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Differences in the Biokinetics of Inhaled Nano- versus Micrometer-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

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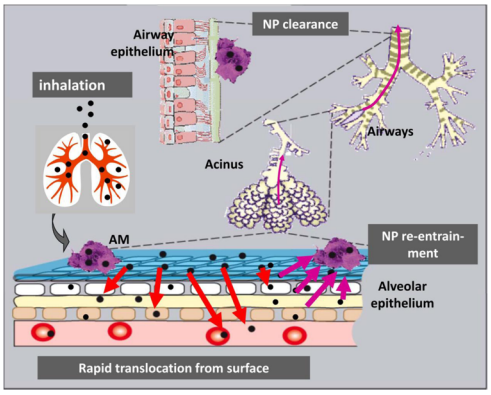
CONSPECTUS

Researchers need to study the biokinetics of inhaled biopersistent nano- and micrometer-sized particles (NPs and μPs) to assess their toxicity and to develop an understanding of their potential risks. When particles are inhaled, they do not necessarily remain at 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 μPs in humans and other animal species is macrophage-mediated particle transport from the peripheral lungs toward ciliated airways and the larynx. However, the transport rate is 10 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 μPs 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 NPs and μPs 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 NPs play an essential role in 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 NPs 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.

1. Introduction

For 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 (NPs, 1–100 nm diameter) versus that of submicrometer and micrometer-sized

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particles (μPs, >100 nm diameter). Dosimetry starts with the estimate of an incorporated dose of particles, which needs to be distinguished and derived from the exposure dose. While μP 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 NPs and μPs are used in a large and rapidly increasing number of products of great diversity in food, pharmacology, personal care, and other day-to-day goods, it appears to be necessary to gain a general estimate of the total NP and μP exposure by oral intake of consumers at large.

Depending on the route of intake, the incorporated dose may be rather complex as is the case for inhaled particles. Their deposition probability in the various regions of the respiratory tract depends on three sets of properties: (1) physicochemical 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) 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 particle and its metabolic compounds in the body over time. In this paper, we focus on the biokinetics of NPs versus μPs administered to lungs and specifically discuss quantitative biokinetics as a comprehensive approach. We will discuss the rather comprehensive data set for rodents and compare it to the scarce but more appropriate data in 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 above 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 tens of milligrams per cubic meter, this is an important limit for NP aerosols. The number concentration limit of 10^6 cm^{-3} of, for example, 20 nm or 100 nm unit-density-NP or 500 nm unit-density-μP corresponds to mass concentrations of 4.2 μg/m^3, 525 μg/m^3, and 66 mg/m^3, respectively. Note the upper mass concentration for 500 nm μPs 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 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 μg/h, 60 μg/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 5×10^{10} epithelial cells.[3] Hence, corresponding in vitro doses to 10^6 cultured cells would be limited to 0.5 ng, 60 ng, and 7.5 μg, respectively, while usually in vitro doses exceed 1−10 μg. Furthermore, particle doses administered as a bolus during <30 s 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 μm in aerodynamic diameter can reach the lungs because these particles deposit already in the nose.[4]

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, because 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.

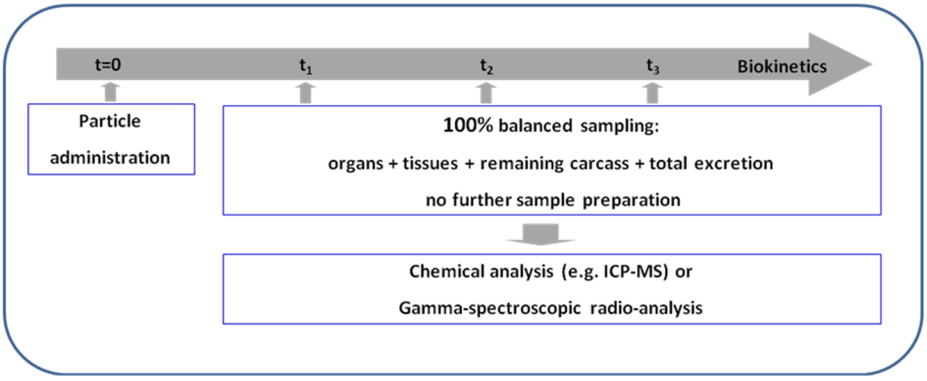

FIGURE 1. Concept of quantitative particle biokinetics assessment. Particles are administered at time $t = 0$. From this time point on, the entire urinary and fecal excretions are collected separately. Animals are euthanized at times t_1, t_2, t_3, etc., and organs and tissues of interest and the entire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

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 with 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 NPs or μPs by any route of delivery: inhalation, intratracheal instillation, or aspiration to the respiratory tract; oral ingestion or gavage to the gastrointestinal 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 and detailed quantitative analysis of their biokinetics is obtained.[5] Note when particles undergo chemical and physical changes such as particle decomposition or disintegration, the biokinetic 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, for example, inductively coupled plasma mass spectroscopy (ICP-MS), provides 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 NPs retained in organs. In addition, endogenous species of the chemical element of the particle may contribute to the natural "background" concentration. γ-Radiospectroscopy may provide an elegant highly sensitive analytical alternative, allowing for a direct analysis of complete organs and tissues without any pretreatment. In this case, the particles need to be labeled using a radio-isotope firmly integrated into the particle matrix without any allowance for leaching. While this requirement is hard to fulfill, when a radioisotope 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 radioactivation of one to a few

atoms per particle. Mostly, the amount of radioactivity is sufficiently high for subsequent radioanalysis, when not more than a single atom per NP was converted by the nuclear reaction while more radioisotopes in μPs 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 radiolabeling may not always be possible with any given particle because of possible nuclear reactions.

However, an important advantage of γ-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 γ 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 an initial particle dose of 10−50 kBq and a mass of 1−10 μg per rodent is administered, 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 5 orders of magnitude.
(b) The administered particle mass is likely not to trigger pathophysiological responses of the organism.

Yet, because the radiospectrometric method similarly to the chemical analytical methods does not distinguish between the particulate state and chemical speciation, those important particle relevant parameters including the firm labeling of the radioisotope 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.

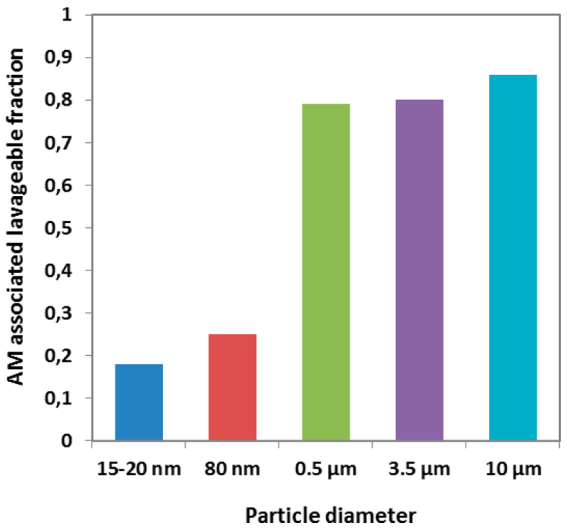

FIGURE 2. Alveolar-macrophage (AM) associated fractions of instilled μPs (0.5, 3, and 10 μm polystyrene particles) and inhaled NPs (radiolabeled 20 and 80 nm iridium NPs) found in BAL of rats 24 h after administration.[13,14] The low fractions of the iridium NPs are similar to those of 20 nm gold NPs, titanium dioxide NPs, and elemental carbon NPs (data not shown).

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 only for μPs of 1−6 μm in diameter, the same is expected for NPs, since this process becomes even more efficient with decreasing particle size.

In general, the majority of μPs remains on the epithelial surface in airways and alveoli of rodent lungs and are accessible to bronchoalveolar lavage (BAL), see Figure 2.[8−12] However, already 24 h after inhalation most NPs 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 toward distal airways. While the retention of μPs generally does not exceed 24−48 h in rodent airways, there is evidence for prolonged retention of NPs and μPs 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 as follows: (1) the particles were no longer accessible to mucociliary clearance either because they penetrated through the mucus layer deep into the periciliary phase,[6] or (2) they deposited in areas without any mucus coverage, since patchy mucus layers can exist in small airways.[18] In both cases, particles can penetrate between cilia to further interact with cells of the inner lung surface, that is, macrophages and dendritic and epithelial cells.[19] 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 gastrointestinal tract; (b) particle transport toward lung-associated lymph nodes; (c) translocation into blood circulation for subsequent accumulation in secondary organs. Note that 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 NPs and μPs. We will not address the second transport pathway toward lymph nodes because 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 NPs and μPs in Rodents. NP relocation into rat epithelial and interstitial tissues (pulmonary tissues) was described after inhalation of aerosolized submicrometer agglomerates of 20 nm primary TiO_2 versus the inhalation of aerosolized submicrometer agglomerates of primary 200−300 nm TiO_2 particles.[11] When compared with submicrometer agglomerates of primary 200−300 nm μPs, an increased NP fraction was determined in pulmonary tissues by chemical analyses. NPs and 200−300 nm μPs samples that had been obtained from exhaustive BAL and the according lavaged lungs were compared. After a 12-week exposure, the authors estimated that about 50% more NP mass than larger particles was

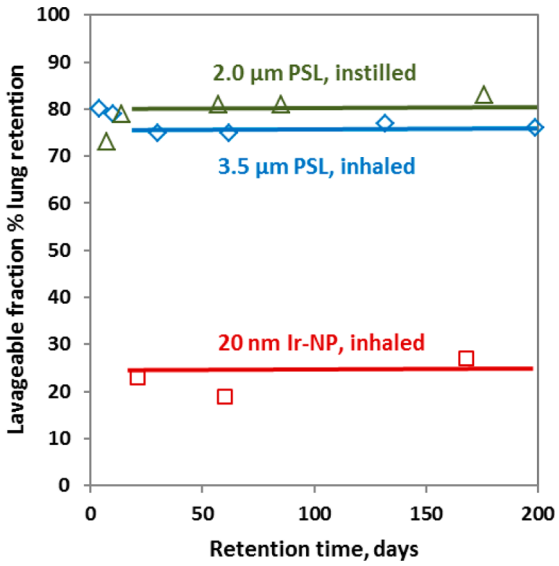

FIGURE 3. Fractions of μPs (either inhaled 3.5 μm ^{85}Sr-labeled polystyrene particles (μPSL) or intratracheally instilled fluorescent 2 μm μPSL versus inhaled NPs (radiolabeled 20 nm iridium NPs) found in bronchoalveolar lavage fluids of rats at various time points from day 3 through 6 months after administration.[8,11,14,21,22] All fractions are relative to the actual lung burden.

relocated into pulmonary tissues. They suggest that disagglomeration of the agglomerates of the primary 20 nm TiO_2 had occurred on the epithelial surface prior to relocation into rat pulmonary tissues, which did not occur to the 200−300 nm TiO_2 particle agglomerates. Similar results were found for intratracheally instilled submicrometer agglomerates of primary 20 nm TiO_2 or Al_2O_3 NPs versus instilled submicrometer agglomerates of primary 200−300 nm TiO_2 or Al_2O_3 particles.[10] However, in these studies, the extent of disagglomeration could not be quantified.

In a more recent rat inhalation study using freshly generated iridium NP aerosols of 20 or 80 nm median diameter (geometric standard deviation (GSD) 1.6) about 80% and 75%, respectively, of the NPs (deposited on the alveolar epithelial surface) were relocated into epithelial and pulmonary tissues within 24 h and no longer accessible to BAL, Figure 2.[21,22] These fractions (relative to the contemporary lung burden) remained unchanged over the next 6 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 25 nm elemental carbon and 20 nm TiO_2 NP aerosols.[23] Three different NP materials (iridium, elemental carbon, and titanium dioxide) of similar size (20 nm), polydispersity, and NP morphology were studied; they all were chain-aggregates/agglomerates of primary particles <5 nm. They were inhaled as 20 nm NP aerosols by rats, and they all

showed rapid internalization into the interstitium, emphasizing the importance of NP size. Note that at a deposited NP dose of about 1 μg per rat lung after the 1-h inhalation a retrograde agglomeration on the alveolar epithelial surface is basically excluded since the projected area of all NPs covers only 10^{-12} of the rat lung surface area.

In contrast, about 80% of inhaled or instilled μPs 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 from the interstitial relocation of NPs, the majority of μPs (fluorescent 2 μm and radiolabeled 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 was collected by exhaustive BAL throughout the entire retention time of six months, Figure 3.[8,11] Similar results were obtained for micrometer-sized radiolabeled fused-clay particles in hamster lungs.[9] 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 that the μP burden as a fraction of the initial dose declines rather rapidly due to continuous alveolar-macrophage mediated clearance toward the ciliated airways and larynx. The initial rate starts at 1−3% per day, but it declines exponentially with time such that after about 200 days >90% of the μPs are cleared.[4,24]

Surprisingly, and very similar to the μP clearance kinetics, more than 90% of the initial 20 nm 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 NPs apparently reappeared from interstitial spaces back onto the epithelium for subsequent clearance by lung-surface macrophages. Although not yet confirmed, the most likely mechanism of NP reappearance 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, that is, the daily cleared NP fractions toward the larynx, were the same 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% per day toward the larynx[4,24] includes not only alveolar macrophages but also macrophages from the interstitium re-entering the epithelium and migrating toward the ciliated airways. This is depicted schematically in Figure 4.

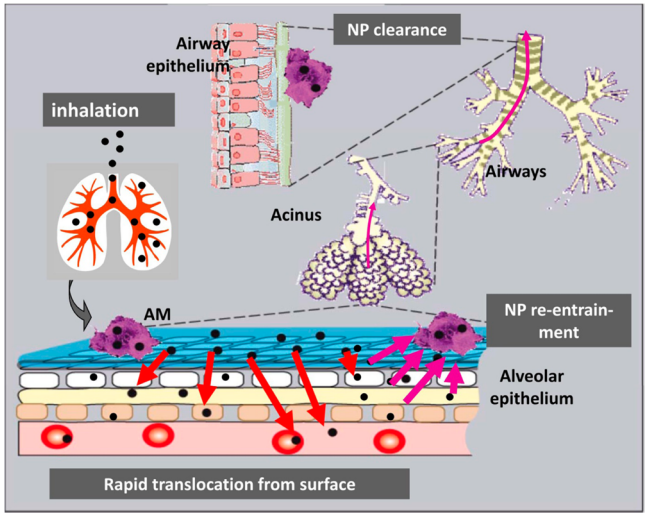

FIGURE 4. Pathways of relocation of inhaled NPs after deposition on the alveolar epithelium of the rodent lungs: rapid transport into the epithelium and interstitial spaces for long-term retention; upon clearance NPs can re-entrain back onto the epithelium for macrophage-mediated transport toward ciliated airways and the larynx.[22]

6.2. Differences in the Biokinetics of Rodents versus Dogs and Monkeys. There seem to be significant differences in μP biokinetics between rodent and human lungs. Yet not all necessary experimental data are available on human μP biokinetics. Therefore 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 1 order of magnitude slower than in rodents; the initial rate is 0.1−0.2% per day of the contemporary lung burden and declines exponentially with time. Furthermore, from early canine biokinetics studies[25−27] on lung retention, clearance and serial, partial bronchoalveolar lavage (BAL) of insoluble, radiolabeled μPs over 1 year, we gained evidence that μPs did not remain on the epithelium as in rodents but had been relocated into the interstitium with a half-life of 150 days. 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 toward 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 microscopy.[28] Indeed μPSL retention in interstitial spaces

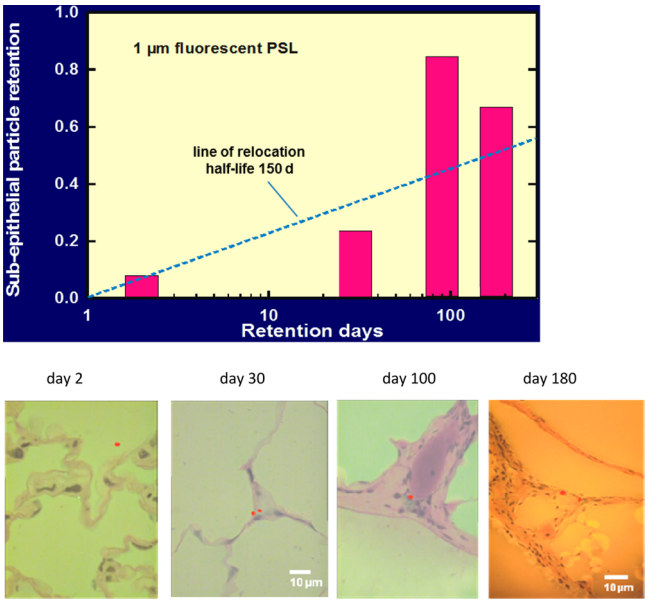

FIGURE 5. Upper panel shows the increasing particle fraction in the canine lung interstitium during a six-month retention period after a short-term inhalation of 1 μm fluorescent polystyrene particles (μPSL).[28] The line corresponds to the particle relocation kinetics with a half-life of 150 days into pulmonary tissues as previously determined in biokinetics studies.[25−27] Lower panels show morphologic locations of μPSL in lung tissues during the 6-month period (μPSL, false color).

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 that increased exponentially with a half-life of 150 days, 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 in monkeys and rats: diesel soot, coal dust, and a combination of both.[29] 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 μm diameter) was micrometer-sized, the diesel soot exhaust consisted of agglomerated submicrometer 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 NPs and submicrometer and micrometer-sized particles to pulmonary tissues. The various data discussed here suggest that NP relocation in pulmonary tissues is similar in rodents, dogs, and monkeys and most likely in man, in contrast to the biokinetics of μPs in rodents with negligible access to pulmonary tissues.

In Figure 6, the long-term relocation and retention of NPs versus μPs are depicted showing the differences between rodent lungs and 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 NPs are retained long-term on the epithelium when μPs 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:

(1) A clear difference in the location of retained μPs between the canine and simian lungs and rodent lungs.
(2) A 10-fold difference in the kinetics of macrophage-mediated clearance from human, canine, and simian lungs versus rodent lungs
(3) 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 toward the Circulation

As discussed above, insoluble NPs in rodent lungs are predominantly retained long-term in interstitial spaces of the alveolar region. Yet there is only limited translocation toward the circulation. This is indeed very surprising since we found significant fractions of 20 nm titanium dioxide NPs in endothelial cells of rat lungs within 24 h after inhalation.[30] 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 co-workers[29] provides indirect evidence as discussed above.

Yet the limited NP translocation in rat lungs strongly depends on the physicochemical NP properties. For instance, after a 1−2-h inhalation of 20 nm NPs of different materials (iridium, elemental carbon, and titanium dioxide) by healthy adult rats the total translocated NP fraction across the air−blood barrier after 24 h varies significantly by 1 order of magnitude from almost 10% for iridium NPs to about 1% for titanium dioxide NPs, see Figure 7. Data on iridium was

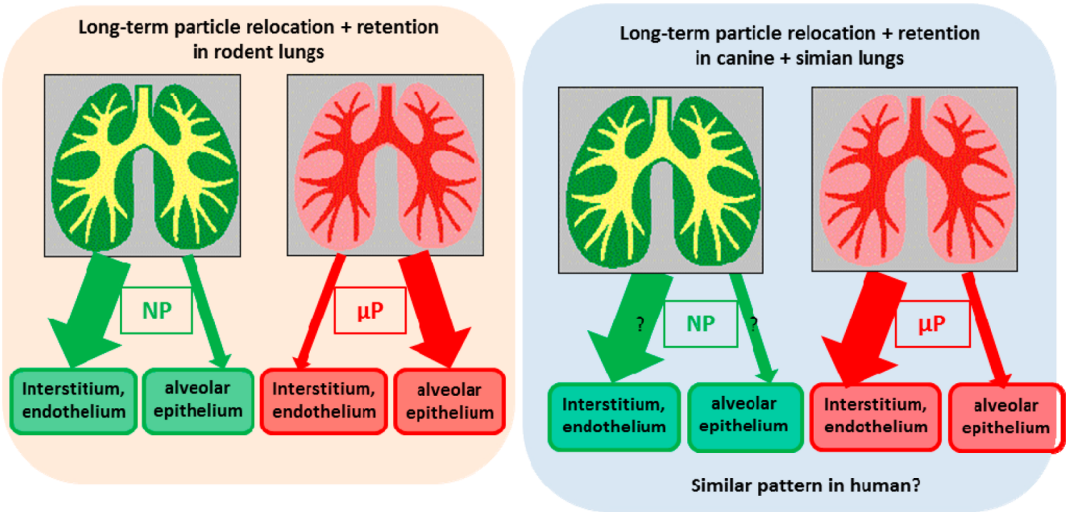

FIGURE 6. Graphical scheme of long-term relocation and retention of NPs versus μPs showing the differences between rodent lungs and canine and simian lungs. We hypothesize that the human pattern may be quite similar to that of the large animal species.

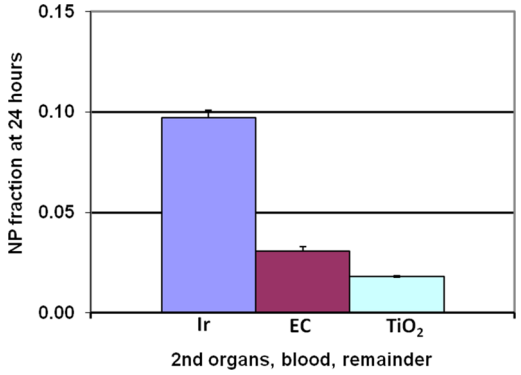

FIGURE 7. Twenty-four-hour translocated fractions of alveolar NP deposit toward blood and subsequent organs and tissues; three different materials (iridium, elemental carbon, and titanium dioxide) were inhaled as freshly generated 20 nm NP aerosols for 1–2 h by healthy adult rats. The iridium NP translocation is significantly higher than those of elemental carbon and titanium dioxide NPs. Iridium data from refs 14 and 21, elemental carbon from ref 23, and titanium dioxide from Kreyling and Semmler-Behnke, personal communication.

taken from refs 14 and 21, for elemental carbon from ref 23, and for titanium dioxide from Kreyling and Semmler-Behnke, personal communication.

Of the translocated NP fractions in Figure 7, 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 NPs. Interestingly even higher fractions of translocated NPs of all three materials than in organs were found in soft tissue (blood content excluded) and the skeleton. Particularly, the latter is of interest because the NPs 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 NPs 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 retranslocation and further accumulation in the various organs, soft tissue, and skeleton.

8. Summary

Biokinetics of inhaled biopersistent NPs and μPs is maintained by a multitude of highly dynamic processes that depend not only on physicochemical 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 μPs prominently stay long-term on the rodent alveolar epithelium but NPs relocate into rodent interstitial spaces, both μPs and NPs gradually relocate into the canine and simian interstitium. Hence, these transport processes and their kinetics may differ between rodents and dogs, monkeys, and probably human. As a result, they significantly affect μP and NP dosimetry and toxicology and may offer new opportunities in the new field of nanomedicine for diagnosis and therapy.

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BIOGRAPHICAL INFORMATION

Wolfgang G. Kreyling (Ph.D.) 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 lung 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 (D.V.M.) 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 and applications after different routes of entry (lungs, blood, and gastrointestinal tract).

Shinji Takenaka (D.V.M., Ph.D.) 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 (Ph.D.) is a biophysicist at the Institute of Lung Biology and Disease (iLBD) of the Helmholtz Zentrum München. He developed the generation of ferromagnetic radiolabeled iron oxide micro- and nanoparticles for human inhalation studies. Using these test aerosols, he conducted several human inhalation studies on deposition and clearance. He currently investigates micro- and nanoparticle-based targeted drug delivery to the upper airways and the paranasal sinuses.

FOOTNOTES

*Corresponding author. Mailing address: 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. E-mail: kreyling@helmholtz-muenchen.de.

The authors declare no competing financial interest.

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