratto and colleagues devised an empirical biokinetic model (compartmental mass transfer model) – which fits the data by using a small number of parameters and it describes adequately the retention time-pattern of the radioactivity in the lungs as a whole. The revised model serves to both increase the calculated retained particle dose in human lungs and correspondingly, reduces the previously estimated lung clearance rates. A simple physiologically-based model developed to predict lung and lymph node particle retention in coal miners was confirmed in these studies (Gregoratto *et al.* 2010). Gregoratto adapted the model originally developed by Kuempel and coworkers (2001) to amend the Human Respiratory Tract

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Model of the International Commission on Radiation Protection (ICRP, 1994) and the model was described and discussed using human data from workers exposed to radioactive aerosols (ECETOC, 2013).

It is important to note that the Gregoratto model findings of particle retention kinetics in the alveolar-interstitial regions of human lungs and corresponding particle sequestration within the interstitium correlate well with the interspecies morphometric/histopathological data differentiating particle disposition, translocation and clearance patterns between rats and nonhuman primates/humans reported by Nikula *et al*. 2001. The retention pattern of retained particles in interstitial sites of coal workers shifts the balance away from tumor development within alveolar regions, as occurs in lungs of PSP-exposed rats. Accordingly, the particle retention pattern occurring in the lungs of coal workers (over a working lifetime exposure) results in a greater likelihood of pulmonary reactions initially occurring within the interstitial compartment, as is evidenced in coal workers pneumoconiosis and pulmonary anthracosis (Figure 7). These patho-physiological findings may also help explain the absence of lung cancer risk in PSP production workers, as evidenced by the abundance of negative epidemiological study results.

4.Morphological/Histopathological Comparisons of Tumor Types as Assessed in Human Lung Cancers vs. Particle-overload related Lung Tumors in rats

Lung cancers in humans arise primarily from cigarette smoking. In some

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cases, workers have developed lung tumors following occupational exposures to some inhaled substance and particles such as asbestos, arsenic, cadmium compounds, coal-tar pitches, and hexavalent chromium compounds. In contrast to those carcinogenic materials, occupational exposures to PSPs such as TiO_2, CB particles, toner particles or coal dust do not result in lung cancers in humans but do produce tumorigenic responses in experimental, chronic, particle overload inhalation studies in rats (Green, 2000). The absence of tumorigenic responses in humans has been confirmed by data reported from a variety of epidemiological studies of > 50,000 occupationally exposed TiO_2 and CB production workers (see below). In addition to the lack of convergence in data generated from epidemiology (humans) vs. two-year inhalation studies (rats), another fundamental difference between rat and human lung tumors is evidenced by the dissimilarities in histological characteristics of the tumor cell types of each species. In this regard, there are four major cell types that are known to occur in the development of human lung cancers: namely, adenocarcinomas, squamous cell carcinomas, as well as small cell and large-cell anaplastic carcinomas (Green, 2000). The majority of rat lung cancers are adeno-carcinomas and squamous cell carcinomas. Non-tumorigenic keratinizing cysts and adenomas are also common in rats exposed chronically to PSPs, but are rare or absent in human lung cancers (Green, 2000). From a histopathology perspective, occupational exposures to TiO_2, CB, or coal dust particles do not produce epithelial hyperplasia, lung inflammation or lung cancer in humans. Alternatively, the pathogenic events in chronically-exposed rats to PSP particle overloading are characterized initially by macrophage accumulation, followed by necrosis of alveolar epithelial cells, granuloma formation lung fibrosis, and bronchiolarization and

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squamous metaplasia of alveoli. In addition, bronchiolar-alveolar adenomas, and squamous proliferative cysts occur as well as keratinizing and squamous cell carcinomas and adenocarcinomas. The squamous lesions represent pathobiological responses which appear to be uniquely developed in alveolar epithelial cells in rats; as they have not been observed or reported in any other species following these chronic exposures (Schultz, 1996). Accordingly, it is imperative to delineate between 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).

Unsurprisingly, characterization and histopathological identification of the lung lesions and tumor types observed in rats following chronic inhalation exposures of PSPs in rats has been somewhat controversial. This was due to the fact that many of the pulmonary lesions that occurred in rats were uncommon and unprecedented and were dissimilar from human pulmonary tumor-types. Accordingly, a pathology working group panel, noted above, of veterinary and human pathologists was convened to gain a better understanding and consensus on one of these lesions, the keratinizing and squamous cell carcinoma. This unusual/rare type of cystic and keratinizing squamous pulmonary lesion that occurred primarily in female rats exposed for two years to particles such as TiO_2 particles and para-aramid fiber-shaped particulates (RFP). Following evaluation of the lesions and extensive discussions, the majority of the pathology panel considered the lesions to be non-neoplastic, keratinizing cysts with diagnostic features as described as 1) an absence of invasion and metastases and 2) orderly squamous metaplasia and keratinization along a thin layer of well-differentiated

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squamous epithelium (not a neoplasm). The panel considered that the most appropriate morphological diagnosis for the lesions was "proliferative keratin cysts" (PKC) (Carleton, 1994; Levy, 1994). None of the participants had seen a similar lesion in humans (Carleton, 1994).

5. Numerous Epidemiological Studies have been published during the past 15 years Following TiO_2, CB, and Toner Production Workers. No Lung Cancer Excess Risks due to Dust Exposures were found in Large Cohorts of Workers.

Epidemiology Studies with TiO_2 Production Workers

There are several primary epidemiological studies that have focused their evaluations on the health of TiO_2 manufacturing workers. None of these studies have reported an association between TiO_2 exposure and lung cancer. A lung cancer case-control epidemiological study of male employees occupationally exposed to TiO_2 was reported by Chen and Fayerweather (1998). A total of 1576 employees were observed from 1956 through 1985 for cancer and chronic respiratory disease incidence and from 1935 through 1983 for mortality. In addition, a cross-sectional sample of 398 employees was assessed for chest roentgenogram abnormalities. The resulting data did not demonstrate an increased risk for lung cancer. In addition, there were no dose-response relationships between TiO_2 exposures and chronic respiratory disease, pleural thickening, pleural plaques, or pleural nodules. The authors concluded that "nested case-

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control analyses found no statistically significant associations between TiO_2 exposure and risk of lung cancer, chronic respiratory disease, and chest roentgenogram abnormalities."

The earlier DuPont TiO_2 epidemiology studies were followed up by Ellis *et al.*, (2010, and 2013 who investigated occupational exposures and mortality among 3607 workers at three TiO_2 plants). Combined and plant-specific cohort mortality was compared with the overall US population and other DuPont employees. The relationships between selected causes of death and annual cumulative exposures to titanium dioxide and chloride were investigated using Poisson regression methods to examine trends with increasing exposure. The authors concluded that there was no indication of a positive association between occupational exposure and death from all causes, all cancers, lung cancers, non-malignant respiratory disease or all heart disease.

Boffetta and colleagues led a multi-centre investigative team to study workers from TiO_2 manufacturing sites throughout Europe (Boffetta *et al.,* 2004). This historical cohort mortality study involved workers from eleven manufacturing sites in Finland, France, Germany, Italy, Norway, and the United Kingdom. Workers employed for at least one year in TiO_2 production plants were assessed using company records and quality controls, taking into account the different manufacturing procedures used at the sites as well as the actual relative levels of exposure to respirable TiO_2 particles (i.e., estimates based on the workers' job descriptions and the time periods for potential exposure). The total cohort population was 15,017 workers. The authors concluded that lung cancer results of the study do not suggest a carcinogenic effect of TiO_2 exposure. Mortality from other non-neoplastic

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pulmonary diseases, was not associated with exposure to TiO_2 dust exposures. In addition, mortality from lung cancer did not increase with either cumulative exposure to TiO_2 dust or duration of employment. The findings of this study included a reduced overall mortality (the so-called healthy worker effect) suggested a better health status of these workers when compared to the health status of their national populations.

Another epidemiological assessment was reported by Fryzek *et al.,* (2003), who conducted an historical mortality study of workers at four TiO_2 manufacturing sites in the US. The cohort size was 4,241 workers. The workers in the study were employed for at least six months since January 1960 at one of the four manufacturing sites. Exposure categories such as job site, title, and calendar years on the job were examined. Similar to the observations of the concurrent European cohort study (Boffetta *et al.*, 2004); significant *decreases* in overall mortality were observed (the healthy worker effect). No impact was reported on the number of deaths from lung cancer, as the rate did not increase at any level of estimated exposure to TiO_2. The authors concluded that the exposures at these US plants were not associated with increases in the risk of death from cancer or other diseases.

Ramanakumar and colleagues (2008) utilized two large population-based, case-control studies in Montreal, Canada to assess the risks of lung cancer following worker exposures to carbon black (CB), titanium dioxide (TiO_2), or talc particles. Detailed lifetime job histories were elicited, and the evidence of exposures to a host of occupational substances were evaluated. Lung cancer risks were analyzed in relation to each exposure, adjusting for several potential confounders, including smoking. The authors concluded

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that subjects with occupational exposures to CB, TiO_2, industrial talc or cosmetic talc did not experience any detectable excess risk of lung cancer.

Epidemiology Studies with Carbon Black Production Workers

Seven studies were considered to be informative for lung cancer by IARC (IARC, 2010), three of which were among carbon black production worker populations. The Working Group considered the studies of CB workers in Germany, the UK and the US to be the most informative for assessing cancer risk (Hodgson and Jones RD,1985; Buchte *et al*, 2006; Dell *et al*., 2006; and Wellmann *et al*., 2006).

The two studies from Germany and the UK indicated an excess lung tumor risk compared with external reference populations. Confounding by tobacco smoking could not be excluded, although it was unlikely to have explained the entire cancer risk. However, in both cohorts, internal analyses by level of exposure to carbon black gave equivocal but mainly null results. The study of the carbon black workers in the US suggested no excess mortality, but did not assess risk by level of exposure (Dell *et al*., 2006). In studies that assessed risks for lung cancer among user industries, the most informative study of German rubber workers showed some indication of excess risk that disappeared when asbestos and talc were adjusted for the analysis (IARC, 2010).

Buchte and coworkers (2006) reported the results of a case-control study of lung cancer in 1528 German carbon black workers from 1976- 1998. The investigators concluded that carbon black particle exposures were not linked to lung cancer risks.

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Dell et al. (2006) followed the historical mortality patterns, in particular lung cancer, of US carbon black workers (5011 workers – 18 facilities) employed for at least one year from the 1930's until 2003. It was concluded that employment in carbon black production plants was not associated with increased mortality, cancer overall, and particularly lung cancer.

In their evaluation of carcinogenic risks to workers, IARC considered the epidemiological evidence for CB as inadequate for lung cancer risks (IARC, 2010).

Since this IARC (IARC 2010) evaluation a further number of studies have been published on CB production workers. In a follow-up of the UK cohort, Sorahan and Harrington (2007) used a novel exposure metric ("lugging"), hypothesizing that carbon black may act as a "late stage" lung carcinogen at the two out of five plants with elevated SMRs and found some suggestive findings (decreasing SMR after cessation of exposure) in these two plants. However, Morfeld and McCunney (2007) tested this hypothesis in the larger German CB cohort and found no decreasing SMR after cessation of exposure, despite the fact that the German cohort showed an elevated lung cancer SMR (Morfeld *at al* 2006).

Additional analysis of the large German carbon black cohort addressed potential "lugging" effects with a multi-model Cox regression approach (Morfeld and McCunney 2009). These studies were designed to explore the impact of cumulative exposure to CB "lugged" at 5, 10, 15, and 20 years and the findings did not support the hypothesis of CB being a late-stage carcinogen. A Bayesian approach was used by Morfeld and McCunney (2010) to investigate potential risk factors and confounders that may have contributed to the SMR lung cancer results but these additional analyses

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provide further support for the lack of an increased risk of lung cancer in CB production workers.

In a follow-up study of the Dell *et al* (2006) investigation, Dell *et al* (2015) evaluated lung cancer and respiratory disease mortality associations with cumulative inhalable carbon black exposure among 6634 US carbon black production workers. No consistent associations were observed between cumulative inhalable CB exposure and respiratory disease mortality and most importantly, quantitative CB exposure estimates were not related to lung cancer or nonmalignant respiratory disease mortality.

Epidemiology Studies with Toner Production Workers

An epidemiological investigation was performed on 33,671 workers with exposure to toner between 1960 and 1982 as manufacturing workers or customer service engineers (Abraham et al., 2010). The investigators concluded that there was an absence of lung cancer excess risk due to dusts exposures. In addition, no evidence of noncancer adverse risks was reported.

Discussion and Conclusions

The Organization for Economic Cooperation and Development (OECD) has defined an Adverse Outcome Pathway (AOP) as a sequential progression of events in an organism dating from the first contact of a toxicant at a molecular level to a final adverse outcome at the individual or population level (OECD, 2013, ECETOC, 2013). Consequently, an AOP can be outlined as a linear cascade of consecutive events wherein one common

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molecular or cellular initiating effect serves as a prerequisite for all subsequent steps leading to a set of toxicity or adverse endpoints. AOPs allow for the different molecular initiating events that can cause the same adverse outcome, and that different modes of actions (MoA) may share common key molecular initiating events (Morfeld *et al*., 2015). When considering pulmonary effects in rats related to particle overload exposures of PSPs, a build-up or accumulation of retained particles is initially required and then progresses to a key set of molecular/cellular initiating events. It is noteworthy that for the rat model, significant impairment of particle clearance by alveolar macrophages recruitment and corresponding persistent neutrophilic pulmonary inflammation are considered to be the defining events that lead to the set of sequential pathological sequelae that ultimately result in lung fibrosis and lung tumors. It should be noted that an AOP approach is utilized to describe an interspecies comparison of pulmonary responses to particle overload. Although particle accumulation in the lung is a common finding for all long-term experimental studies, it is clear that significant differences in the phenotypic “adverse outcome” occurs between rats and all other mammalian species, including humans (Morfeld *et al.,* 2015). A conceptual AOP map of key events leading to lung fibrosis and tumors in particle-overloaded rats is described in Figure 8 (ECETOC, 2013). In this scenario, continued high dose exposures to PSP ultimately result in impaired particle clearance and persistent neutrophilic inflammation in alveolar regions of the lung. Continual particulate exposures stimulate increased levels of reactive oxygen species, augmented cell proliferation effects, secondary genotoxic effects and corresponding cell mutations, metaplastic responses, hyperplastic responses, septal fibrosis and ultimately lung tumors. Many of these pulmonary effects (short of course of the lung

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tumors) were documented during the subchronic 90-inhalation studies with pigment-grade and ultrafine TiO_2 particles; as well as with carbon black particles. Curiously, particle-overload exposed mice develop similar initial particle-overload effects (i.e., inhibitions of particle clearance and sustained lung inflammation), however, the cascade of sequential pathological effects demonstrated in the rat is either diminished or absent in the mouse model. One possible explanation stems from the findings of Carter *et al.*, (2006) in interspecies measurements from the subchronic 90-day inhalation study with carbon black particles. Following 90-day exposures of female rats, mice and hamsters to identical particle exposures, the investigators conducted comparative, sequential bronchoalveolar lavage (BAL) studies. The recovered BAL fluids from each of the species were assessed for indices including recovered cell number and type, quantification of reactive oxygen and nitrogen species, and cytokine levels. In addition, *ex vivo* mutational analysis of inflammatory cells was evaluated by co-incubating recovered inflammatory cells *ex vivo* with lung epithelial cells. Finally, lung tissue was evaluated for gene expression of various anti-inflammatory mediators. Many of the endpoint findings were consistent within a given species (when comparing rat lung responses vs. mouse or hamsters). The investigators concluded that rats demonstrated the greatest propensity for generating pro-inflammatory pulmonary mediator levels, whereas mice and hamsters demonstrated increased anti-inflammatory mediators. Importantly, it was postulated that these differences in pro- and anti-inflammatory responses may contribute and strongly influence the apparent species differences in pulmonary responses following particle-overload inhalation exposures.

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In contrast to the inter-rodent species differences, the fundamental differences in distribution patterns between particle-exposed rats and monkeys/coal miners – appear to lead to differential pulmonary responses among the several species studied. As discussed above, inhaled particles are deposited and retained within the alveolar duct compartments of exposed rats. In contrast, to a much greater extent, inhaled particles that deposit in the distal lung or respiratory bronchiole transmigrate across alveolar epithelial cell compartments to interstitial sites in the respiratory tracts of nonhuman primates or humans. The interstitialization of particles appears to serve as a repository in these species, is less reactive, and the comparative data demonstrate a reduced distal lung/alveolar hyperinflammatory pulmonary response following high dose exposures in the monkeys and coal miners. Over a working lifetime, coal workers exposed to high dust concentrations may develop interstitial-based coal workers pneumoconiosis/progressive massive fibrosis (PMF) (see Figure 7), but the risk of developing pulmonary tumors seems to be non-existent or immeasurable, as demonstrated by the vast amount of negative epidemiological data in these workers.

A recent discussion (Morfeld *et al* 2015) adds to the assessments and perspectives given in this current review. The authors analyzed the current approach of the German MAK Commission applying translational toxicology to derive a cancer classification and an occupational limit value for granular biopersistent particles without specific toxicity (covering TiO_2 and CB as examples) using rat study findings. We also refer to the letter exchange that followed (Hartwig 2016, Morfeld *et al* 2016).

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From another perspective, Pauluhn has suggested that particle retention kinetics and pulmonary inflammation were the main drivers of particle overload and were consequences primarily of the volumetric particle dose and the macrophage pool. According to the author, these factors determine the percentage of macrophage volume that is displaced by particles leading to particle overload and corresponding impaired clearance function. As a consequence, Pauluhn has concluded that the particle displacement volume is the most prominent unifying denominator linking the retained dose of particles in the lung with toxicity (Pauluhn, 2011; Pauluhn 2014).

In conclusion, five important factors are again detailed and discussed which provide compelling evidence that the rat lung tumor response to PSP-related particle overload is not relevant to lung cancer risks for human production workers.

These are summarized below:

1) Following identical subchronic exposures of rats, mice and hamsters to aerosols of PSPs, markedly different responses were observed in the different species. The results of these comparative studies better explain the sensitivity and sequence of events or pathological sequelae of exposed rats which does not occur in similarly exposed mice or hamsters. Based on these and other comparative studies a conceptual AOP has been proposed by an ECETOC Task force (2013) for the rat model. It should be noted that the proposed AOP model for the particle-overload exposed rat represents a species-specific set of pathological sequelae and is not consistent with the

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pulmonary effects documented in PSP exposed mice/hamsters or in particle-exposed nonhuman primates or coal miners; [see Tables 1 and 3]

2) better clarity and articulation of the fundamental interspecies differences in particle kinetics and pulmonary responses when comparing chronically exposed rats to nonhuman primates or coal workers. Unlike the rodent species, a large proportion of inhaled particles which deposit in the distal lung translocate across alveolar epithelial barriers to interstitial sites to a significant degree in humans and monkeys. This sequestration of particles in the interstitial (monkeys) vs. alveolar (rat) compartments is most likely to have an important impact on the reported subsequent development of adverse pulmonary effects in humans and rats. In addition, numerous comparative long-term inhalation study results have clearly demonstrated the characteristic, hyperactive pulmonary responses in exposed rats when compared to the relatively normal physiological lung responses in monkeys and humans exposed to high dust burdens. [See Tables 2-3]

3) Updated information is now available on the ICRP Human Respiratory Tract Model (HRTM) which formerly underestimated the long-term particle retention in the lungs of humans. The new model by Gregoratto and colleagues, estimates substantially greater translocation of inhaled particles into the interstitial compartment, and this finding correlates well with the experimental/morphometric findings of pulmonary responses reported in studies on particle-exposed monkeys and post-mortem evaluations of particle kinetics/responses in the lungs of long-term coal miners;

4) Differences in the morphology and characterization of lung tumor types in PSP particle-overload exposed rats vs. human lung tumors (occurring

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primarily as a result of cigarette smoke and/or asbestos fiber exposures). The pulmonary tumors characterized in PSP-exposed rats differ in cell type and anatomical location when compared to human lung neoplasms.

5) Updated epidemiology data on PSP production workers (e.g., TiO_2, CB, and toner - > 50,000 workers) demonstrate no correlation between workplace exposures to PSPs and either lung cancer and/or noncancer respiratory disease.

The data focusing on these five individual factors which are interrelated in many ways have been discussed in detail. They clearly demonstrate a lack of relevance between the experimental findings of particle-overload related, PSP-induced lung tumors in overload-exposed rats when evaluating and formulating human risk assessment issues relative to lung cancers in PSP production workers. The most reasonable conclusion that can be reached, based on all the available relevant data, is that lung tumor results from chronic particle-overload inhalation studies with PSPs in rats have no relevance for determining lung cancer risks in occupational production workers exposed for a working lifetime to these PSPs and perhaps to other PSPs for which no epidemiological studies are available.

Therefore, the development of lung tumors following overload exposures to PSPs is unique to rats and occurs only under the circumstances of sustained particle overload in the lungs. In contrast, overload particle exposures to PSPs do not produce neoplastic responses in mice or hamsters, or larger mammals such as humans or non-human primates. Clearly, there is not an adverse outcome pathway scenario for these particular species that progresses to the development of lung tumors.

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Declaration of interests

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).

Acknowledgments

The authors acknowledge Dr. Gregoratto for providing important insights into the manuscript. Dr.Jerrold Abraham is also acknowledged for providing a micrograph demonstrated Coal Workers Pneumoconiosis. Dr. Scott Brown is acknowledged for making important suggestions in the preparation of this manuscript.

ACCEPTED MANUSCRIPT

References

Baan RA [2007]. Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. Inhal Toxicol 19 (Suppl. 1):213–228.

Bermudez E, Mangum JB, Asgharian B, Wong BA, Reverdy EE, Janszen DB, Hext PM, Warheit DB, Everitt JI. 2002. Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol.Sci. 70, 86-97.

Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI. 2004. Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol. Sci. 77, 347-357.

Boffetta P, Soutar A, Cherrie JW, Granath F, Andersen A, Anttila A, Blettner M, Gaborieau V, Klug SJ, Langard S, Luce D, Merletti F, Miller B, Mirabelli D, Pukkala E, Adami HO, Weiderpass E. 2004. Mortality among workers employed in the titanium dioxide production industry in Europe. *Cancer Causes Control.* 15, 697-706

Bolton RE, Vincent JH, Jones AD, Addison J and Beckett ST. 1983. An overload hypothesis for pulmonary clearance of UICC amosite fibres inhaled by rats. Br. J. Industrial Med. 40, 264-272.

Boorman GA, Brockmann M, Carlton WW, Davis JM, Dungworth DL, Hahn FF, Mohr U, Reichhelm HB, Turusov VS, Wagner BM. 1996. Classification of cystic keratinizing squamous lesions of the rat lung: report of a workshop. Toxicol. Pathol. 24, 564-72.

Buchte SF, Morfeld P, Wellmann J, Bolm-Audorff U, McCunney RJ, Piekarski C. 2006. Lung cancer mortality and carbon black exposure: a nested case-control study at a German carbon black production plant. J. Occup. Environ. Med, 48, 1242-1252.

ACCEPTED MANUSCRIPT

Carleton WW. "Proliferative keratin Cyst" a lesion in the lungs of rats following chronic exposure to para-aramid fibrils. 1994. Fundamental Appl.Toxicol. 23,304-307.

Carter JM, Corson N, Driscoll KE, Elder A, Finkelstein JN, Harkema JN, Gelein R, Wade-Mercer P, Nguyen K, Oberdorster G. 2006. A comparative dose-related response of several key pro- and antiinflammatory mediators in the lungs of rats, mice, and hamsters after subchronic inhalation of carbon black. J.Occup. Environ. Med. 48, 1265-1278.

CASAC/Clean Air Science Advisory Committee/US EPA Letter to CM Browner US EPA Administrator (1995)

Chen JL, Fayerweather WE. 1988. Epidemiologic study of workers exposed to titanium dioxide. J.Occup. Med. 30, 937-942.

Dell LD, Mundt KA, Luippold RS et al.,2006. International Carbon Black Association A cohort mortality study of employees in the U.S. carbon black industry. J. Occup. Environ. Med. 48, 1219-1229.

Dell LD, Gallagher AE, Crawford L, Jones RM, Mundt KA. 2015. Cohort Study of Carbon Black Exposure and Risk of Malignant and Nonmalignant Respiratory Disease Mortality in the US Carbon Black Industry. J. Occup.. Environ. Med. 57, 984-997.

ECETOC -European Centre for Ecotoxicology and Toxicology of Chemicals. Poorly Soluble Particles/Lung Overload. 2013. Technical Report No., 122

Elder A, Gelein R, Finkelstein JN, Driscoll KE, Harkema J, Oberdörster G. 2005. Effects of subchronically inhaled carbon black in three species. I. Retention kinetics, lung inflammation, and histopathology. Toxicol. Sci. 88, 614-629.

Ellis ED, Watkins J, Tankersley W, Phillips J, Girardi D. 2010. Mortality among titanium dioxide workers at three DuPont plants. J. Occup. Environ. Med. 52, 303-9.

Ellis ED, Watkins JP, Tankersley WG; Phillips JA, Girardi DJ. 2013. Occupational exposure and mortality among workers at three titanium dioxide plants. Am. J. Ind. Med. 56, 282-291.

ACCEPTED MANUSCRIPT

Fryzek JP, Chadda B, Marano D, White K, Schweitzer S, McLaughlin JK, Blot WJ. 2003. A Cohort Mortality Study Among Titanium Dioxide Manufacturing Workers in the United States. J Occup. Environ. Med. 45, 400-409.

Green FHY. 2000. Pulmonary responses to inhaled poorly soluble particulate in the human. Inhal. Toxicol 12, 59-95.

Gregoratto D, Bailey MR, Marsh JW. 2010. Modelling particle retention in the alveolar-interstitial region of the human lungs. Journal of radiological protection. J Radiol Prot. 30, 491–512.

Gregoratto D, Bailey MR, Marsh JW. 2011. Particle clearance in the alveolar-interstitial region of the human lungs: model validation. Radiat. Prot. Dosimetry 144, 353–356.

Hartwig A. Reply on behalf of the 'Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area' (MAK Commission). 2015. Available from: http://particleandfibretoxicology.biomedcentral.com/articles/10.1186/s12989-015-0079-3/comments.

The Health Effects Institute. 1995. Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects. A Special Report of the Institute's Diesel Working Group. Health Effects Institute, April, 1995.

Heinrich U, Peters L, Creutzenberg O, Dasenbrock C, and Hoymann H-G 1994. Inhalation exposure of rats to tar/pitch condensation aerosol or carbon black alone or in combination with irritant gases. In Mohr U, Dungworth DL, Mauderly JL, and Oberdörster G (editors), Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract, pp. 433-441 (Cited in IARC, 1996).

Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, and Levsen K. 1995. Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal. Toxicol. 7, 533-556.

Hodgson JT, Jones RD. 1985. A mortality study of carbon black workers employed at five United Kingdom factors between 1947 and 1980. Arch. Environ. Health. 40, 261-268.

ACCEPTED MANUSCRIPT

ILSI Risk Science Institute. 2000. The relevance of the rat lung response to particle overload for human risk assessment: a workshop consensus report. ILSI Risk Science Institute. Inhal Toxicol 12, 1-17.

International Agency for Research on Cancer [IARC] 1996. IARC Monograph Series, Volume 65, pp. 149-262.

International Agency for Research on Cancer [IARC] 2010. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans, Volume 93 – Carbon Black, Titanium Dioxide and Talc, pp. 43-191.

ICRP. 1994. Human respiratory tract model for radiological protection. In: Smith H (ed) Annals of the ICRP, ICRP Publication No. 66, International Commission on Radiological Protection, Tarrytown, New York.

Klonne DR, Burns JM, Halder CA, Holdsworth CE, and Ulrich CE. 1987. Two-year inhalation toxicity study of petroleum coke in rats and monkeys. Am. J. Ind. Med. 11, 375-389.

Knaapen AM, Borm PJA, Albrecht C, Shins RPF [2004]. Inhaled particles and lung cancer. Part A: Mechanisms. Int J Cancer 109:799–809

Kuempel ED, Tran CL, Smith RJ, Bailer AJ. 2001. A biomathematical model of particle clearance and retention in the lungs of coal miners. II. Evaluation of variability and uncertainty. Regul. Toxicol. Pharmacol. 34, 88–101.

Lee KP, Trochimowicz HJ, Reinhardt CF. 1985. Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. Toxicol. Appl. Pharmacol. 79, 179-182.

Lee KP, Henry NW 3rd, Trochimowicz HJ, Reinhardt CF. 1986. Pulmonary response to impaired lung clearance in rats following excessive TiO2 dust deposition. Environ. Res. 41, 144-167.

Levy LS.1994. Squamous lung lesions associated with chronic exposure by inhalation of rats to p-Aramid fibrils (fine fiber dust) and to titanium dioxide: Findings of a pathology workshop. In: Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract. DL Dungworth, JL Mauderly and G Oberdorster, eds. International Life Sciences Institute/ILSI Press, 1994.

Levy LS. 1995. Review: The 'Particle Overload' phenomenon and human risk assessment. Indoor and Built Environment 4, 254-262.

ACCEPTED MANUSCRIPT

Lewis TR, Green FHY, Moorman WJ, Burg JR, and Lynch DW. 1989. A chronic inhalation toxicity study of diesel engine emission and coal dust, alone and combined. J. Am. Coll. Toxicol 8, 345-375.

MacFarland HN, Coate WB, Disbennett DB and Ackerman LJ. 1982. Lon-term inhalation studies with raw and processed shale dusts. Ann. Occup. Hyg. 26, 213-224.

Mauderly JL, Jones RK, Griffith WC, Henderson RF, McClellan RO. 1987. Diesel exhaust is a pulmonary carcinogen in rats exposed chronically by inhalation. Fundamental Appl. Toxicol. 9, 208-221.

Mauderly JL, Banas DA, Griffith WC, Hahn FF, Henderson RF, and McClellan RO. 1996. Diesel exhaust is not a pulmonary carcinogen in CD-1 mice exposed under conditions carcinogenic to F344 rats. Fundamental and Applied Toxicology 30, 233 – 242.

Mauderly JL. Relevance of particle-induced rat lung tumors for assessing lung carcinogenic hazard and human lung cancer risk. 1997. Environ Health Perspect. 105 Suppl 5, 1337-46.

McClellan RO. 1997. Use of mechanistic data in assessing human risks from exposure to particles. Environ Health Perspect. 105 Suppl 5:1363-72.

Morfeld P, Büchte SF, McCunney RJ, Piekarski C. 2006. Lung cancer mortality and carbon black exposure: uncertainties of SMR analyses in a cohort study at a German carbon black production plant. J. Occup Environ. Med. 48, 1253–64.

Morfeld P, Büchte SF, Wellmann J, McCunney RJ, Piekarski C. 2006. Lung cancer mortality and carbon black exposure: cox regression analysis of a cohort from a German carbon black production plant. J. Occup. Environ. Med. 48, 1230–41.

Morfeld P, McCunney RJ. 2007. Carbon black and lung cancer: testing a new exposure metric in a German cohort. Am. J. Ind. Med. 50, 565–7.

Morfeld P, McCunney RJ. 2009. Carbon black and lung cancer-testing a novel exposure metric by multi-model inference. Am. J. Ind Med.
52, 890–9.

ACCEPTED MANUSCRIPT

Morfeld P, McCunney RJ. 2010. Bayesian bias adjustments of the lung cancer SMR in a cohort of German carbon black production workers. J Occup. Med. Toxicol. 2010; Available from: http://www.ncbi.nlm.nih.gov/pubmed/20701747.

Morfeld P, Bruch J, Levy L, Ngiewih Y, Chaudhuri I, Muranko HJ, Myerson R, and McCunney RJ. 2015. Translational toxicology in setting occupational exposure limits for dusts and hazard classification - a critical evaluation of a recent approach to translate dust overload findings from rats to humans. Part Fibre Toxicol. 2015 Apr 23;12:3. doi: 10.1186/s12989-015-0079-3.

Morfeld P, Bruch J, Levy L, Ngiewih Y, Chaudhuri I, Muranko HJ, Myerson R, McCunney RJ. 2016. Response to the Reply on behalf of the 'Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area' (MAK Commission) by Andrea Hartwig Karlsruhe Institute of Technology (KIT). Particle and Fibre Toxicology. 13(1):1-6.

Morrow PE. 1988. Possible mechanisms to explain dust overloading of the lungs. Fundam. Appl. Toxicol. 10, 279-290.

Nikula KJ, Snipes MV, Barr EB, Griffith WC, Henderson RF, and Mauderly JL 1995. Comparative pulmonary toxicities and carcinogenicity of chronically inhaled diesel exhaust and carbon black in F344 rats. Fundam. Appl. Toxicol. 25, 80-94.

Nikula KJ. 2000. Rat lung tumours induced by exposure to selected poorly soluble nonfibrous particles. Inhal Toxicol 12, 97-119.

Nikula KJ, Avila KJ, Griffith WC, Mauderly JL. 1997. Lung tissue responses and sites of particle retention differ between rats and cynomolgus monkeys exposed chronically to diesel exhaust and coal dust. Fundam. Appl. Toxicol. 37, 37-53.

Nikula KJ, Vallyathan V, Green FH, Hahn FF. 2001. Influence of exposure concentrations or dose on the distribution of particulate material in rat and human lungs. Environ. Health Perspect 109, 311-318.

NIOSH 2011. Current Intelligence Bulletin 63 – Occupational exposure to Titanium Dioxide. NIOSH Dept. of Health and Human Services.

ACCEPTED MANUSCRIPT

Oberdörster G. 1995. Lung particle overload: Implications for occupational exposures to particles. Regul Toxicol Pharmacol. 21, 123-35.

Pauluhn J. Poorly soluble particulates: Searching for a unifying denominator of nanoparticles and fine particles for DNEL estimation. Toxicology 279 (2011) 176-188.

Pauluhn J. Review: Derivation of occupational exposure levels (OELs) of low-toxicity isometric biopersistent particle: how can the kinetic lung overload paradigm be used for improved inhalation toxicity study design and OEL-derivation? Part and Fibre Toxicology (2014) 11:72

PCRARM, Presidential/Congressional Commission on Risk Assessment and Risk Management. 1997. Final Report, Risk Assessment and Risk Management in Regulatory Decision Making. Volume 2, 1997. Pp. 65 and 67; http://www.riskworld.com/Nreports/1997/risk-rpt/volume2/pdf/v2epa.pdf

Ramanakumar AV, Parent ME, Latreille B, Siemiatycki J. 2008. Risk of lung cancer following exposure to carbon black, titanium dioxide and talc: Results from two case-control studies in Montreal. Int. J. Cancer 122,183-189.

Schultz M. 1996. Comparative pathology of dust-induced pulmonary lesions: Significance of animal studies to humans. Inhalation Toxicology 8,433-456, 1996.

Sorahan T, Hamilton L, van Tongeren M, Gardiner K, Harrington JM. 2001. A cohort mortality study of U.K. carbon black workers, 1951–1996. Am J Ind Med. 39, 158–70.

Sorahan T, Harrington JM. 2007. A “lugged” analysis of lung cancer risks in UK carbon black production workers, 1951–2004. Am J Ind Med.
50, 555–64.

Wagner WD, Groth DH, Holtz JL, Madden GE and Stokinger HE 1969. Comparative chronic inhalation toxicity of beryllium ores, bertrandite and beryl, with production of pulmonary tumors by beryl. Toxicol. Appl. Pharmacol. 15, 10-29.

Warheit DB, Hansen JF, Yuen IS, Kelly DP, Snajdr SI, Hartsky MA. 1997. Inhalation of high concentrations of low toxicity dusts in rats results in

ACCEPTED MANUSCRIPT

impaired pulmonary clearance mechanisms and persistent inflammation. *Toxicol Appl Pharmacol* 145, 10-22.

Warheit DB and Frame SR. 2006 characterization and reclassification of titanium dioxide-related pulmonary lesions. J Occup Environ Med. 48, 1308-13.

Wehner AP, Stuart BO, Sanders CL. Inhalation studies with Syrian golden hamsters. In: Progress in Experimental Tumor Research. Vol. 24 (Homburger F, ed) Switzerland: Karger, Basel 1979; 177-196.

Wellmann J, Weiland SL, Neiteler G et al., 2006. Cancer morality in German carbon black workers 1976-98. Occup Environ Med, 63, 513-521

Wolff RK, Henderson RF, Snipes MB, Griffith WC, Mauderly JL, Cuddihy RG, McClellan RO. 1987. Alterations in particle accumulation and clearance of rats chronically exposed to diesel exhaust. Fundam. Appl. Toxicol. 9, 154-166.

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Figures 8-17a-2016

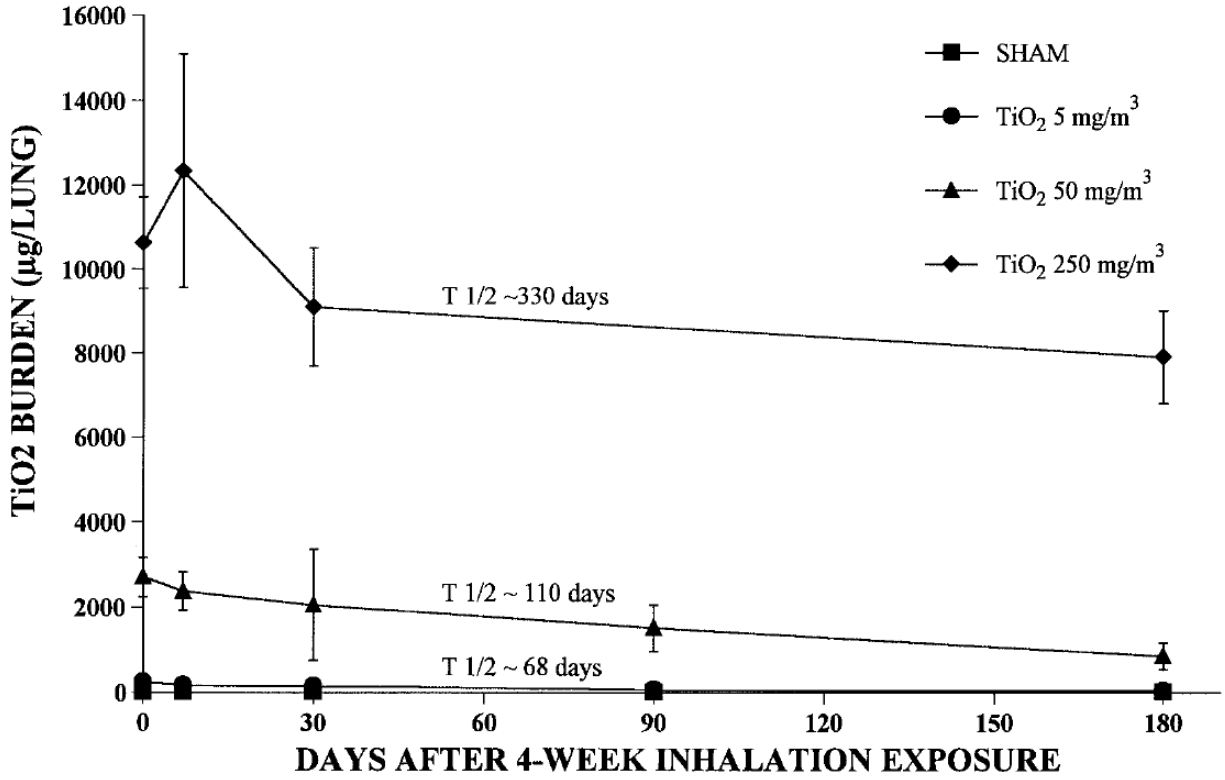

Fig. 1. Lung burden and clearance of inhaled pigment-grade TiO_2 particles in rats. Rats were exposed for 4 weeks and lung burden/clearance kinetics were followed over a 6-month postexposure period. Clearance of TiO_2 particles was significantly retarded following exposures to 250 mg/m^3. Copied from Warheit et al. 1997.

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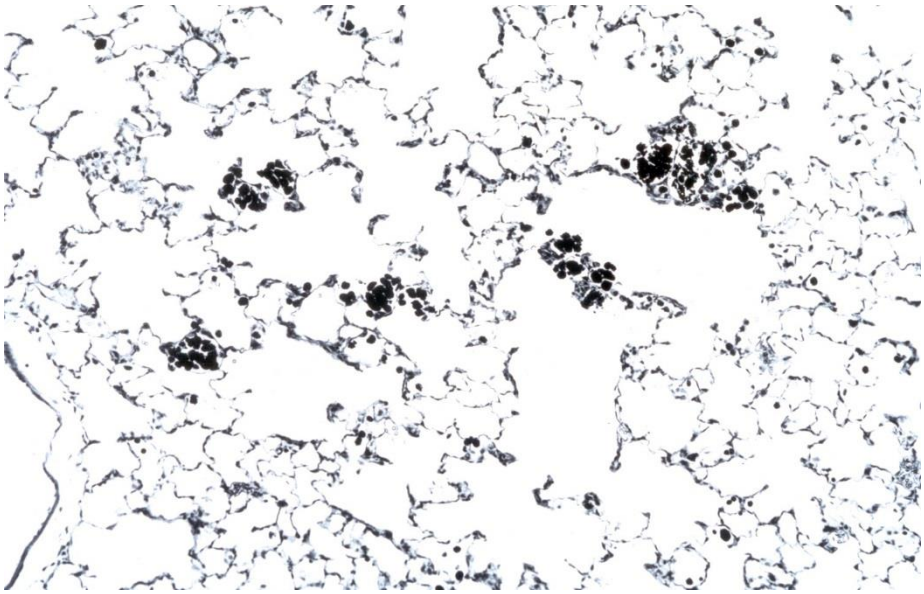

Figure 2.

Low magnification light micrograph (LM) - lung section of a rat following a 4-week inhalation exposure (and 1-week recovery) to 250 mg/m^3 Pigment-grade TiO_2 particles. Note that most of the deposit particles remain within the alveolar ducts regions

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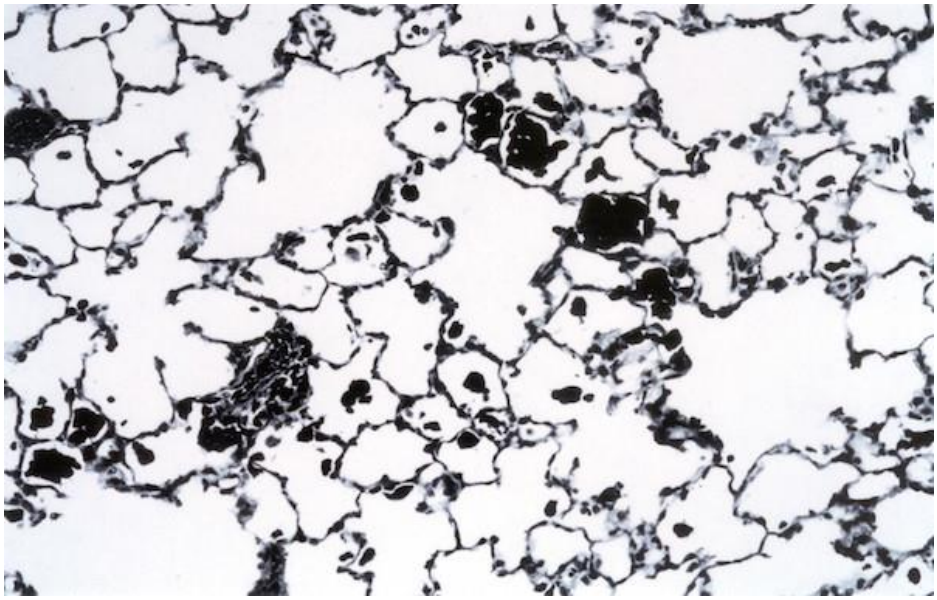

Figure 3. Higher magnification light micrograph (LM) of a lung section of a rat following a 4-week inhalation exposure (and 1-week recovery) to 250 mg/m^3 Pigment-grade TiO_2 particles. Note that most of the deposited particles remain in the alveolar duct regions.

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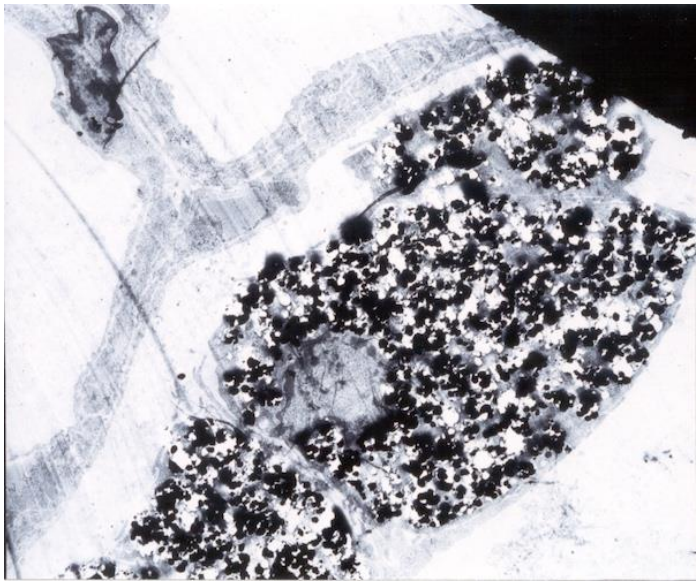

Figure 4. Transmission electron micrograph of an alveolar macrophage containing numerous phagocytized TiO_2 particles – adjacent to a Type I alveolar epithelial cell.

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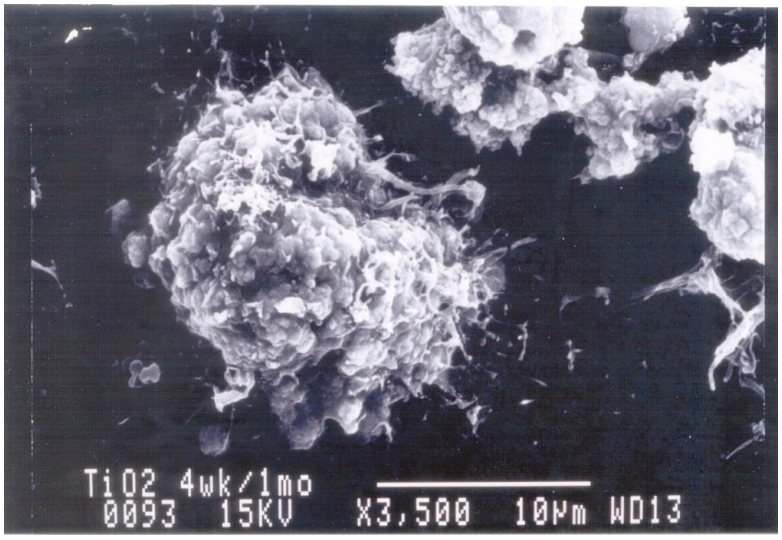

Figure 5a. Scanning electron micrograph (secondary image) of a TiO_2-particle containing macrophage lavaged from the lungs of a rat and placed in cell culture. The macrophage was subsequently subjected to an *in vitro* phagocytosis assay to determine phagocytic capacity of in vitro administered carbonyl iron particles (see the backscatter image below)

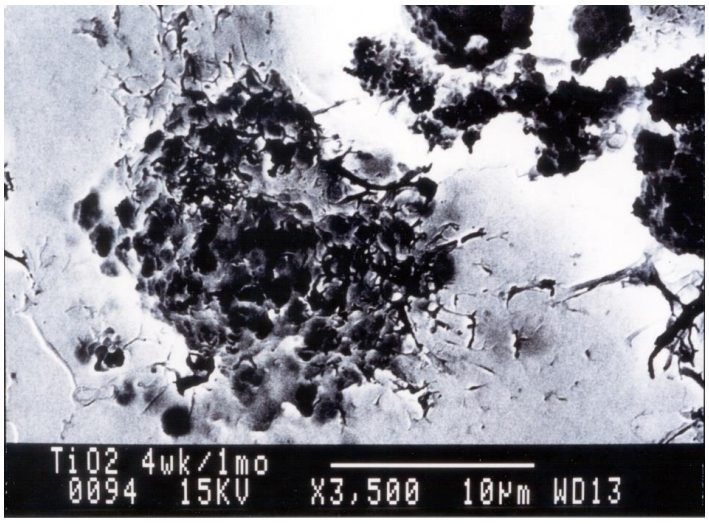

Figure 5b. Scanning electron micrograph (backscatter image) of a TiO_2-particle containing macrophage lavaged from the lungs of a rat and placed in cell culture. The macrophage was subsequently subjected to an in vitro phagocytosis assay to determine phagocytic capacity of *in vitro* administered carbonyl iron particles (see the backscatter image below)

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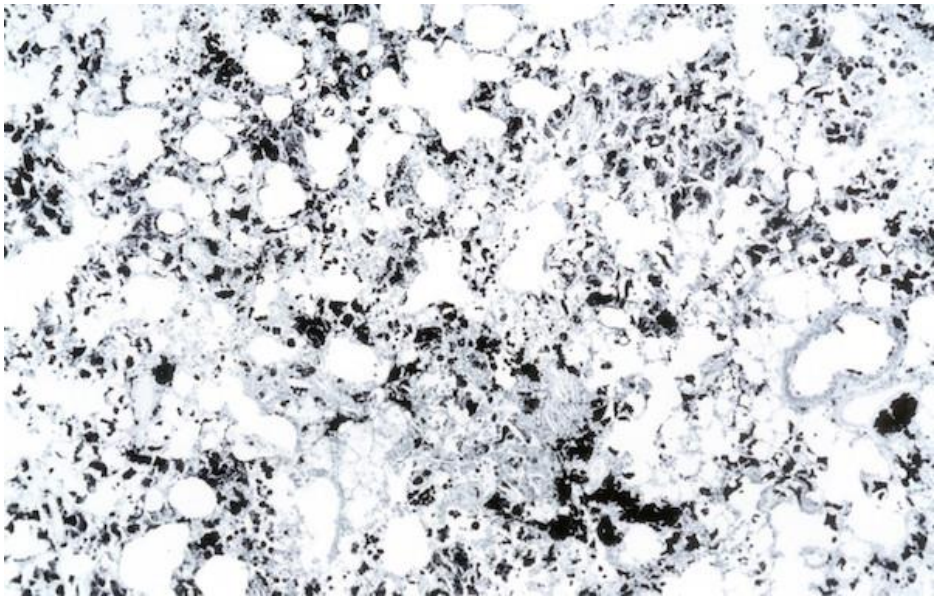

Figure 6. Micrograph of rat lung following 2-year inhalation exposure to 250 mg/m3 Pigment-grade TiO2. Note the retention pattern of particles – both alveolar and interstitial.

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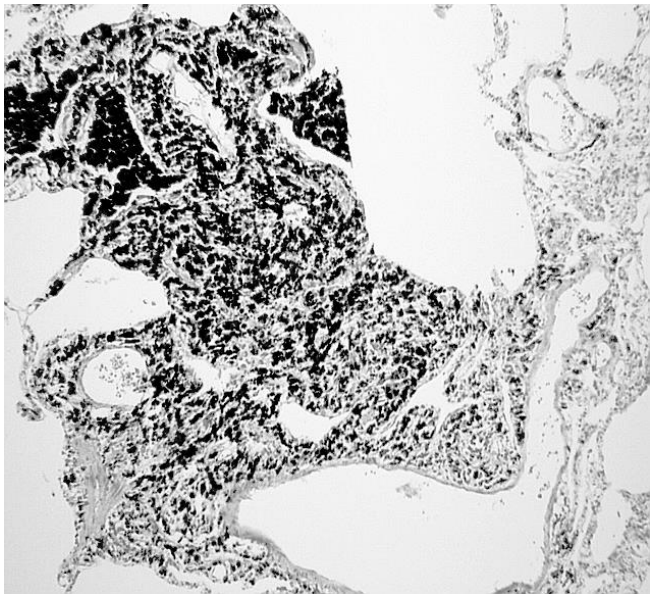

Figure 7. Light Micrograph of a human lung post-mortem demonstrating Coal workers Pneumoconiosis. Most of the particulate material has translocated to the interstitium.

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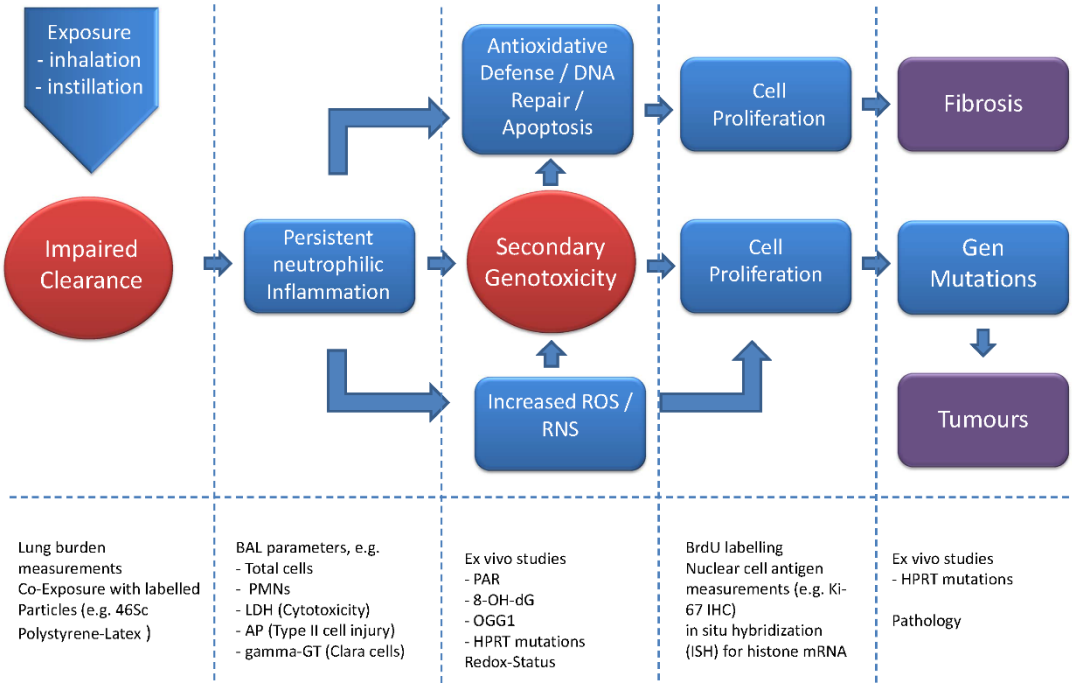

Figure 8. : Conceptual AOP model of `lung overload` pathological sequelae in rats chronically exposed to aerosols of PSPs - related key events and possible investigative methods [copied from ECETOC 2013 reference]

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Table 1

Species Comparison of Rodent Lung Responses to Inhaled Poorly-Soluble Particles

Likelihood for Developing Particle Overload

- Rat – high degree
- Mouse – high degree
- Hamster – low degree

Alveolar Macrophage Responses following long-term high-dose exposures

- Rat – high but accumulation primarily in alveolar ducts
- Mouse – high but accumulation primarily in alveolar ducts
- Hamster – high but favors more rapid lung clearance

Pulmonary inflammatory Responses

- Rat – high degree
- Mouse – high degree
- Hamster – low degree

Alveolar Epithelial Cell Proliferation

- Rat – high degree
- Mouse – medium to low degree
- Hamster – low degree

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Development of Fibroproliferative Effects including Lung Fibrosis

- Rat – high and sustained effects
- Mouse – moderate to low degree
- Hamster – low degree

Anatomical location of Retained Particles in the Lung

- Rat – primarily an accumulation of particles in alveolar ducts
- Mouse – primarily an accumulation of particles in alveolar ducts
- Hamster – comparatively less accumulations of particles in alveoli – as a consequence of more rapid particle clearance

Development of Particle Overload-related lung tumors

- Rat – yes
- Mouse – No
- Hamster – No

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Table 2.

Comparisons of Pulmonary Distribution Patterns and Lung Effects following Long-term Inhalation Exposures in Rats vs. Nonhuman Primates/Humans

Nikula et al., (1997)

Male cynomolgus monkeys and F344 rats exposed to aerosols of diesel exhaust (DEEP); coal dust or coal dust + DEEP for 24 months. Morphometric analysis of distribution of retained particles in the selected anatomic compartments in the lung; and histopathology:

Rats → retention in alveolar ducts; significant alveolar hyperplasia, inflammation and septal fibrosis

Monkeys → retention primarily in pulmonary interstitium; very limited pulmonary responses

Wagner et al., 1969

Two strains of rats and squirrel monkeys were chronically exposed to identical concentrations of betrandite or beryl ore.

Rats responded with chronic pulmonary inflammation with granulomas and alveolar epithelial hyperplasia concomitant with an increased incidence of lung tumors in beryl-exposed rats. The lung response in monkeys was described as the absence of lesions other than macrophage accumulations in the location of the respiratory bronchioles and blood vessels

Klonne et al. (1987)

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Cynomolgus monkeys and SD rats exposed to aerosols of petroleum coke (0, 10, or 31 mg/m^3) 6 hr/d, 5 d/wk for 24 months. Histopathological assessment of respiratory tract.

Rats → chronic inflammation, focal fibrosis, bronchiolization, alveolar metaplasia, keratin cyst formation, increased lung wts., black discoloration.

Monkeys → increased lung wts, black discoloration, deposition and phagocytosis of test material.

MacFarland et al., (1982)

Cynomolgus monkeys and F344 rats exposed to aerosols of raw or processed shale (0, 10, or 30 mg/m^3) 6 hr/d, 5 d/wk for 24 months.

Rats → proliferative bronchiolitis and alveolitis, nonprogressive fibrosis, cholesterol clefts and microgranulomas.

Monkeys →pigment-laden macrophages; majority had little or no reaction to accumulated material.

Lewis et al (1989); Nikula et al., (1997)

Monkeys and rats exposed to aerosols of diesel exhaust or coal dust (2 mg/m^3) 7 hr/d, 5 d/wk for 24 months.

Morphometric analysis of distribution of retained particles in the selected anatomic compartments in the lung; and histopathology.

Rats → retention in alveoli and alveolar ducts; significant alveolar epithelial hyperplastic and inflammatory responses.

Monkeys → retention primarily in pulmonary interstitium; very limited pulmonary responses.

Nikula et al., (1997)

Male cynomolgus monkeys and F344 rats exposed to aerosols of diesel exhaust (DEEP); coal dust or coal dust + DEEP for 24 months.

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Morphometric analysis of distribution of retained particles in the selected anatomic compartments in the lung; and histopathology:

Distribution of inhaled particles in lungs following long-term exposures – Humans vs. Rats

Nikula et al., (2001)

F344 rats exposed to aerosols of diesel exhaust (DEEP) 0.35, 3.5 or 7 mg/m^3; Nonsmoking coal miners [2 mg/m^3 standard or < 10 mg/m^3 for mean working life of 40 years].

Morphometric analysis of distribution of retained particles in the selected anatomic compartments in the lung; and histopathology:

Rats → 82 – 85% of retained particles in alveoli and alveolar ducts – primarily in macrophages;

Humans → chronically inhaled particulate material retained primarily in pulmonary interstitium;

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Table 3

Comparisons of Rodent Lung Responses versus Human/Primate Responses to Inhaled Poorly Soluble Particulates

Likelihood for Developing Particle Overload

- Rat – high degree
- Human/Primate – not determined

Alveolar Macrophage Responses to Particle Overload

- Rat – high accumulation in alveolar ducts
- Human/Primate – not extensive due to greater particle translocation to interstitium

Lung Inflammatory Responses

- Rat – high degree
- Human/Primate – low degree

Alveolar Epithelial Cell Proliferation

- Rat- high degree
- Human/Primate – low degree

Development of Fibroproliferative Effects Including Lung Fibrosis

- Rat – high and sustained effects
- Human/Primate – low degree

Location of Retained Particles in the Lung

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- Rat – primarily accumulation of particles in alveolar ducts
- Human/Primate – primarily interstitial

Development of Particle Overload-related Lung Tumors

- Rat – Yes
- Human/Primate - No

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Relevance of the rat lung tumor response to particle overload for human risk assessment—Update and interpretation of new data since ILSI 2000

Warheit, D. B.; Kreiling, R.; Levy, Len

2016-11-19
Elsevier
http://dx.doi.org/10.1016/j.tox.2016.11.013
D.B. Warheit R. Kreiling L.S. Levy, Title: Relevance of the Rat Lung Tumor Response to Particle Overload for Human Risk Assessment—Update and Interpretation of new data since ILSI 2000, Toxicology, Volume 374, 30 December 2016, Pages 42–59

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