Section 6B: Health Session

Dosimetry, Dose-metrics, Bioprocessing and Human Risk Extrapolation

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REACTIVITY OF ULTRAFINE PARTICLES

High specific surface area: m²/g

Surface Reactivity as Dose-Metric,
e.g., ROS inducing potential to determine response per unit particle surface area

- DTT (dithiothreitol) assay
- DCFH-DA (2’-7’ dichlorofluorescin-diacetate) assay
- FRAS (ferric reducing ability of serum) assay
- Vit C assay
- ESR
- others...

as screening tool for categorization of UFPs based on reactivity
in cellular or cell free assays for Hazard Ranking
[Bello et al., 2009; Rushton et al., 2010]
Noncellular Nanoparticle ROS Summary (Carbon Particles)

Particle Mass Correlation

- PALAS fresh: 41nm
- PALAS aged: 41nm
- Sevacarb: 300nm
- Printex-90: 14nm
- Sterling-V: 70nm
- C-13: 42nm
- CB+25% Fe: 40nm

Equivalent H$_2$O$_2$ conc. (µM) vs. µg
Noncellular ROS Summary  (Carbon Particles)

Particle Surface Area Correlation

<table>
<thead>
<tr>
<th>Particle Type</th>
<th>Surface Area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PALAS fresh</td>
<td>700 m²/g</td>
</tr>
<tr>
<td>PALAS aged</td>
<td>700 m²/g</td>
</tr>
<tr>
<td>Sevacarb</td>
<td>7 m²/g</td>
</tr>
<tr>
<td>Printex-90</td>
<td>300 m²/g</td>
</tr>
<tr>
<td>Sterling-V</td>
<td>37 m²/g</td>
</tr>
<tr>
<td>C-13</td>
<td>~700 m²/g</td>
</tr>
<tr>
<td>CB+25% Fe</td>
<td>~700 m²/g</td>
</tr>
</tbody>
</table>

Equivalent H₂O₂ conc. (μM) vs. cm²
CONCLUSIONS, UF PARTICLE BOUND ROS (oxidative potential)

- ROS activity/m$^3$ of ambient UFP can vary widely

- ROS activity of lab-generated nanoparticles seems to reasonably well predict acute in vivo responses

- ROS activity of ambient UFP does not necessarily parallel their number or mass concentration

- Do results of epidemiological studies indicate association between UFP reactivity and effects at organ of entry or secondary organs?
Challenges re UFP Standard:

Physico-chemical properties of UFP are different from different sources:

- Elemental carbon
- Organic carbon compounds
- Inorganics (metals)
- Agglomeration/aggregation
- Surface properties
  - Solubility
  - Volatility

UFP source A ≠ UFP source B → different biol./toxicol. effects
Proposed Concept for Standard

• UFP standard should best be source-specific

• Based on number concentration of emitted UFP

• Need to identify sources that emit most reactive UFP

• Regulate these sources (rather than all UFP) by introducing a number emission standard, based on UFP risk assessment
What is surface reactivity of Coal/Biomass Combustion Generated Nanoparticles?
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Chem. Characterization by HR-TEM/STEM/EELS
Coal Combustion Ash

Si-Al-rich Sphere

Nano-sized Carbon deposits With Heavy Metals (Fe/Zn/Pb/As/Co/Cu etc.)

200 nm
Case study with inhaled Nano SiO$_2$ NPs:

Exposure-Dose-Response Inhalation Study in Rats to determine No Effect Level
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Exposure-Dose-Response Inhalation Study in Rats to determine No Effect Level

Dosimetric extrapolation modeling to assess human risk
**Silica/SiO\textsubscript{2} Starting Materials**

**TEM:**
Exposure SiO\textsubscript{2} Material

- **SiO\textsubscript{2}-NPs “agglomerates”**
- Majority of the NPs are spherical or semi-spherical and ~ 20-40 nm in size.
- Nanoparticles tend to form dense agglomerated aggregates.
- Nanoparticles have smooth surfaces without etching or dissolution patterns.
- Particles are not zoned or show different densities (core to surface).
- Particles are amorphous
SiO$_2$ Starting Nanoparticles

This HR-TEM shows the amorphous nature of the supplied SiO$_2$ NPs.

Aggregation and Agglomeration is part of NPs Formation.
Pulmonary Inflammation in Rats After 4 Weeks of Exposure to Silica NP-Containing Slurry

Lung Lavage Analysis, (mean ± SD)

Means ± SD

- 0.22 mg/m³ as SiO₂ [NOAEC]
- 0.98 mg/m³ as SiO₂
- 4.66 mg/m³ as SiO₂
- Filtered air control
Dosimetric Extrapolation of Inhaled Particles from Rats to Humans

HEC, Human Equivalent Concentration

Rat

Exposure \( [\text{mg} (\text{m}^3)^{-1}] \)

Inhaled Dose \( [\text{mg} (\text{kg})^{-1}] \)

Deposited Dose \( \left[ \frac{\mu g (\text{cm}^2)^{-1}}{\mu g (\text{g})^{-1}} \right] \)

Retained (Accumulated) Dose \( \left[ \mu g (\text{g})^{-1} ; \mu g (\text{cm}^2)^{-1} \right] \)

Effects

Human

Exposure (HEC) \( [\text{mg} (\text{m}^3)^{-1}] \)

Inhaled Dose \( [\text{mg} (\text{kg})^{-1}] \)

Deposited Dose \( \left[ \frac{\mu g (\text{cm}^2)^{-1}}{\mu g (\text{g})^{-1}} \right] \)

Assumption: If retained dose is the same in rats and humans, then effects will be the same.
Estimation of Chronic NOAEL from Subchronic Rodent Study using MPPD Model

Lung burden not causing adverse effects:
- ○ after exposure for 3 months to "Subchronic" NOAEL
- ● after exposure for 2 years to "Chronic" NOAEL
("chronic" NOAEL < "subchronic" NOAEL)

--- hypothetical accumulation in lung at continued "subchronic" NOAEL exposure
--- predicted accumulation in lung during "chronic" NOAEL exposure

From: Oberdörster, 2002
Impact of Aerosol Density on Lung Deposition of Inhaled Agglomerated Particles: MPPD Prediction, Rat, 4 hour Inhalation

2.5mg/m$^3$; MMAD = 1.4$\mu$m; GSD = 2.9

Deposited Dose as Function of Agglomerate Density
Determining Aerosol Density for Input into MPPD Model

Several terms and meanings for density \((\rho = \text{mass}/\text{volume})\): specific; material; packing, effective, relative ….

Deposition of airborne particles in the respiratory tract is affected by effective or actual density of aerosols

A number of suggested methods to determine aerosol density:
Charvet et al., 2014, 2015; Maricq et al., 2004, Spencer et al., 2007
Miller et al., 2013; Park et al., 2003; Hering and Stolzenburg, 1995; Wang et al., 2015
Result of MPPD derived $\rho_{\text{eff}}$ for SiO$_2$ slurry aerosols using data of 4-hr. rat inhalation study:

$$\rho_{\text{eff}} = 0.165 \text{ g/cm}^3$$

Compare to SiO$_2$ material density of 2.65 g/cm$^3$!
Determining in vivo SiO$_2$ dissolution

$b_{tot}$ of biosoluble particles in the lung is the sum of mechanical (AM-mediated) removal and of dissolution:

$$b_{tot} = b_{mech} + b_{diss}$$

$$b_{diss} = b_{tot} - b_{mech}$$

**Note:**

*Species differences of particle clearance rates in rat and human lung:*

*mechanical clearance rate:* very different ($T_{1/2}$ rat ~ 70 days, human ~400-700 days)

*dissolution clearance rate:* assumed to be the same in mammalian lungs
Accumulation in rat lungs of inhaled SiO$_2$ NPs vs PSPs

(modeled from 4 week inhalation study)

SiO$_2$, $T_{1/2} = 23.5$ days; $\rho_{eff} = 0.165$

PSP, $T_{1/2} = 70$ days

measured at end of 4 week
SiO$_2$ inhalation (*NOAEL*)

Days of exposure

μg/lung

4 wk exposure

4 wk exposure

Days of exposure

equilibrium: 73 ug

equilibrium: 25 ug
Verifying in vivo dissolution of SiO$_2$ NPs by HR-TEM/STEM/EELS analysis
Silica/SiO$_2$ Starting Materials

- Majority of the NPs are spherical or semi-spherical and ~20-40 nm in size.
- Nanoparticles tend to form dense agglomerated aggregates.
- Nanoparticles have smooth surfaces without etching or dissolution patterns.
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- Particles are amorphous
After 27 days, STEM shows many areas that are enriched in Si, but the nanoparticle size is so small that at the depicted Magnification, the particles appear like clouds (outlined with the yellow lines).

The Si-enriched zones have very small nanoparticles that could be identified to have Si, but it is not determined whether they are SiO$_2$, or Si-phosphates (see next slide).

The formation of the Si-enriched areas is a clear indication, that after 27 days, the original SiO$_2$ NPs have undergone at least partial in vivo processing. We observe dissolution patterns (rough surfaces, pore formation in the starting materials, edge pits with areas of high solubility) In addition we see formation of precipitates that are $<< 2$ nm and are part of what appears as Si-clouds. The Si-nanoparticles inside clouds are well dispersed suggesting, that there is some in-situ mechanism that prevents particle agglomeration. More work needed to identify coronas.
HEC Calculation from 4 week rat inhalation study with SiO\textsubscript{2} slurry aerosol:

**Environmental Exposure**

Deposition in **human lung** of inhaled SiO\textsubscript{2} aerosol of same particle size as in rat study, predicted by MPPD model with MMAD = 0.38 µm, GSD = 2.0, ρ = 0.165:

- 6.3 % deposition in alveolar region,
- 3.75 % in tracheo-bronchial region

Environmental setting: TV 625 ml; BF 12 min\textsuperscript{-1} *(resting breathing)*

**Alveolar Surface Area Basis**

**NOAEL rat: 13.6 µg** *(retained lung dose at end of 4 week exposure in rats)*

Normalized by alveolar surface area: 5.97 ng/cm\textsuperscript{2} surface area

Equivalent total retained dose in humans’ lungs to be reached after continuous exposure:

**3,787 µg**

**HEC** to reach this lung burden over total life at

- 24 hours/day, 7 days/week, resting breathing, 365 days/year:

**304 µg/m\textsuperscript{3}**

Daily deposited dose in humans: 208 µg/day

*(additional safety/assessment factors to define Reference value?)*
Risk Assessment and Risk Management Paradigm For Engineered Nanoparticles (NPs)

Hazard Identification

- Physico-chemical Properties!
- Adverse NP Effect: at portal of entry and remote organs
  - Experimental Animals
  - Humans

Exposure Assessment

- Inhalation
  - Ingestion, Dermal
- Biological Monitoring (markers of exposure)
- Occupational/Environmental Monitoring

Risk Management

- Public health/social/economical/political consequences
- Regulations
  - Expos. Standards
- Prevention/Intervention Measures
  - Biomed./Engineering

Risk Characterization

- Exposure-Dose-Response Data
  - In Vivo Studies (acute; chronic)
  - In Vitro Studies (non-cellular)
    - (animal/human cells)
    - (subcellular distribution)

Dose-Metric!

Biokinetics!

Alternative Models

- In Vivo Studies (acute; chronic)
- In Vitro Studies (non-cellular)
  - (animal/human cells)
  - (subcellular distribution)

Mechanistic Data

Risk Calculation

- Susceptibility Extrapolation Models
  - (high → low)
  - (animal → human)

Dosimetry

LCA!

Assessment Factors

Modified from Oberdörster et al., 2005
$Risk = f(\text{hazard}; \text{exposure})$