Refining Dosimetric Extrapolation Modeling of Inhaled Nanoparticles for Deriving a Human Equivalent Concentration

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Dosimetric Extrapolation of Particle Exposures from Rats to Humans

Rat

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

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

Deposited Dose \([\mu g(cm^2)^{-1}; \mu g(g)^{-1}]\)

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

Effects

Breathing

Minute Volume

for

rat \(\longrightarrow\) human

Tidal Volume, Resp. Rate

Resp. Pause

Particle characteristics

Anatomy

for

rat \(\longrightarrow\) human

Clearance

Retention

Regional Uptake

(Metabolism, \(T\ 1/2\))

for

rat \(\longrightarrow\) human

Deposit Dose \([\mu g(cm^2)^{-1}; \mu g(g)^{-1}]\)

Human

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

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

Modified from: Oberdörster, 1998
Dosimetric Extrapolation of Particle Exposures from Rats to Humans

**Concept:** HEC is defined as the Exposure Concentration resulting in Humans in the same normalized lung burden as measured in rats after acute, subchronic or chronic inhalation.

Effects may be different for both species.
Factors Involved in Respiratory Tract Dosimetry

**Exposure**

Aerosol Concentration \((\text{mg/m}^3; \text{n/cm}^3)\)

Minute Ventilation

**Dose**

Inhaled Dose \((\mu\text{g}; \mu\text{g/kg})\)

\{Tidal Volume, Resp. Rate, Resp. Pause, Particle Characteristics, Anatomy\}

Deposited Dose \((\mu\text{g}; \mu\text{g/cm}^2; \mu\text{g/kg})\)

Local, Systemic Uptake, Clearance, Retention

Retained Dose \((\mu\text{g}; \mu\text{g/cm}^2; \mu\text{g/g}; \mu\text{g/cell})\)

Accumulation

**Response**

Effect

\((Upper \text{ and lower respiratory tract; secondary target organs)\)
INFORMATION NEEDED TO ANALYZE EXPOSURE-DOSE-RESPONSE OF INHALED NANOMAERIALS FOR RISK EXTRAPOLATION MODELING

- **Aerosol specifics** (agglomerate/aggregate; MMAD; GSD)
- **Resp. tract geometry** (species specific branching pattern)
- **Resp. tract physiology** (species specific breathing parameters)
- **NM properties** (physico-chemical [intrinsic]; functional [extrinsic])
- **Exposure duration**
Physico-Chemical and **Functional** NP Properties of Relevance for Toxicology

**Size** (*aerodynamic, hydrodynamic*)

Size distribution

**Shape**

Agglomeration/aggregation

**Density** (*material, bulk*)

**Surface properties:**
- area (*porosity*)
- charge
- chemistry (*coatings, contaminants*)
- defects

**Crystallinity**

**Biol. contaminants** (e.g. endotoxin)

**Solubility/dissol-rate** (*physiol. fluid, in vivo*)

**Surface reactivity** (*ROS inducing capacity*)

Properties can change
- with: method of production preparation process storage
- when introduced into physiol. media, organism

Key parameter: Dose!
**Surface Reactivity as Dose-Metric,**

*e.g.*, ROS inducing potential expressed 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 hazard ranking of NPs based on their reactivity in cell free or cellular assays*

*Bello et al., 2009; Rushton et al., 2010*
Cell-free Assay, NP bound ROS, Summary (Carbon Particles)

Particle Mass Correlation

Equivalent H$_2$O$_2$ conc. (µM)

- PALAS fresh: 41nm
- PALAS aged: 41nm
- Sevacarb: 300nm
- Printex-90: 14nm
- Sterling-V: 70nm
- C-13: 42nm
- CB+25% Fe: 40nm
Cell-free Assay, NP bound ROS, Summary (Carbon Particles)

Particle Surface Area Correlation

Equivalent H$_2$O$_2$ conc. (µM)

- PALAS fresh: 700 m$^2$/g
- PALAS aged: 700 m$^2$/g
- Sevacarb: 7 m$^2$/g
- Printex-90: 300 m$^2$/g
- Sterling-V: 37 m$^2$/g
- C -13: ~700 m$^2$/g
- CB+25% Fe: ~700 m$^2$/g
Dissolution as one Determinant of Pulmonary Biopersistence of Inhaled Particles

**Biopersistence** = \( f \) (Physiological Clearance; Biodurability)

Overall clearance rate = \( AM\)-mediated clearance rate + dissolution* rate

(*may be masked due to prolonged retention of bioprocessed particles/ions)
Acellular solubility/dissolution assays with simulated lung fluids

Static

equilibrium solubility (g/L) + agitation

Flow-through

full dissolution rate ng/cm²/day

Courtesy of Potter, 2015
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
MPPD Model

Input Choices
Allometric Scaling of Respiratory Parameters to Bodyweight of Rat, for Input into MPPD

### Tidal Volume

- **Stahl, 1968; Guyton 1947**
- **Miller et al, 2014**
- **Costa & Tepper, 1988**
- **MPPD 2013 default (Mauderly, 1979)**

![Tidal Volume Graph](image)

### Respiratory Rate

- **Piccione et al, 2005**
- **MPPD 2013 default (Mauderly, 1979)**

![Respiratory Rate Graph](image)

### Functional Residual Capacity (FRC)

- **Takezawa et al, 1980**
- **MPPD 2013 default**

![Functional Residual Capacity Graph](image)

### Upper Respiratory Tract Volume (URT)

- **Menache et al, 1997**
- **MPPD 2013 default**

![Upper Respiratory Tract Volume Graph](image)
Impact of Aerosol Density on Lung Deposition of Inhaled Agglomerated Particles: MPPD Prediction, Rat, 4 hour Inhalation

2.5mg/m³; MMAD = 1.4μm; GSD = 2.9

Deposited Dose as Function of Agglomerate Density

Deposition, μg

Density, g/cm³
Case Study: 28 Day Nano-Silica Rat Inhalation Study as basis for human extrapolation modeling

**Objective/Questions:**

Determine aerosol characteristics, effects and fate of amorphous SiO$_2$ NPs in a short (4 hr) and a repeat (4-wk) rat inhalation study: (suspended as slurry used for CMP in electronics industry)

— What are results telling us in terms of hazard extrapolation to humans?

— Is a 4-week exposure duration sufficient for risk characterization?

— What is a safe level for worker exposure?
Silica/SiO$_2$ Starting Materials

TEM: Exposure SiO$_2$ Material

- 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.
Outline, study plan of SiO$_2$ NP inhalation:

– **4 hour acute inhalation in rats:**
  - to determine effective density $\rho_{\text{eff}}$ of SiO$_2$ aerosol “in vivo”

– **4 week inhalation in rats:**
  - three concentrations to determine NOAEC
  - estimate overall lung clearance rate ($b_{\text{tot}}$) of SiO$_2$ NPs
  - compare to normal clearance rate ($b_{\text{mech}}$) for insoluble particles
  - derive in vivo dissolution rate ($b_{\text{diss}}$) of SiO$_2$ NPs: $b_{\text{tot}} = b_{\text{mech}} + b_{\text{diss}}$
  - dosimetric extrapolation to human exposure

– **verify in vivo dissolution by HRTEM analysis**
  - bioprocessing in phagolysosome of macrophages
  - analyzing chemistry of subcellular interactions of NPs
### 4 Week Study: Exposure Characteristics and Retained Doses (µg) Using Silica Nanoparticle-Containing CMP Slurries

**Fisher-344 Male Rats**

<table>
<thead>
<tr>
<th></th>
<th>High Dose</th>
<th>Mid Dose</th>
<th>Low Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SiO₂ Aerosol (mg/m³)</strong></td>
<td>4.66</td>
<td>0.98</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Lung Retained Dose</strong> (µg, as SiO₂, at 4 wks expos)</td>
<td><strong>196 ± 7</strong></td>
<td><strong>47 ± 9</strong></td>
<td><strong>13.6 ± 3.3</strong> NOAEL</td>
</tr>
<tr>
<td><strong>MMAD, µm (GSD)</strong></td>
<td>0.5 (2.4)</td>
<td>0.4 (1.8)</td>
<td>0.4 (2.0)</td>
</tr>
</tbody>
</table>
Pulmonary Inflammation in Rats After 4 Weeks of Exposure to Silica NP-Containing Slurry

Lung Lavage Analysis, (mean ± SD)

- 0.24 mg/m³ (0.2 mg/m³ as SiO₂)
- 1.08 mg/m³ (0.9 mg/m³ as SiO₂)
- 5.18 mg/m³ (4.6 mg/m³ as SiO₂)
- filtered air control

Means ± SD
Effective Density of SiO$_2$ Aerosols

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$^3$!
Verifying in vivo dissolution of SiO₂ NPs by HR-TEM/STEM/EELS analysis
Starting Materials

TEM:

SiO₂-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
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.
Agglomerate 27 Days p.E.
- SiO$_2$ NPs show significant in vivo processing.

Most SiO$_2$ NPs lost original spherical morphology. NPs show dissolution patterns, void/pore formation and outward growth (secondary growth)
Dosimetric Extrapolation of Particle Exposures from Rats to Humans

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Modified from: Oberdörster, 1998
HEC Calculation from 4 week rat inhalation study with SiO$_2$ slurry aerosol for Occupational Exposure:

Deposition in **human lung** of inhaled SiO$_2$ aerosol of same particle size as in rat study, predicted by MPPD model with MMAD = 0.38 µm, GSD = 2.0, $\rho = 0.165$:

- **5.5 %** deposition in alveolar region,
- **3.75 %** in tracheo-bronchial region

Occupational setting: TV 1025 ml; BF 20 min$^{-1}$ (light exercise)

**Normalizing per Unit Alveolar Surface Area of Human and Rat**
## Surface Areas of Respiratory Tract Regions at FRC

<table>
<thead>
<tr>
<th></th>
<th>Rat</th>
<th>Human</th>
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<tbody>
<tr>
<td></td>
<td>cm²</td>
<td>% of total</td>
</tr>
<tr>
<td>Nasal</td>
<td>18.5</td>
<td>0.75</td>
</tr>
<tr>
<td>Trach-bronch</td>
<td>24</td>
<td>1.00</td>
</tr>
<tr>
<td>Alveolar</td>
<td>2422</td>
<td>98.25</td>
</tr>
</tbody>
</table>

*Keyhani et al., 1997; Kimbell et al., 1997; Miller et al., 2011*
CONCLUSIONs, re:
REFINING DOSIMETRIC EXTRAPOLATION MODELING

— Appropriate allometric adjustment of respiratory parameters as input into MPPD is critical for
  • determining effective aerosol density during exposure
  • separating biosoluble from biopersistent NPs (non-inflammatory conditions)

— NP in vivo dissolution rate is important for NP characterization
  • dynamic “in vitro” dissolution as surrogate?
  • contrast with static solubility
  • desirable: retention/clearance kinetic in post-exposure period
  • need to study composition and fate of newly found secondary NPs

— When to use alv. surface area vs. lung weight for extrapolation of retained lung burden?

— A well-designed 4-week inhalation study may be sufficient for risk characterization

(based on Kuempel et al, 2001, model)

Clear. Rate = 0.0017/day
$T^{1/2} = 400$ days (~1 year, ~60%)

Clear. Rate = 0.00003/day
$T^{1/2} = 23,000$ days (~63 years)

Clear. Rate = 0.001/day
$T^{1/2} = 700$ days (~2 years, ~40%)

Combined alveolar clearance: rate = 0.0027/day
$T^{1/2} = 250$ days (~0.7 years, 100%)
Amorphous and Crystalline Silica Types

- Trydimite
- Cristobalite
- Quartz
- Porosil
- Amorphous Silica particles
- Precipitated silica
- Sol, Colloidal silica
- Stöber silica
- Monomer: Si(OH)$_4$, SiCl$_4$
- Pyrogenic or Fumed Silica
- Ordered mesoporous materials
- Aerogel
- Xerogel

Modified from Napierska et al., 2010