Biokinetics and effects of titania nano-material after inhalation and i.v. injection

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Abstract. Within NanoSafe2 we developed a special inhalation model to investigate deposition of inhaled particles in the lung and the further distribution in the body after. Concurrently, the effects of the inhaled materials in the lung were examined. The results for nano-Titania were compared to results from inhalation studies with micron-sized (non-nano) Titania particles and to quartz particles (DQ12, known to be potent lung toxicants). To build a PBPK model for nano-Titania the tissue distribution of the material was also examined following intravenous (i.v.) administration.

1. Material and methods
The materials tested were nano-Titania, micrometer-seized Titania and quartz dust DQ12 (Figure 1).

![Image of transmission electron micrographs of a) nano-Titania, b) micron-sized Titania, c) quartz dust DQ12.](image)

- a) Nano-Titania
  - Primary particle: 20-50 nm
  - BET surface area: 48.6 g/m²

- b) Micron-sized Titania
  - Primary particle: 225-500 nm
  - BET surface area: 6.0 mg/m²

- c) Quartz dust DQ 12
  - Primary particle: 225-2000 nm
  - BET surface area: 5.9 mg/m²

Figure 1. Transmission electron micrographs of a) nano-Titania, b) micron-sized Titania, c) quartz DQ 12.

Male Wistar (strain Crl:WI (Han)) rats (7 weeks of age) were obtained from Charles River Laboratories. All procedures for care an exposure of the animals were conducted in an AAALAC-approved laboratory in accordance with the German Animal Welfare Act and the European Council Directive 86/609/EEC.
Groups of male rats were head-nose exposed to dust aerosols for 6 h a day on five consecutive days. The amount of the test materials in the lung, mediastinal lymph nodes, liver, kidney, spleen and basal brain with olfactory bulb was determined (organ burden) in 3 rats per group and time point. The lungs of rats exposed to nano-Titania were evaluated by electron microscopy (3 rats per time point). The examinations were carried out shortly after the exposure and after an exposure-free period 14 days.

To generate the test atmosphere of nano-Titania, the material was dispersed in highly deionised water (0.5% by weight) and nebulised by a two-component atomizer (stainless steel, Schlick mod. 970). The micron-sized Titania and the quartz dust were dispersed by brush generators (developed by the Technical University of Karlsruhe in cooperation with BASF, Germany). The targeted atmospheric concentrations were 100 mg/m$^3$ for nano-Titania, 250 mg/m$^3$ for micron-sized Titania and 100 mg/m$^3$ for quartz dust. Technical details of the generation and characterization of the atmospheres were described elsewhere [1].

The application suspension consisted of nano-Titania dispersed in rat serum (0.5%). The Titania particle size distribution was determined by analytical ultracentrifugation of ~500 µl of a test substance application preparation that was made in parallel with that used for the i.v. administration.

A total of 12 male healthy Wistar rats, with a narrow range of body weights, were randomly assigned to three experimental groups, with one control and three treated animals in each group. The control animals were injected intravenously via the tail vein with ~1 ml of sterile saline, and the treated animals were injected i.v. via the tail vein with the test substance preparation. The dose was 5 mg/kg body weight (~1 ml of test substance preparation/kg of rat body weight). At 1, 14, and 28 days post i.v. injection rats were sacrificed by cervical dislocation under isoflurane anesthetises. Blood samples were taken, lung, liver, kidney, spleen and brain were collected and weighed immediately after sacrifice of the animals. The amounts of the test materials in these organs as well as in blood cells, plasma and popliteal lymph nodes were determined. Following this 28-day study [3], a 90-day study with i.v. injection was performed in the same manner.

To determine Ti and Si content in the tissues, wet tissue samples were digested and the solutions were analysed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) at wavelength of 338.376 nm for Ti and 288.15 nm for Si.

2. Results

The data of the target inhalation atmosphere concentrations, the actual analyzed concentrations and the particle size distribution measurements are presented in Table 1. The MMADs were between 1.0 and 1.2 µm, which is highly respirable for all three materials without any differences between those of the nanomaterial and the micron-sized materials. The calculated respirable fraction (MMAD < 3 µm) ranged from 81.7% to 93.1%. Only approximately 10% of the particles measured by the SMPS were smaller than 100 nm for all three exposure atmospheres (Table 1). The mass concentration of this fraction (<100 nm) was calculated (Table 1). For atmospheres with nano Titania the number concentrations of particles <100 nm represented only 0.5% of the total particle mass.

We used an analytical ultracentrifuge to measure both the highly concentrated and very turbid samples used for the i.v. injections and a sample that was diluted with FBS gold. The particle size of the Titania used for the i.v. injection was mostly in the fine fraction with components of up to 1 µm in size. Approximately 10 wt% of the particles were found in the nano-size range (< 100 nm).

After the 5-day inhalation exposure, Ti or Si was only detectable in the lung and mediastinal lymph nodes of the exposed animals (Table 2). The limit of quantification for Ti was 0.3 µg per tissue sample, corresponding to 0.5 µg Titania. The detection limit for Si was 5 µg per tissue sample, corresponding to 11 µg SiO$_2$.
Table 1. Particle size and concentration in the test atmospheres.

| Variable                                | Test substance       |
|-----------------------------------------|----------------------|
|                                          | Nano-Titania         | Micron-Titania       | Quartz DQ 12         |
| Target concentration (mg/m³)            | 100                  | 250                  | 100                  |
| Measured concentration (mg/m³)          | 88.0 ± 6.4           | 274.0 ± 30.5         | 96.0 ± 5.4           |
| MMAD (µm) / GSD                         | 1.0 / 2.2            | 1.1 / 2.2            | 1.2 / 2.1            |
| OPC (µm): count median diameter (Q₀)    | 0.6                  | 1.5                  | 0.4                  |
| SMPS (µm): count median diameter (Q₀)   | 0.2                  | 0.2                  | 0.2                  |
| Count concentration of particles in SMPS (number particle/cm³) | 88 x 10⁴            | 13 x 10⁵             | 33 x 10⁴             |
| Count concentration (number particle/cm³) of particles < 100 nm | 205 920             | 54 600               | 21 292               |
| Calculated* mass fraction < 100 nm      | 0.5 %                | 0.05 %               | 0.03 %               |

* assuming all particles are spherical and have a diameter of 100 nm, the mass fraction (%) was calculated by multiplying the volume of a particle with particle count concentration (<100 nm) and physical density 4.2 g/cm³ for Titania and 2.65 g/cm³ for quartz dust DQ 12.

Table 2. Organ distribution after 5-day inhalation exposure (mean values of 3 animals per group and time point in µg per organ)

| Organ                                      | n-Titania | p-Titania | Quartz DQ 12 |
|--------------------------------------------|-----------|-----------|--------------|
|                                            | Day 5     | Day 19    | Day 5        | Day 19    |
| Liver, kidney, spleen, basal brain with olfactory bulb (µg) | < 0.5*    | < 0.5*    | < 11**       | <11**     |
| Lung (µg)                                  | 2025      | 1547      | 9182         | 7257      |
| Lymph nodes (µg)                           | 2.2       | 8.5       | 8.2          | 108       |

* 0.5 µg/organ was the detection limit by ICP-AES for Titania.
** 11 µg/organ was the detection limit by ICP-AES for quartz.

Figure 2. Transmission electron micrographs from the lungs of rats exposed to aerosol of nano-scale Titania.
Left: agglomerates located free in the alveolar space. Right: alveolar macrophages with agglomerates in the cytoplasm.

Electron microscopy was used to characterize the particles deposited in the tissues (Figure 2). The particles from both the micron- and the nano-scale Titania were mainly located extra-cellularly in the lumen of the alveoli and bronchi. Moreover, particles were detected in the cytoplasm of alveolar macrophages. The particles from nano-scale material found in the lung, were mostly agglomerates of about the same size as found in the atmosphere; there were no signs of desagglomeration of the inhaled agglomerates. There was an inflammatory response in the lung and sensitive parameters in the 5-day inhalation study correlated well with results from previous subchronic inhalation study. [2]
After i.v. administration of nano-Titania, there were no detectable levels of Titania (<0.5 µg Ti per sample) in blood cells, plasma, brain, or lymph nodes (mediastenal, mesenteric, popliteal) at any of the three time points tested. The average distributions of Titania in the liver, spleen, lung, and kidney are shown in Table 3. The Ti levels were highest in the liver, followed in decreasing order by the levels in the spleen, lung, and kidney (very low levels of <0.7 µg). In kidney, a clearance could be demonstrated. The residue concentrations of Ti in liver, lung and spleen showed a high variability with a decreasing trend over time in the 28 day study, the data after 90 days, however, demonstrated that in these organs, no clearance occurred during the 3 month observation period. Ti was not detectable in urine. Ti in feces of treated animals was not significantly higher than in feces of untreated animals.

The use of this biokinetic studies for PBPK modeling and is presented at this NanoSafe2008 Conference [4].

There were no clinical changes and no changes in blood parameters at any timepoint, indicating that there was no detectable inflammatory response or organ toxicity.

### Table 3. Titania distribution in organs after i.v. injection of 5 mg nano-Titania/kg body weight.

| Organ      | Titania on day 1 | Titania on day 14 | Titania on day 28 |
|------------|------------------|-------------------|------------------|
|            | Control a        | Treated b         | Control c        | Treated d        | Control e        | Treated f        |
| Liver      | 0.5              | 133.8±26.0        | 0.5              | 99.5±55.8        | 0.5              | 111.3±27.3       |
| Spleen     | 0.8              | 78.7±19.3         | 0.9              | 48.8±27.8        | 0.7              | 33.3±28.8        |
| Lung       | 1.5              | 8.8±0.6           | 1.7              | 2.8±1.4          | 1.5              | 2.3±1.0          |
| Kidney     | < Loq            | 0.67±0.06         | < Loq            | < Loq            | < Loq            | < Loq            |

*Values expressed as mean ± SD (µg/g wet tissue)  a n=1  b n=3.

3. Conclusion

Due to comparable aerodynamic particle size, inhaled nano- and micron-sized Titania as well as quartz (DQ12) particles deposited similarly in the lung, but had significantly different effects. Translocation to other organs than the lung-draining lymph nodes was not observed. nano-Titania administered by i.v. injection did not cause toxic effects; it was mainly found in the liver and no clearance was detected within 90 days.

References

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