

Published in final edited form as:

Acc Chem Res. 2013 March 19; 46(3): 714–722. doi:10.1021/ar300043r.

Differences in the biokinetics of inhaled nano- versus micron-sized particles

Wolfgang G. Kreyling*, **Manuela Semmler-Behnke**, **Shinji Takenaka**, and **Winfried Möller**
 Comprehensive Pneumology Center - Institute of Lung Biology and Disease, and Focus Network
 Nanoparticles and Health, Helmholtz Zentrum München – German Research Center for
 Environmental Health, Neuherberg, Germany

Keywords

quantitative biokinetics; biodistribution; nanoparticle; micron-sized particle; particle inhalation; lung retention; particle clearance; particle translocation; particle relocation towards interstitium

1. Introduction

Assessing the potential risk of incorporated particles to human health, an important part within hazard identification and exposure is their dosimetry. Dosimetry represents the rational base for any subsequent toxicological evaluations. Only sufficient knowledge of both dosimetry and toxicological responses allows for comprehensive hazard identification. Here we will compare the current knowledge on the dosimetry of inhaled engineered nanoparticles (NP; 1-100nm diameter) versus that of submicrometer and micron-sized particles (μ P; > 100nm diameter). Dosimetry starts with the estimate of an incorporated dose of particles which needs to be distinguished and derived from the exposure dose. While μ P exposure has extensively been discussed previously^{1,2}, NP exposure during the entire lifetime is still under discussion. Currently occupational NP exposure during NP generation and processing dominates the debate. This is particularly true for the inhalation route. However, since many NP and μ P are used in a large and increasing number of products of large diversity in food, pharmacology, personal care and other day-by-day goods with rapidly growing tendency, it appears to be necessary to gain a general estimate of the total NP and μ P exposure by oral intake of consumers at large.

Depending on the route of intake the incorporated dose may be rather complex as it is the case for inhaled particles. Their deposition probability in the various regions of the respiratory tract depends on three sets of properties: (1) physico-chemical parameters of the inhaled particles themselves, (2) the breathing conditions and (3) the geometry of the respiratory tract including changes in diseased lungs. Once particles have been deposited onto the respiratory tract epithelia, multiple serial interactions and mechanisms with (1) proteins and biomolecules of body and cellular fluids, and (2) with cellular membranes and organ barriers will be initiated. These will determine the fate of a particle and its chemical and physical modifications (including protein binding to the surface and metabolic reactions) within the lungs as the organ of intake (primary organ), as well as after translocation into circulation and accumulation in secondary organs or excretion out of the body. The multitude of actions determines the biokinetics of the initially incorporated

*Corresponding Author Comprehensive Pneumology Center - Institute of Lung Biology and Disease Helmholtz Zentrum München Ingolstädter Landstraße 1 85764 Neuherberg, Germany Tel.: +49/(0)89/3187-2309 Fax: +49/(0)89/3187-3397 kreyling@helmholtz-muenchen.de.

particle and its metabolic compounds in the body over time. In this paper we focus on the biokinetics of NP versus μP administered to lungs and specifically discuss quantitative biokinetics as a comprehensive approach. We will discuss the rather comprehensive data set for rodents and compare to the scarce, but more appropriate data of dogs and monkeys as human models.

2. Particle characterization and administration to the lung

The generation of a well characterized aerosol containing the selected model particles and their inhalation during a well-controlled exposure study provide the gold standard of particle dosimetry and toxicology to the respiratory tract. Since often neglected it needs to be emphasized that there is a natural dose limit on the particle number concentration of a sufficiently stable aerosol during exposure due to thermodynamic properties of the aerosol. At particle concentrations beyond 10^6 cm^{-3} , coagulation dynamics under inhalable aerosol conditions causes rapid decrease of the particle number concentration accompanied by a shift of the aerosol size distribution to larger sizes. While this limit is usually not reached for μP aerosols even at mass concentrations of up to several 10 mg/m^3 , this is an important limit for NP aerosols. The number concentration limit of 10^6 cm^{-3} of e.g. 20nm, or 100nm unit-density-NP or 500nm unit-density- μP corresponds to mass concentrations of $4.2 \mu\text{g/m}^3$, $525 \mu\text{g/m}^3$ and 66 mg/m^3 , respectively. Note the upper mass concentration for 500nm μP occurs only very seldom. Accordingly the dose rate to the respiratory tract is determined by this physical phenomenon and the breathing parameters: breathing frequency and tidal volume. In contrast, particle doses used for intratracheal instillation etc. allow for extremely high dose rates conflicting with maximally possible dose rates during inhalation. For the above mentioned aerosols the maximum hourly dose rate is $0.5 \mu\text{g/h}$, $60 \mu\text{g/h}$ and 60 mg/h , respectively; (presuming an hourly inhaled volume of 360 L/h and a deposition probability of 0.3. This hourly dose deposits on about $5 \cdot 10^{10}$ epithelial cells³. Hence, corresponding in vitro doses to 10^6 cultured cells would be limited to 0.5 ng , 60 ng and $7.5 \mu\text{g}$, respectively, while usually in vitro doses exceed $1\text{-}10 \mu\text{g}$. Furthermore, particle doses administered as a bolus during $<30 \text{ sec}$ frequently exceed drastically the maximal hourly inhaled dose by a factor 120.

Another limit is the particle size: for rodents which are obligatory nose breathers virtually no particles larger than $3 \mu\text{m}$ in aerodynamic diameter can reach the lungs because these particles deposit already in the nose⁴.

3. Methodologies for determining biokinetics of inhaled particles

Many of the recent biokinetics studies attempted to administer a given particle dose followed by time-sequential analyses of particle retention in a few selected organs and tissues of interest. This selective approach provides limited information on the overall biokinetics of the administered particle material, as it usually describes the fate of only a small fraction of the administered particles – sometimes $< 30\%$. In fact, it leaves important open questions about the excreted fraction and eventually major accumulated fractions in sites like the skeleton or soft tissue or other organs not analyzed. Therefore, a comprehensive understanding of the entire biokinetics is hampered by this reductionist analysis. In addition, biokinetics studies are commonly embedded in larger toxicological studies which should be based on a broad range of administered particle doses. In these cases even the lower dose of the selected particle dose range already causes significant biological and eventually toxic responses, which may affect the biokinetic fate of the particles. While these biokinetics studies are important for dosing and evaluation of toxic responses, they need to be compared to a baseline study under physiological (healthy) conditions. Therefore, administered particle doses used in biokinetics studies should aim for

as low levels as possible, being necessary for a careful analysis in all relevant target organs and the remaining body (including excretion) to determine the particle biokinetics under physiological conditions. Such dose considerations are often neglected.

4. Quantitative biokinetics

To overcome the issues discussed above quantitative biokinetics may be an attractive and comprehensive alternative. It aims for an overall quantitative estimate of the total amount of the administered particles within and out of the body. Quantitative biokinetics can be performed after the administration of NP or μP by any route of delivery: inhalation, intratracheal instillation or aspiration to the respiratory tract; oral ingestion or gavage to the gastro-intestinal tract; intravenous or intra-arterial injection to the blood circulation, or dermal applications to the skin. The concept is simple, aiming to estimate the total amount of particles administered and retained in the entire body at certain time points after exposure including total excretion, as shown in Figure 1. Hence, not only the distributions of particles in organs and tissues of interest are determined, but particles in the organs and tissues of the remaining carcass and those excreted are also included. With this, a 100% balance of the administered particles is achieved and a complete, yet detailed, quantitative analysis of their biokinetics is obtained⁵. Note when particles undergo chemical and physical changes such as particle decomposition and/or disintegration, the biokinetics fate of the various fractions may differ from each other and they may lose their particulate structure into molecules and ions and newly formed complexes. Although this approach of quantitative biokinetics is principally applicable to any animal species, ethical, economic and practical reasons usually limit the analytical attempt to small laboratory animals.

Analytical chemistry e.g. inductively coupled plasma mass spectroscopy (ICP-MS) provide suitable methods to quantify chemical elements of particles in the collected specimens. Thereby, special attention has to be paid to sample preparation, because of the extremely low mass of NP retained in organs. In addition, endogenous species of the chemical element of the particle may contribute to the natural “background” concentration. Gamma radio-spectroscopy may provide an elegant highly sensitive analytical alternative, allowing for a direct analysis of complete organs and tissues without any pre-treatment. In this case, the particles need to be labeled using a radio-isotope being firmly integrated into the particle matrix without any allowance for leaching. While this requirement is hard to fulfill, when a radio-isotope is not blended into the particles during production but chemically engineered onto the particle surface, stable labeling is obtained, when the radio-isotope belongs to the same chemical element as the particle matrix. This, however, is often difficult to achieve. Nuclear reactions within already existing particles upon neutron, proton or any other ion beam irradiation, allow radio-activation of one to a few atoms per particle. Mostly, the amount of radioactivity is sufficiently high for subsequent radio-analysis, when not more than a single atom per NP was converted by the nuclear reaction while more radio-isotopes in μP are required due to their larger particle mass. Note however, that usually the ratio between the radioactive and the stable isotopes of a particle is much less than 10^{-5} , representing a negligible “impurity” fraction by the radioisotope, which is unlikely to affect the chemical properties and stability of the particle matrix. Firm radio-labeling may not always be possible with any given particle because of possible nuclear reactions. However, an important advantage of the gamma-spectrometric analysis is the option of using extremely low particle masses necessary for determining particle biokinetics at the physiological background level. The use of high sensitivity, low-background gamma detectors of well-type geometry allows the radioactivity determination of about 0.1 Bq in a total organ or the entire remaining carcass. Such low detection limits allow for analyses of particle doses in the picogram-range per organ or tissue. Furthermore, when administering

an initial particle dose of 10-50 kBq and a mass of 1-10 μg per rodent then two important aims can be achieved:

- (a) The dose range from administration to organs of lowest radioactivity can cover a dynamic range of more than five orders of magnitude and
- (b) The administered particle mass is likely not to trigger patho-physiological responses of the organism.

Yet, since the radio-spectrometric method similarly like the chemical analytical methods do not distinguish between the particulate state and chemical speciation, those important particle relevant parameters including the firm labeling of the radio-isotope need to be estimated by additional (auxiliary) studies specifically designed to answer these questions. Since particles being cleared out of the lungs by mucociliary transport may enter the gastrointestinal tract additional auxiliary studies need to cover particle biokinetics after oral ingestion.

Total organs and all body fluids are collected without any cross contamination. Tissue samples, the remaining carcass and the entire excretion are collected such that the entire organism is sampled and weighed in wet state. The application of clean dissection techniques is essential to avoid cross contamination, in particular in inhalation studies, where fur contamination occurs upon whole body exposures. Systematic changes of dissection tools and equipment are mandatory. In addition, whole body vascular perfusion is recommended to empty the blood vessels within the organs to solely determine particle retention in the parenchyma.

5. Current knowledge of particle biokinetics

5.1. Particle retention in the lungs

After particle inhalation and deposition on the lung epithelium the retention of particles starts with their wetting by surfactant and the epithelial lining fluid and subsequent displacement from the air into the aqueous phase regardless of particle shape, surface topography and surface free energy^{6,7}. Though experimentally demonstrated yet for μP of 1 - 6 μm in diameter, the same is expected for NP, since this process becomes even more efficient with decreasing particle size.

In general, the majority of μP remains on the epithelial surface in airways and alveoli of rodent lungs and are accessible to broncho-alveolar lavage (BAL), see Figure 2⁸⁻¹². However, already 24 hours after inhalation most NP are no longer accessible to BAL (Figure2).

Their retention time in the lung depends on the deposition site and on the interaction of the particles with the inner lung surface. It is short for particles deposited in conducting airways due to efficient mucociliary and cough clearance (time span hours up to one day), but it increases with airway generation number, as a consequence of increasing pathway length and decreasing mucus transport velocity towards distal airways. While the retention of μP generally does not exceed 24–48 h in rodent airways, there is evidence for prolonged retention of NP and μP in airways of dogs and man^{4,15,16}. The probability of long term particle retention in the airways is inversely correlated to particle size and a maximal fraction of up to 80% was found for particles 100 nm^{16,17}.

Possible explanations for the long term retained particles in the airways are: (1) the particles were no longer accessible to mucociliary clearance either because they penetrated through the mucus layer deep into the periciliary phase⁶, or (2) they deposited in areas without any

mucus coverage, since patchy mucus layers can exist in small airways¹⁸. In both cases, particles can penetrate between cilia to further interact with cells of the inner lung surface, i.e. macrophages, dendritic and epithelial cells¹⁹. Hence, the probability for particle relocation beyond the epithelial barrier is enhanced.

5.2. Particle clearance from the peripheral lungs

There are three major transport pathways of biopersistent particles out of the peripheral lungs: (a) alveolar-macrophage-mediated transport from the alveoli to the ciliated airways for mucociliary transport to the larynx and subsequent swallowing into the gastro-intestinal tract, (b) particle transport towards lung-associated lymph nodes; (c) translocation into blood circulation for subsequent accumulation in secondary organs. Note, the clearance of rapidly or moderately soluble particles is not addressed but may be an important clearance mechanism for many particles^{4,13}. In the following we will discuss the first clearance pathway in detail distinguishing between NP versus μ P. We will not address the second transport pathway towards lymph nodes as we and others have reported on that previously^{4,5,13,20}.

6. Particle relocation from the alveolar epithelium into the pulmonary tissues of rodents

6.1. Differences in biokinetics between NP and μ P in rodents

NP relocation into rat epithelial and interstitial tissues (pulmonary tissues) was described after inhalation of aerosolized submicrometer agglomerates of 20nm primary TiO₂ versus the inhalation of aerosolized submicrometer agglomerates of primary 200-300nm TiO₂ particles¹¹. When compared to submicrometer agglomerates of primary 200-300nm μ P an increased NP fraction was determined in pulmonary tissues by chemical analyses. NP versus 200-300nm μ P samples were compared which had been obtained from exhaustive BAL and the according lavaged lungs. After the 12-week exposure the authors estimated that about 50% more NP mass than larger particles were relocated into pulmonary tissues. They suggest that dis-agglomeration of the agglomerates of the primary 20nm TiO₂ had occurred on the epithelial surface prior to relocation into rat pulmonary tissues which did not occur to the 200-300nm TiO₂ particle agglomerates. Similar results were found for intratracheally instilled submicrometer agglomerates of primary 20nm TiO₂ or Al₂O₃ NP versus instilled submicrometer agglomerates of primary 200-300nm TiO₂ or Al₂O₃ particles¹⁰. However, in these studies the extent of dis-agglomeration could not be quantified.

In a more recent rat inhalation study using freshly generated iridium NP aerosols of 20nm or 80nm median diameter (geometric standard deviation (GSD) 1.6) about 80% and 75%, respectively, of the NP (deposited on the alveolar epithelial surface) were relocated into epithelial and pulmonary tissues within 24 hours and no longer accessible to BAL, Figure 2^{21,22}. These fractions (relative to the contemporary lung burden) remained unchanged over the next six months since only about 20% were eliminated by BAL, Figure 3.

Twenty-four hours after inhalation we found similar BAL versus lavaged lungs fractionations of freshly generated 25nm elemental carbon and 20nm TiO₂ NP aerosols²³. Three different NP materials (iridium, elemental carbon, titanium dioxide) of similar size (20nm), similar polydispersity and NP morphology were studied – they all were chain-aggregates/agglomerates of primary particles <5nm. They were inhaled as 20nm NP aerosols by rats and they all showed rapid internalization into the interstitium, emphasizing the importance of NP size. Note, at a deposited NP dose of about 1 μ g per rat lung after the 1-hour inhalation a retrograde agglomeration on the alveolar epithelial surface is basically excluded since the projected area of all NP covers only 10⁻¹² of the rat lung surface area.

In contrast, about 80% of inhaled or instilled μP were accessible to BAL during months after administration and the remaining small fraction of 20% not accessible to BAL was relocated into pulmonary tissues, Figure 3. Different to the interstitial relocation of NP, the majority of μP (fluorescent 2 μm and radio-labeled 3.5 and 10 μm polystyrene particles) remained on the alveolar epithelial surface of rodent lungs and more than 80% of the lung-retained fraction were collected by exhaustive BAL throughout the entire retention time of six months, Fig 3^{8,11}. Similar results were obtained for micron-sized radio-labeled fused-clay particles in hamster lungs⁹. All these studies confirmed that until more than six months after μP instillation or inhalation an 80%-fraction of the contemporary rodent lung particle burden remains on the alveolar epithelium being accessible to BAL throughout this period.

Note the μP burden as a fraction of the initial dose declines rather rapidly due to continuous alveolar-macrophage mediated clearance towards the ciliated airways and larynx. The initial rate starts at 1-3%/d but it declines exponentially with time such that after about 200 days >90% of the μP are cleared^{4,24}.

Surprisingly, and very similar to the μP clearance kinetics, more than 90% of the initial 20nm iridium NP deposit had been cleared from the alveolar region during the six month retention period. The NP fraction was also cleared by alveolar-macrophage-mediated transport as confirmed by BAL analysis^{21,22}. So, these long-term retained NP apparently re-appeared from interstitial spaces back onto the epithelium for subsequent clearance by lung-surface macrophages. Although not yet confirmed, the most likely mechanism of NP re-appearance on the epithelial surface is presumed to be via macrophage-migration in the interstitium. Even more surprising, despite the high interstitial NP retention, the clearance kinetics, i.e. the daily cleared NP fractions towards the larynx followed the same kinetics as those of the daily cleared μP fractions, which were primarily retained on the epithelium^{5,21}. So the earlier discussed clearance rate mediated by rodent lung-surface macrophages of 1-3%/d towards the larynx^{4,24} does not only include alveolar macrophages but also macrophages from the interstitium re-entering the epithelium and migrating towards the ciliated airways. This is depicted schematically in Figure 4.

6.2. Differences in the biokinetics of rodents versus dogs and monkeys

There seem to be significant differences in μP biokinetics between rodents and human lungs. Yet not all necessary experimental data are available on the human μP biokinetics. Therefore the canine and simian biokinetics may serve as more appropriate models since the kinetics of μP lung retention and clearance is very similar in these three species as discussed earlier^{4,24}. Macrophage-mediated μP clearance kinetics from the human, simian and canine lungs was shown to be one order of magnitude slower than in rodents; the initial rate is 0.1 – 0.2%/d of the contemporary lung burden and declines exponentially with time. Furthermore, from early canine biokinetics studies²⁵⁻²⁷ on lung retention, clearance and serial, partial broncho-alveolar lavage (BAL) of insoluble, radio-labeled μP over one year we gained evidence that μP did not remain on the epithelium like in rodents but had been re-located into the interstitium with a half-life of 150 d. Yet long-term μP clearance was dominated by macrophage-mediated transport to the ciliated airways and larynx – similar to the long-term NP clearance from the rodent lungs.

In order to identify μP transport from the epithelium towards interstitial spaces in dogs we performed a morphological study: after a short-term inhalation of 1 μm fluorescent polystyrene particles (μPSL) we analyzed the morphological location of the μPSL in lung tissues by fluorescence microscopy²⁸. Indeed μPSL retention in interstitial spaces increased during six months to about 70% of all lung retained μPSL , as shown in Figure 5. These morphologic data are in reasonable agreement with the previous biokinetics data. These had

shown a μP relocation kinetics into pulmonary tissues which increased exponentially with a half-life of 150 d, see Figure 5.

Further evidence for species differences in particle relocation and accumulation in pulmonary tissues comes from a long-term exposure study to various dusts to monkeys and rats: Diesel soot, coal dust and a combination of both²⁹. Though a similar retention pattern as in canine pulmonary tissues (see above) was observed in monkeys, particle retention in rats was much more prominent on the alveolar epithelium, similar to the data shown in Figure 3. While the coal dust aerosol ($<7\mu\text{m}$ diameter) was micron-sized, the Diesel soot exhaust consisted of agglomerated sub-micron and nanoparticles. The fact that the coal dust particles and the Diesel exhaust particles were retained similarly in pulmonary tissues of monkeys demonstrates the access of NP, submicron and micron-sized particles to pulmonary tissues. The various data discussed here, suggest that the NP relocation towards pulmonary tissues is similar in rodents, dogs and monkeys and most likely in man, - in contrast to the biokinetics of μP in rodents with negligible access to pulmonary tissues.

In Figure 6 the long-term relocation and retention of NP versus μP are depicted showing the differences between rodent lungs versus canine and simian lungs. We speculate that the human pattern may be quite similar to the latter.

As mentioned above we hypothesize that the canine and simian are valid models for the biokinetics of inhaled particles in humans because of the congruent human, simian and canine data. Unfortunately no detailed long-term NP clearance kinetics data are known for the human, simian or canine lungs. In fact, it appears unlikely that NP are long-term retained on the epithelium when μP are relocated to the interstitium. Yet the kinetics of macrophage-mediated clearance seems to be independent of the location of the retained particle as the rodent data showed.

So, we can summarize the various findings on particle retention and biokinetics:

A clear difference in the location of retained μP between the canine and simian versus rodent lungs,

A ten-fold difference in the kinetics of macrophage-mediated clearance from human, canine and simian versus rodent lungs,

But long-term macrophage-mediated clearance seems not to depend on the particle retention site either on the epithelium or in the interstitium of all species studied.

7. Predominant NP lung retention and limited translocation towards the circulation

As discussed above insoluble NP in rodent lungs are predominantly long-term retained in interstitial spaces of the alveolar region. Yet there is only limited translocation towards the circulation. This is indeed very surprising since we found significant fractions of 20nm titanium dioxide NP in endothelial cells of rat lungs within 24 hours after inhalation³⁰. Unfortunately there is no direct proof for this NP accumulation in endothelial cells of large animal species and man but the study of Nikula and coworkers²⁹ provides indirect evidence as discussed above.

Yet the limited NP translocation in rat lungs strongly depends on the physico-chemical NP properties. For instance, after a 1-2-hour inhalation of 20nm NP of different materials (iridium, elemental carbon and titanium dioxide) by healthy adult rats the totally translocated NP fraction across the air-blood-barrier after 24 hours varies significantly by

one order of magnitude from almost 10% for iridium NP to about 1% of for titanium dioxide NP; see Figure 7. Iridium:^{14,21}; elemental carbon:²³; titanium dioxide: Kreyling & Semmler-Behnke, personal communication.

Of the translocated NP fractions in Figure 6 the highest organ fraction was found in the liver followed by spleen and kidneys followed by the heart, uterus and brain. This was true for all three NP. Interestingly even higher fractions of translocated NP of all three materials than in organs were found in soft tissue (blood content excluded) and the skeleton. Particularly, the latter is of interest since the NP were transported via blood circulation such that the skeletal NP retention is expected to be directly in the bone marrow. In addition, low but detectable NP concentrations in the blood over several weeks after inhalation of iridium and titanium dioxide NP suggest not completely stationary conditions of the translocated and accumulated NP in the organism but some clearance from the primary and secondary organs needs to be taken into account resulting in re-translocation and further accumulation in the various organs, soft tissue and the skeleton.

8. Summary

Biokinetics of inhaled biopersistent NP and μP is maintained by a multitude of highly dynamic processes which depend not only on physico-chemical properties of the particles but also on a manifold of cellular and molecular responses and interactions. As a result particles do not necessarily remain on their site of deposition after inhalation but undergo numerous transport processes within the various anatomical sites of the lungs and out of the lungs. While μP prominently stay long-term on the rodent alveolar epithelium but NP relocate into rodent interstitial spaces, both μP and NP gradually relocate into the canine and simian interstitium. Hence, these transport processes and their kinetics may differ between rodents versus dogs and monkeys and probably human. As a result, they significantly affect μP and NP particle dosimetry and toxicology, and may offer new opportunities in the new field of nano-medicine for diagnosis and therapy.

Acknowledgments

This work was partially supported by the European Union (EU) FP5 FIGD-CT-2000-00053, EU FP6 PARTICLE_RISK 012912 (NEST), EU FP7 NeuroNano (NMP4-SL-2008-214547) and ENPRA (NMP4-SL-2009-228789), US-NIH RO1HL070542.

BIOGRAPHICAL INFORMATION

Wolfgang G. Kreyling (PhD) is a biophysicist at the Institute of Lung Biology and Disease of the Helmholtz Center Munich (HMGU). His research interests range from aerosol sciences and nanoparticle technology to lungs biophysics reaching from the characterization of ambient aerosols to particle dosimetry and nanoparticle lung interactions on the level of the entire organism, cells like alveolar macrophages, and molecular compounds.

Manuela Semmler-Behnke (DVM) was a postdoctoral research fellow at the Institute of Lung Biology and Disease of the Helmholtz Center Munich (HMGU). Currently she works at the Bavarian Health and Food Safety Authority. Her research interests are deposition and biokinetics of inhaled engineered nanoparticles in rodents from neonates to adults or applications after different routes of entry (lungs, blood, gastrointestinal tract).

Shinji Takenaka (DVM, PhD) is a pathologist at the Institute of Lung Biology and Disease, CPC, Helmholtz Center Munich, Germany. His research focuses on health effects of air pollutants in experimental animals after long-term exposure to common air pollutants or industrial products. Formerly he worked at the National Institute for Environmental Studies,

Tsukuba, Japan, and the former Fraunhofer Institute of Toxicology and Aerosol Research, Schmallenberg/Hannover, Germany. At present, he studies the ultrastructural localization of inhaled nanoparticles in the lungs of rodents.

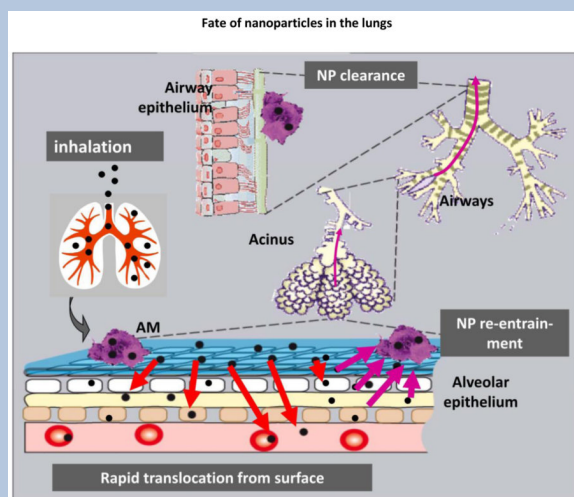
Winfried Möller (PhD) is a biophysicist at the Institute of Lung Biology and Disease (iLBD) of the Helmholtz Zentrum München. He developed the generation of ferromagnetic radio-labelled iron oxide micro and nano particles for human inhalation studies. Using these test aerosols he conducted several human inhalation studies on deposition and clearance. He currently investigates micro- and nano-particle based targeted drug delivery to the upper airways and the paranasal sinuses.

References

- (1). ICRP Human respiratory tract model for radiological protection. A report of a Task Group of the International Commission on Radiological Protection. *Ann. ICRP.* 1994; 24:1–482.
- (2). US-EPA Particulate Matter (PM) Standards Revision - 2006. Vol. Vol. 2006. US Government; 2006.
- (3). Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. *Am. J. Respir. Cell Mol. Biol.* 1992; 6:235–243. [PubMed: 1540387]
- (4). Kreyling, WG.; Scheuch, G. Clearance of particles deposited in the lungs. In: Gehr, P.; Heyder, J., editors. *Particle-Lung Interactions.* Marcel Bekker, Inc.; New York; Basel: 2000. p. 323–376.
- (5). Geiser M, Kreyling W. Deposition and biokinetics of inhaled nanoparticles. *Part. Fibre Toxicol.* 2010; 7(No. 2)
- (6). Schürch S, Gehr P, Im Hof V, Geiser M, Green F. Surfactant displaces particles toward the epithelium in airways and alveoli. *Respir. Physiol.* 1990; 80:17–32. [PubMed: 2367749]
- (7). Geiser M, Matter M, Maye I, Im Hof V, Gehr P, Schurch S. Influence of airspace geometry and surfactant on the retention of man-made vitreous fibers (MMVF 10a). *Environ. Health Perspect.* 2003; 111:895–901. [PubMed: 12782489]
- (8). Lehnert BE, Valdez YE, Tietjen GL. Alveolar macrophage-particle relationships during lung clearance. *Am. J. Respir. Cell Mol. Biol.* 1989; 1:145–154. [PubMed: 2620000]
- (9). Ellender M, Hodgson A, Wood KL, Moody JC. Effect of bronchopulmonary lavage on lung retention and clearance of particulate material in hamsters. *Environ. Health Perspect.* 1992; 97:209–213. [PubMed: 1396460]
- (10). Ferin J, Oberdörster G, Soderholm SC, Gelein R. Pulmonary tissue access of ultrafine particles. *J. Aerosol Med.* 1991; 4:57–68.
- (11). Oberdörster G, Ferin J, Lehnert BE. Correlation between particle size, in vivo particle persistence, and lung injury. *Environ. Health Perspect.* 1994; 102(Suppl 5):173–179.
- (12). Oberdörster G, Ferin J, Soderholm SC, Gelein R, Cox C, Baggs R, Morrow PE. Increased Pulmonary Toxicity of Inhaled Ultrafine Particles: Due to Lung overload Alone? *Ann. Occup. Hyg.* 1994; 38(Suppl.1):295–302.
- (13). Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles. *Environ. Health Perspect.* 2005; 113:823–839. [PubMed: 16002369]
- (14). Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdörster G, Ziesenis A. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J. Toxicol. Environ. Health.* 2002; 65:1513–1530.
- (15). Kreyling WG, Blanchard JD, Godleski JJ, Haeussermann S, Heyder J, Hutzler P, Schulz H, Sweeney TD, Takenaka S, Ziesenis A. Anatomic localization of 24- and 96-h particle retention in canine airways. *J. Appl. Physiol.* 1999; 87:269–284. [PubMed: 10409585]
- (16). Möller, W.; Kreyling, WG.; Schmid, O.; Semmler-Behnke, M.; Schulz, H. Deposition, Retention and Clearance, and Translocation of Inhaled Fine and Nano-Sized Particles in the Respiratory Tract. In: Gehr, P.; Mühlfeld, C.; Rothen-Rutishauser, B.; Plank, F., editors. *Particle-Lung Interactions.* 2nd ed. Vol. Vol. 241. Informa Healthcare USA; New York: 2009. p. 79–107.

- (17). Möller W, Felten K, Sommerer K, Scheuch G, Meyer G, Meyer P, Haussinger K, Kreyling WG. Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. *Am. J. Respir. Crit. Care Med.* 2008; 177:426–432. [PubMed: 17932382]
- (18). Van As A, Webster I. The morphology of mucus in mammalian pulmonary airways. *Environ. Res.* 1974; 7:1–12.
- (19). Blank F, Rothen-Rutishauser B, Gehr P. Dendritic cells and macrophages form a transepithelial network against foreign particulate antigens. *Am. J. Respir. Cell Mol. Biol.* 2007; 36:669–677. [PubMed: 17272826]
- (20). Donaldson K, Murphy F, Duffin R, Poland C. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part. Fibre Toxicol.* 2010; 7(No. 5)
- (21). Semmler M, Seitz J, Erbe F, Mayer P, Heyder J, Oberdorster G, Kreyling WG. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. *Inhal. Toxicol.* 2004; 16:453–459. [PubMed: 15204761]
- (22). Semmler-Behnke M, Takenaka S, Fertsch S, Wenk A, Seitz J, Mayer P, Oberdorster G, Kreyling WG. Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent reentrainment onto airways epithelium. *Environ. Health Perspect.* 2007; 115:728–733. [PubMed: 17520060]
- (23). Kreyling WG, Semmler-Behnke M, Seitz J, Scymczak W, Wenk A, Mayer P, Takenaka S, Oberdorster G. Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. *Inhal. Toxicol.* 2009; 21:55–60. [PubMed: 19558234]
- (24). Kreyling WG. Interspecies comparison of lung clearance of “insoluble” particles. *J. Aerosol Med.* 1990; 3:S93–S110.
- (25). Heyder J, Beck-Speier I, Ferron GA, Josten M, Karg E, Kreyling WG, Lenz AG, Maier KL, Reitmeier P, Ruprecht L, Takenaka S, Wohland T, Ziesenis A, Schulz H. Long-term responses of canine lungs to acidic particles. *Inhal. Toxicol.* 2009; 21:920–932. [PubMed: 19681732]
- (26). Kreyling WG, Dirscherl P, Ferron GA, Heilmann P, Josten M, Miaskowski U, Neuner M, Reitmeier P, Ruprecht L, Schumann. Health effects of sulfur-related environmental air pollution. III. Nonspecific respiratory defense capacities. *Inhal. Toxicol.* 1999; 11:391–422. et, a. [PubMed: 10380176]
- (27). Kreyling WG, Ferron GA, Fürst G, Heilmann P, Neuner M, Ruprecht L, Schumann G, Takenaka S, Heyder J. Long-term exposure of dogs to a sulfite aerosol: III. Macrophage mediated long-term particle clearance. *Inhal. Toxicol.* 1992; 4:197–233.
- (28). Kreyling WGT, S. Schumann G, Ziesenis A. Particles are predominantly transported from the canine epithelium towards the interstitial spaces and not to larynx! Analogy to human lungs? *Am. J. Respir. Crit. Care Med.* 2001; 163:A166.
- (29). Nikula KJ, Avila KJ, Griffith WC, Mauderly JL. Sites of particle retention and lung tissue responses to chronically inhaled diesel exhaust and coal dust in rats and cynomolgus monkeys. *Environ. Health Perspect.* 1997; 105(Suppl. 5):1231–1234. [PubMed: 9400729]
- (30). Geiser M, Rothen-Rutishauser B, Kapp N, Schurch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J, Gehr P. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ. Health Perspect.* 2005; 113:1555–1560. [PubMed: 16263511]

Conspectus



Researchers need to study the biokinetics of inhaled biopersistent nano- and micron-sized particles (NPs and μ Ps) to assess their toxicity and to develop understand their potential risks. When particles are inhaled, they do not necessarily remain on their sites of deposition in the respiratory tract. Instead they can undergo numerous transport processes within the various tissues of the lungs, including clearance from the lungs. In this context we would like to understand how the biokinetic studies performed in animals can be extrapolated to humans. Interestingly, the particle retention is much shorter in rodent lungs and declines much faster than it does in human, simian and canine lungs.

The predominant long-term clearance pathway for both NPs and μ Ps in humans and other animal species is macrophage-mediated particle transport from the peripheral lungs towards ciliated airways and the larynx. However, the transport rate is ten times higher in rodents than in other species. In addition to particle clearance out of the lung, we also observe particle redistribution from the epithelium toward and within the interstitium and lymph nodes of the lung and particle translocation to blood circulation leading to subsequent accumulation in secondary organs. While μ Ps have limited access to interstitial spaces in the rodent lungs, NPs rapidly relocate in the epithelium and the underlying interstitium. By contrast, indirect evidence shows that both NP and μ P are relocated into the epithelium and interstitial spaces of the human, simian and canine lungs.

Only NPs translocate into the circulatory system and subsequently accumulate in the secondary organs and tissues of the body. Translocated NP fractions are rather low but they depend strongly on the physicochemical properties of the NP and their surface properties. Growing evidence indicates that the binding and conjugation of proteins to NP play an essential role on the translocation across cellular membranes and organ barriers.

In summary, particle biokinetics result from a multitude of highly dynamic processes which depend not only on physicochemical properties of the particles but also on a multitude of cellular and molecular responses and interactions. Given the rather small accumulation in secondary organs after acute inhalation exposures, it appears likely that adverse effects caused by NP accumulated in secondary organs may only occur after chronic exposure over extended time periods. Therefore adverse health effects in secondary organs such as the cardiovascular-system that are associated with chronic

exposure of ambient urban air pollution are less likely to result from particle translocation. Instead, chronic particle inhalation could trigger or modulate the autonomous nervous system or the release of soluble mediators into circulation leading to adverse health effects.

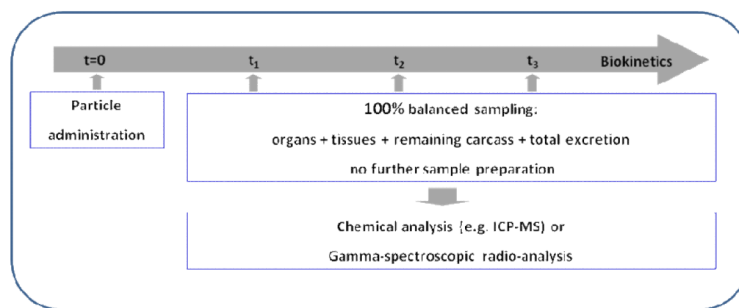


Figure 1. Concept of quantitative particle biokinetics assessment. Particles are administered at time $t=0$. From this time point on, the entire urinary and fecal excretions are collected separately. Animals are euthanized at times t_1 , t_2 , t_3 , etc., and organs and tissues of interest and the entire remaining carcass are sampled (100% balanced sampling). Special sample preparation is required before chemical analysis. When particles are radio-labeled, total organ samples will be analyzed directly using gamma spectrometry without any further preparation.

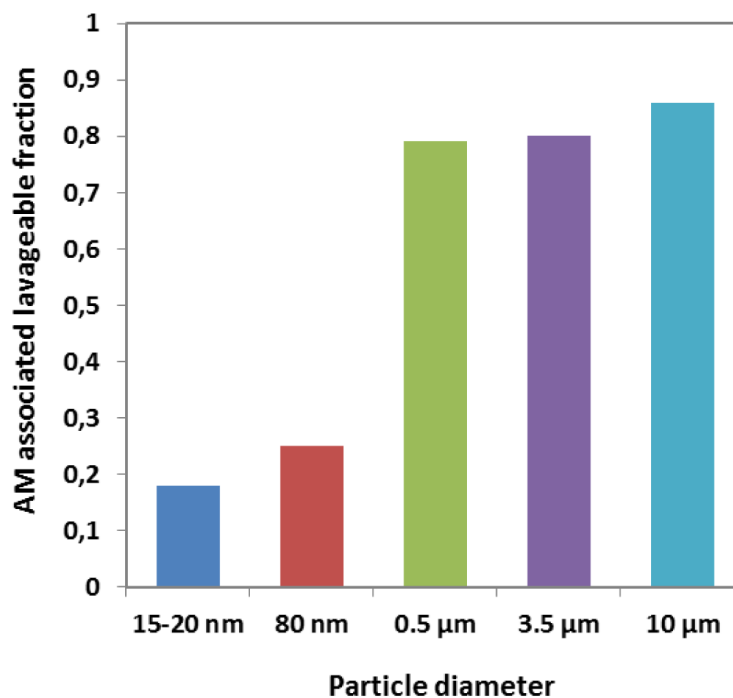


Figure 2. Alveolar-macrophage (AM) associated fractions of instilled μ P (0.5, 3 and 10 μ m polystyrene particles) and inhaled NP (radio-labeled 20 and 80nm iridium NP) found in BAL of rats 24 hours after administration^{13,14}. The low fractions of the iridium NP are similar to those of 20nm gold NP, titanium dioxide NP and elemental carbon NP (data not shown).

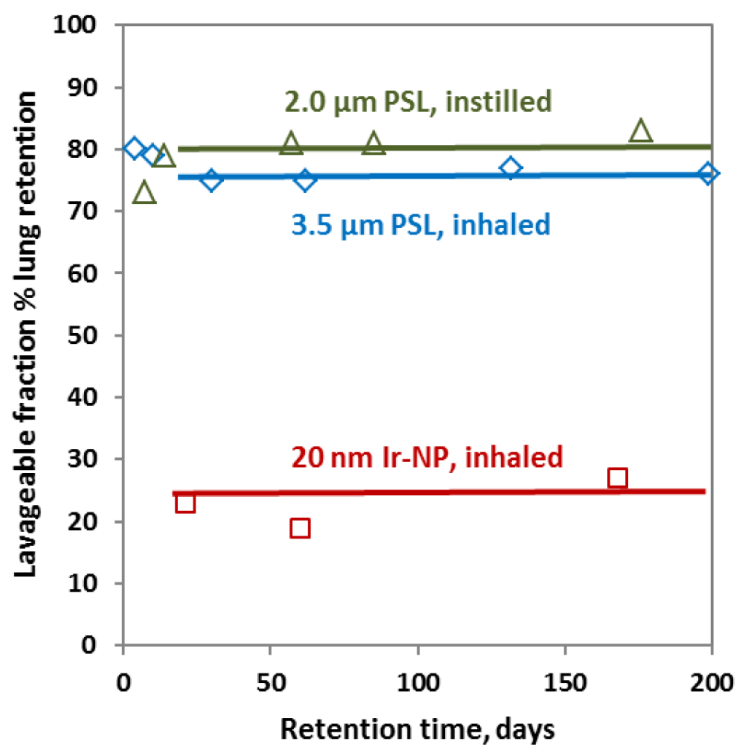


Figure 3. Fractions of μP (either inhaled $3.5\ \mu\text{m}$ ^{85}Sr -labeled polystyrene particles (μPSL) or intratracheally instilled fluorescent $2\ \mu\text{m}$ μPSL versus inhaled NP (radio-labeled 20nm iridium NP) found in broncho-alveolar lavage fluids of rats at various time points from day 3 through six months after administration^{8,11,14,21,22}. All fractions are relative to the actual lung burden.

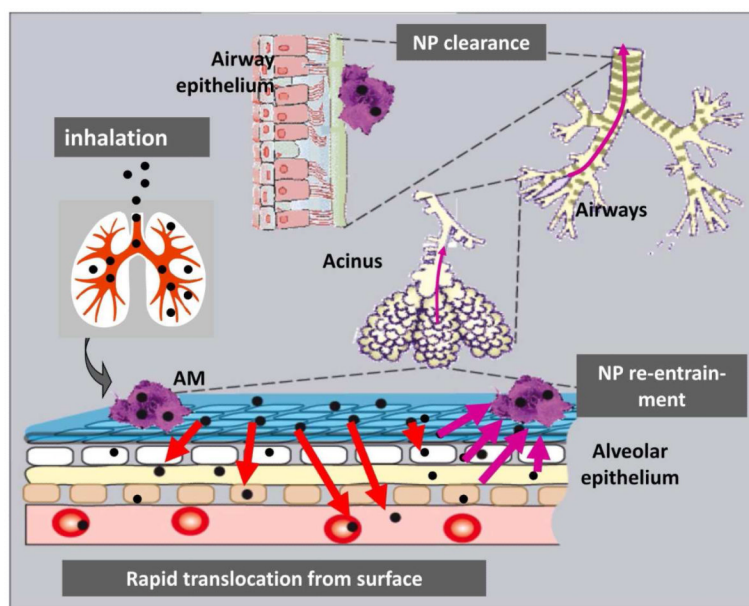


Figure 4. Pathways of relocation of inhaled NP after deposition on the alveolar epithelium of the rodent lungs: rapid transport into the epithelium and interstitial spaces for long-term retention; upon clearance NP can re-entrain back onto the epithelium for macrophage-mediated transport towards ciliated airways and the larynx²².

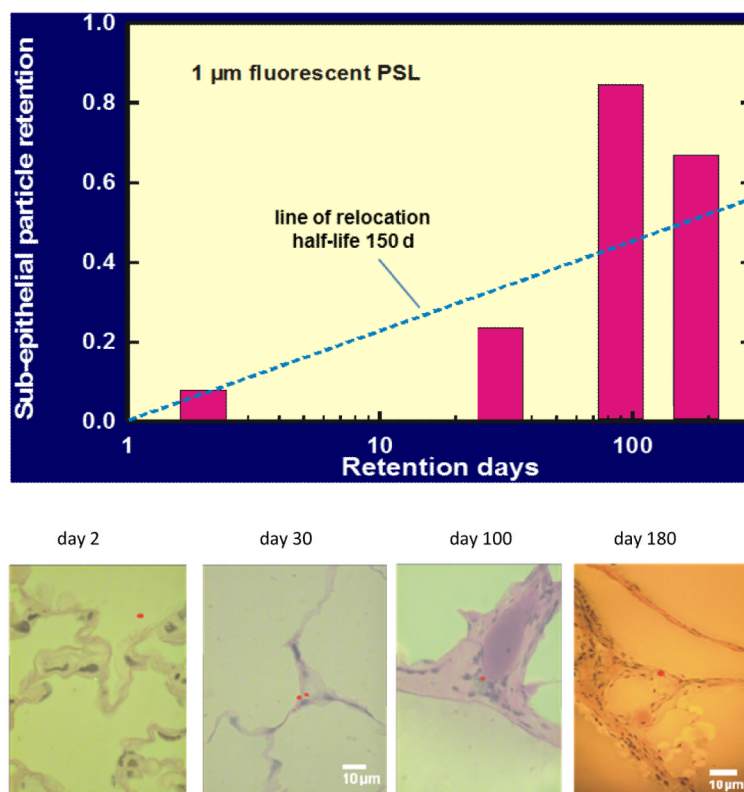


Figure 5. Upper panel shows the increasing particle fraction in the canine lung interstitium during a six-month retention period after a short-term inhalation of 1 μ m fluorescent polystyrene particles (μ PSL)²⁸. The line corresponds to the particle relocation kinetics with a half-life of 150 d into pulmonary tissues as previously determined in biokinetics studies²⁵⁻²⁷. Lower panels show morphologic locations of μ PSL in lung tissues during the six-month period (μ PSL, false color).

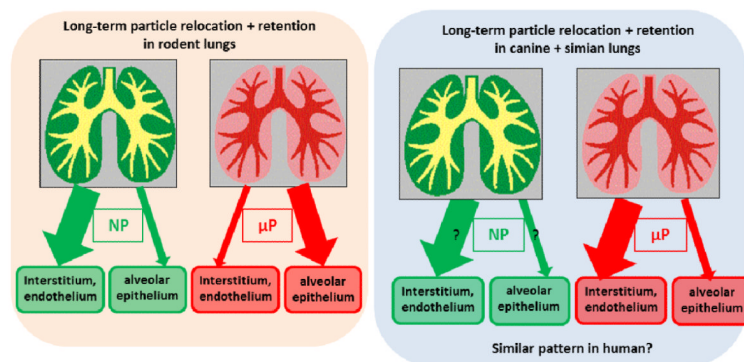


Figure 6. Graphical scheme of long-term relocation and retention of NP versus μ P showing the differences between rodent lungs versus canine and simian lungs. We hypothesize that the human pattern may be quite similar to that of the large animal species.

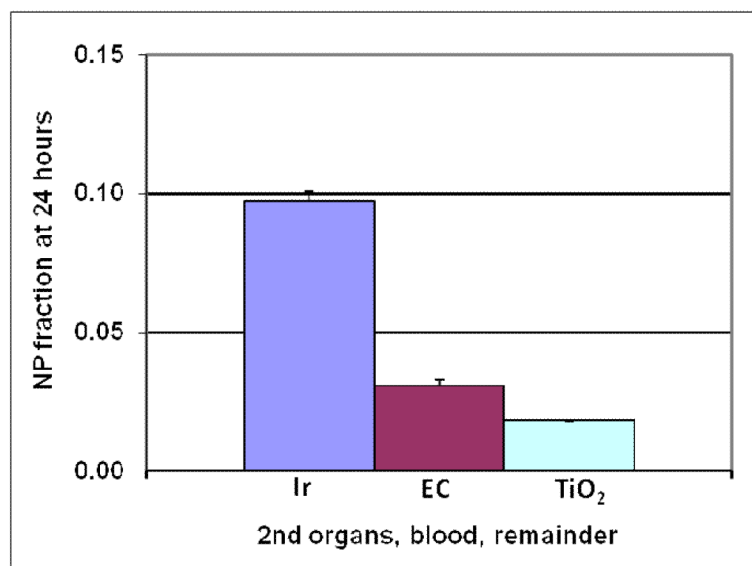


Figure 7.

Twenty-four-hour translocated fractions of alveolar NP deposit towards blood and subsequent organs and tissues; three different materials (iridium, elemental carbon and titanium dioxide) were inhaled as freshly generated 20nm NP aerosols for 1-2 hours by healthy adult rats. The iridium NP translocation is significantly higher than those of elemental carbon and titanium dioxide NP. Iridium:^{14,21}; elemental carbon:²³; titanium dioxide: Kreyling & Semmler-Behnke, personal communication.