How Does One Interpret the Relevance of Particle Overload/ Rat Lung Tumor Findings in Chronic Inhalation Studies with PSPs for Assessing Human Occupational Health Risks?

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Granular Biopersistent Dusts (GBS) and Translational Toxicology: Deriving HECs/ Occupational Limit Values
Berlin, Germany
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Background

It has been known for some time that some poorly soluble particles (PSPs) can cause lung cancer in rats due to chronic inflammation as a result of “lung overload” in the alveolar region of the lung. [The lung cancer findings appear to be unique to the rat.]

These findings will affect classification and the setting of occupational exposure limits for many such PSP including many metals and their inorganic compounds.
Recent developments

• Recently, French ANSES in their CLH report to ECHA concluded that TiO$_2$ particles should be classified as Carc. 1B- H350i by the inhalation route. Category 1B is applicable to the substance presumed to have carcinogenic potential for humans, based largely on animal evidence.

• “although no definitive conclusion can be drawn about the carcinogenic effect after inhalation of TiO$_2$ based on human data” (despite the negative epidemiological data on > 24,000 production workers) “lung tumours were reported in 2 inhalation studies in animals with fine rutile TiO$_2$ (Lee et al., 1985”) and nano anatase/rutile P25 TiO$_2$ (Heinrich et al, 1995). “In the Lee (1985) study, performed with a protocol similar to OECD guideline, increases of adenomas were reported in both sexes”. [comment: Although NIOSH is heavily cited in the CLH document]
Responses to the CLH Proposal - Fundamental differences in pulmonary responses between particle-exposed rats and occupationally exposed humans

• The formation of tumours upon inhalation exposure to TiO$_2$ is considered specific to rats and limited to conditions of particle overload.

• This species specificity manifests itself by a complete absence of similar responses in all other tested species, coupled with negative human epidemiology, suggesting a lack of relevance of this observation to humans.

• In addition, the disposition of such inhaled particles has been shown to be substantially different between nonhuman primates and rats, with the latter being particularly sensitive.

• The lung cellular responses of rats exposed chronically to particles is hyper-inflammatory and hyperplastic, which primates show normal physiological reactions such as particle accumulation and macrophage responses to inhaled particles.
The Dilemma

Whilst we very much welcome the use of modelling and translational toxicology from rat data to assist prediction to human hazard and risk assessment; we would argue that more consideration should be given to using both a weight of evidence and fit for purpose approach which take into account the totality of the data; in particular species differences and human findings from working populations exposed to PSPs under relevant occupational conditions that have been extensively investigated.
Outline of Evidence

• Interspecies differences in lung responses of rats vs. other rodents
• Interspecies differences in particle kinetics of rats vs. nonhuman primates and coal miners
• Advanced and updated human respiratory tract retention models demonstrating particle retention patterns similar to morphometric studies in monkeys and coal miners.
• Differences in morphologies and locations of rat lung tumors exposed to overload concentrations of PSPs vs. human lung cancers to asbestos and cigarette smoke.
• Comprehensive epidemiology studies in PSP production workers and coal miners that demonstrate no correlation between lifetime working exposures and lung cancer.
Outline of Evidence - 1

• Interspecies differences in lung responses of rats vs. other rodent species
Species Comparisons of Rodent Lung Responses to Inhaled Poorly-Soluble Particles

Development of Particle Overload-related lung tumors

• Rat – yes
• Mouse – No
• Hamster – No
Species Comparisons of Rodent Lung Responses to Inhaled Poorly-Soluble Particles

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Mouse</th>
<th>Hamster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likelihood for Developing</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Particle Overload</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar Macrophage Responses</td>
<td>High (accumulation</td>
<td>High (accumulation</td>
<td>High (with more</td>
</tr>
<tr>
<td>(long-term)</td>
<td>alveolar ducts)</td>
<td>alveolar ducts)</td>
<td>rapid clearance)</td>
</tr>
<tr>
<td>Pulmonary inflammatory</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Responses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degree of alveolar epithelial</td>
<td>High</td>
<td>Medium to Low</td>
<td>Low</td>
</tr>
<tr>
<td>cell proliferation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroproliferative Effects</td>
<td>High and sustained</td>
<td>Moderate to Low</td>
<td>Low</td>
</tr>
<tr>
<td>Location of Retained Particles</td>
<td>Alveolar ducts</td>
<td>Alveolar ducts</td>
<td>Less on alveolar</td>
</tr>
<tr>
<td>in the Lung</td>
<td>primarily</td>
<td>primarily</td>
<td>ducts, faster</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>clearance</td>
</tr>
</tbody>
</table>
Particle Overload – Rats exposed to TiO$_2$ aerosolized particles – 4 wks
Light Micrograph of a rat lung section after 4-week exposure to 250 mg/m³ TiO₂ particles
TEM of a lung section containing a highly phagocytic alveolar macrophage adjacent to a Type I epithelial cell (TiO$_2$ 250 mg/m$^3$)
Conceptual AOP Model of lung overload sequelae in Rats following chronic PSP exposures (ECETOC 2013)

**Exposure**
- Inhalation
- Instillation

**Impaired Clearance**

**Persistent neutrophilic inflammation**

**Secondary Genotoxicity**

**Increased ROS / RNS**

**Antioxidative Defense / DNA Repair / Apoptosis**

**Cell Proliferation**

**Fibrosis**

**Gen Mutations**

**Tumours**

- Lung burden measurements
- Co-Exposure with labelled Particles (e.g. 46Sc Polystyrene-Latex)

- BAL parameters, e.g.
  - Total cells
  - PMNs
  - LDH (Cytotoxicity)
  - AP (Type II cell injury)
  - gamma-GT (Clara cells)

- Ex vivo studies
  - PAR
  - 8-OH-dG
  - OGG1
  - HPRT mutations
  - Redox Status

- BrdU labelling
  - Nuclear cell antigen measurements (e.g. Ki-67 IHC)
  - in situ hybridization (ISH) for histone mRNA

- Ex vivo studies
  - HPRT mutations
  - Pathology
Conceptual AOP Model of lung overload sequelae in Mice and Hamsters following chronic PSP exposures Differs from Rats – lacks pathological sequelae to Tumor formation

Exposure - inhalation - instillation

Impaired Clearance

Persistent neutrophilic inflammation

Secondary Genotoxicity

Increased ROS / RNS

Antioxidative Defense / DNA Repair / Apoptosis

Cell Proliferation

Fibrosis

Cell Proliferation

Gen Mutations

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Lung burden measurements
Co-Exposure with labelled Particles (e.g. 46Sc Polystyrene-Latex)

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in situ hybridization (ISH) for histone mRNA

Ex vivo studies
- HPRT mutations
Pathology
Outline of Evidence - 2

• Interspecies differences in particle kinetics of rats vs. nonhuman primates and coal miners
## Comparisons of Rat Lung Responses vs. Human/Primates to Inhaled PSPs

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Human/Primate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Likelihood for Developing Particle Overload</strong></td>
<td>High</td>
<td>Not determined</td>
</tr>
<tr>
<td><strong>Alveolar Macrophage Responses (long-term)</strong></td>
<td>High (accumulation alveolar ducts)</td>
<td>Not extensive due to greater particle translocation to interstitium</td>
</tr>
<tr>
<td><strong>Pulmonary inflammatory Responses</strong></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Fibroproliferative Effects</strong></td>
<td>High and sustained</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Location of Retained Particles in the Lung</strong></td>
<td>Alveolar ducts primarily</td>
<td>Interstitium primarily</td>
</tr>
<tr>
<td><strong>Development of Particle Overload-related Lung Tumors</strong></td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>
Comparisons of Pulmonary Distribution Patterns and Lung Effects following long-term exposures of rats vs. Nonhuman Primates [likely non-overload expts]


- MacFarland et al. (1982) Monkeys/Rats exposed to raw or processed shale 24 mos. Rats -> prolif. bronchiolitis/alveolitis/fibrosis; microgranulomas. Monkeys -> pigment-laden macrophages; little or no adverse reaction.

Comparisons of Pulmonary Distribution Patterns and Lung Effects following long-term exposures of rats vs. Humans

- **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 particle in the selected anatomical compartment in the lung + 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.
Higher mag. LM of rat lung section after 4-week exposure to 250 mg/m³ TiO₂ particles. Most of the particles remain in alveolar ducts.
TEM of a lung section containing two highly phagocytic alveolar macrophages adjacent to a Type I epithelial cell ($\text{TiO}_2$ 250 mg/m$^3$)
Light Micrograph of a human lung post-mortem demonstrating interstitial-based Coal workers Pneumoconiosis
Outline of Evidence - 3

• Advanced and updated human respiratory tract retention models demonstrating particle retention patterns similar to morphometric studies in monkeys and coal miners.

• Recent studies by Gregoratto et al. – to update the ICRP Model – demonstrating greater translocation of inhaled radionuclides – also correlates with the morphometric studies demonstrating greater interstitial particle distribution in human lungs.
• Differences in morphologies and locations of rat lung tumors exposed to overload concentrations of PSPs vs. human lung cancers to asbestos and cigarette smoke.
Schematic of the Airway generations in the adult human lung – particles generally deposit on the respiratory bronchiole
Rat Lung Microdissection
Rat Lung Tissue Dissected to Demonstrate the Junction of the Terminal Airway and Proximal Alveolar Region
Lung Dissection – Alveolar Duct Bifurcation in a Rat
Iron Particle (↑) Deposition in the Lungs of Exposed Rats
Iron Particle Deposition at Bronchoalveolar Junction
(Backscatter Image)
Inhaled Chrysotile Asbestos Fibers (↑) Deposit on Alveolar Duct Bifurcations in the Lungs of Exposed Rats
Alveolar Macrophage Clearance of Inhaled Iron Particles
Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis
Alveolar Macrophage Migration to Iron Particle Deposition and Phagocytosis
(Backscatter Image)
Alveolar Macrophage Clearance of Inhaled Particles at Sites of Deposition
Clearance of Iron Particles by Alveolar Macrophages on the Airway Mucociliary Escalator
Clearance of Iron Particles on the Airway Mucociliary Escalator (BS image)
TEM of the Interstitial Space of the Lung

Figure 1. Electron micrograph showing a pulmonary capillary (C) in the alveolar wall. Note the extremely thin blood gas barrier of less than 0.5 microns. The arrow indicates the diffusion path from alveolar gas to the interior of the erythrocyte (EC) and includes the layer of surfactant (not shown in the preparation), alveolar epithelium (EP), interstitium (IN) capillary endothelium (EN) and plasma. Parts of structural cells called fibroblasts (FB), basement membrane (BM) and a nucleus of an endothelial cell are also seen. (From Weibel, E. R. Respirat. Physiol. 11: 54, 1970.)
Lung Dissection – Alveolar Duct Bifurcation in a Silica-exposed Rat
Alveolar Duct Bifurcation Post Silica Exposure – Neutrophil (P) Recruitment
Lung Tissue Sections – Control (A); Min-U-Sil (B); NanoQ II (C); Fine Quartz (D).
Morphometry at Bronchoalveolar Junctions
Critical Differences between Rats and Nonhuman Primates/Humans

• Particle Distribution patterns for Nonhuman Primates/Humans – Significant Translocation of inhaled particles to Interstitium

• Particle Distribution patterns for Rats – Mainly Macrophage Phagocytosis within alveolar ducts and neutrophilic inflamm. → greater likelihood for enhanced epithelial cell proliferation response

• Hyperplastic responses in rats to high dose particle exposures

• Normal physiological (macrophage phagocytic) responses in nonhuman primates + interstitialization of particles
Rat lung micrograph after 2-year inhalation exposure to 250 mg/m³ TiO₂ particles
Keratin cysts arising from pulmonary parenchyma ($\text{TiO}_2$ 250 mg/m$^3$)
Differences between human and rat lung tumors

- Humans – Lung Tumors are primarily located in bronchi/bronchioles
- Humans – Lung tumors following exposures to cigarette smoke or asbestos but not PSPs

- Rats – lung tumors occur following chronic particle overload exposures to PSPs
- Rats – Tumors are of alveolar origin
- Rats – adaptive feature of keratinizing squamous cell response
Outline of Evidence - 5

• Comprehensive epidemiology studies in PSP production workers and coal miners that demonstrate no correlation between lifetime working exposures and lung cancer.
Epidemiology Studies of TiO₂ Production Workers

• DuPont Studies – Chen and Fayerweather 1998; Ellis et al., 2010; 2013; 3607+ workers at 3 TiO₂ plants – no indication of a positive association between occupational exposure and lung cancer or non-malignant respiratory/heart disease.

• Boffetta et al., 2004 European studies – total cohort population > 15,000 workers. Authors concluded that TiO₂ exposures not correlated with lung cancer.


• Ramanakumar et al., 2008 - 2 population-based case-control occupational studies in Montreal (TiO₂, CB, or talc). No excess risk of lung cancer.
Epidemiology Studies with other PSP workers

• Carbon Black
• Talc
• Coal Dust

• No correlation with occupational exposures to PSP and development of lung cancers

- 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.
Rats vs. Humans

There are thus Five Comparative Factors that provide important insights on fundamental differences in pulmonary responses between rats chronically exposed to high concentrations of PSPs and developing lung tumors vs. occupationally-exposed humans.
Five Key Factors distinguishing rats vs. nonhuman primates/humans

1. Interspecies differences in particle kinetics of rats vs nonhuman primates and humans triggering differential particle-related pulmonary responses.

2. Interspecies differences in lung responses of rats vs. other rodents, triggering different adverse outcome pathways (AOPs);

3. Advanced and updated human respiratory tract deposition and retention models allowing more realistic particle translocation/retention estimates.
Five Key Factors distinguishing rats vs. nonhuman primates/humans (cont.)

4. Comprehensive in-depth analysis of available epidemiological data from PSP production workers and coal miners that demonstrate no correlation between particle exposures and lung cancers or other non-malignant respiratory diseases.

5. Differences in morphologies and characterization of rat vs. human pulmonary tumor types and locations (alveolar vs bronchial) within the respiratory tract.
Conclusions

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.
Review
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 tumours in rats for human risk assessment following chronic inhalation exposures to poorly soluble particulates (PSPs) 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 particulate 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.

- Interspecies differences in lung responses of rats vs. other rodents, triggering different adverse outcome pathways (AOPs);
- Interspecies differences in inhaled particle kinetics in rats vs. nonhuman primates and humans triggering different pathobiological pathways (e.g., primary to secondary);
- Advanced and updated human respiratory tract deposition and retention models allowing more realistic particle translocation/retention estimates;
- Differences in morphologies and characterizations of rat vs. human pulmonary tumor types and locations within the respiratory tract;
- 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-inflamatory respiratory diseases.

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.
Questions for the Audience

• What does it take to prove a negative response in humans – when the epidemiology studies are negative for lung cancer and non-cancer respiratory disease – yet the findings are positive – only in rats exposed to overload concentrations of PSPs?
• How should Hazard vs. Risk Issues be handled
• What if the Mechanisms of Action are different between rats vs. Humans/Primates?
• How should the Precautionary Principle be Implemented – Is it appropriate?
Thank you for your attention
Disclosure

• DBW is employed by a company (Chemours) that produces and sells titanium dioxide particles.