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Long-Term Clearance Kinetics of Inhaled Ultrafine Insoluble Iridium Particles from the Rat Lung, Including Transient Translocation into Secondary Organs

M. Semmler, J. Seitz, F. Erbe, P. Mayer, J. Heyder
GSF National Research Center, Institute for Inhalation Biology, Neuherberg/Munich, Germany

G. Oberdörster
University of Rochester, Department of Environmental Medicine, Rochester, New York, USA

W. G. Kreyling
GSF National Research Center, Institute for Inhalation Biology, and Focus Network: Aerosols and Health, Neuherberg/Munich, Germany

Recently it was speculated that ultrafine particles (UFP) may translocate from deposition sites in the lungs to systemic circulation and whether long-term clearance differs between ultrafine and micrometer-sized particles. We have studied lung retention and clearance kinetics in 12 healthy male adult WKY rats up to 6 mo after an inhalation of ^{192}Ir-radiolabeled, insoluble, ultrafine 15- to 20-nm iridium particles. Whole-body retention was followed by external gamma counting, and particle clearance kinetics were determined by excretion radioanalysis. Four rats each were sacrificed after 3 wk and 2 and 6 mo; all organs as well as tissues and the carcass were radioanalyzed to balance the entire deposited radioactivity of the particles. The most prominant fraction was retained in the lungs at each time point of sacrifice (26%, 15%, 6%, respectively), and clearance out of the body was solely via excretion. Extrapulmonary particle uptake did not continue to increase but decreased with time in liver, spleen, heart, and brain when compared to previous data obtained during the first 7 days after inhalation (Kreyling et al., 2002). UFP long-term lung retention derived from whole-body measurements was comparable to previously reported data using insoluble micrometer-sized particles (Bellmann et al., 1994; Lehnert et al., 1989). In addition, differential analysis including daily excretion data revealed a pattern of fractional particle clearance rate of the ultrafine iridium particles similar to that of micrometer-sized particles reported by Snipes et al. (1983) and Bailey et al. (1985).

Recent epidemiological studies provide evidence that an increase in ultrafine particles is associated with adverse cardiovascular effects (Wichmann et al., 2000; Wichmann & Peters, 2000; Ibald-Mulli et al., 2002). These findings pertain not only to elderly people but also to susceptible persons with underlying diseases of various origins (U.S. EPA, 1996). Currently there is concern about translocation of ultrafine particles (UFP) from the lungs into systemic circulation and uptake in transpulmonary organs causing adverse cardiovascular effects. While there are conflicting reports about short-term translocation and accumulation in the liver ranging in human studies from about 7% (Nemmar et al., 2001) to <1–2% (Brown et al., 2002) and in animal studies from about 50% (Oberdörster et al., 2002) to 0.5% (Kreyling et al., 2002), nothing is known about long-term translocation and whether clearance kinetics of ultrafine particles differ from that of larger particles, as one may expect from differing clearance mechanisms for micrometer-sized particles versus UFP.

Translocation pathways of UFP are assumed to be different from those of larger particles (>100 nm diameter); for example, lung retention and clearance of UFP are less alveolar macrophage-mediated than are those of larger particles (Kreyling & Scheuch, 2000). Although alveolar macrophages do not phagocytize a prominent fraction of UFP—as they do in

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the case of particles larger than 100 nm—they are still involved in phagocytic and pinocytic processes of UFP uptake and secretion of mediators (Kreyling & Scheuch, 2000; Donaldson et al., 2001).

A common approach to investigating the dosimetry of particles in the lungs is the use of metal or metal oxide particles labeled with a convenient radioactive gamma emitter (Kreyling & Scheuch, 2000). ^{192}Ir-UFP turned out to be an appropriate material to study clearance kinetics, although we are aware that iridium is not a relevant component of the ambient aerosol. In a prior study (Kreyling et al., 2002), a predominant retention of ultrafine insoluble ^{192}Ir particles was shown in the lung for 1 wk after inhalation. The UFP deposited in both the conducting airways and lung periphery were predominantly cleared via airways and larynx into the gastrointestinal tract (GIT) and feces. There also was a small but detectable fraction translocated to secondary target organs (liver, spleen, heart, brain, <1%) and carcass (<5%).

The first aim of this follow-up study was to determine whether translocated fractions in secondary target organs continue to increase. To focus the analysis on the kinetics of particle translocation from the lungs, we attempted to avoid pelt contamination and particle deposition in extrathoracic airways. Therefore, ultrafine iridium particles radiolabeled with ^{192}Ir were inhaled by rats via intratracheal intubation. Second, we aimed to study whether there is a difference in long-term particle clearance towards the larynx and GIT between ultrafine insoluble ^{192}Ir particles and micrometer-sized particles. Ferin et al. (1992) and Oberdörster et al. (1994) also had shown the uptake of 20-nm ultrafine TiO_2 particles in epithelial cells and in the interstitial space. More recently, Takenaka et al. (2003) had shown that ultrafine Ag particles translocated from the luminal side into and beyond the epithelial membrane of the lung, including uptake in type I epithelial cells, endothelial cells, and the alveolar septum. In contrast, micrometer-sized particles were shown to stay retained on the epithelium (Lehnert et al., 1989). The different sites of retention led us to hypothesize that long-term clearance kinetics should differ between UFP and micrometer-sized particles.

MATERIAL AND METHODS

Animals

Young, adult, healthy, male Wistar-Kyoto rats (WKY/Kyo@ Rj rats, Janvier, France) were housed in pairs in a humidity-controlled (55% relative humidity) and temperature-controlled (22°C) room in individually ventilated cages (VentiRack, cage type CU-31), maintained on a 12-h day/night cycle prior to the studies. Laboratory animal diet and water were provided ad libitum. The studies were conducted under federal guidelines for the use and care of laboratory animals and were approved by the Regierung von Oberbayern (District of Upper Bavaria, approval number 211-2531-108/99) and by the GSF Institutional Animal Care and Use Committee.

Aerosol Production and Characterization

The ultrafine ^{192}Ir aerosol was produced with a spark generator as described previously (Kreyling et al., 2002). The spark frequency was 3 Hz in an argon stream of 3 L min^{-1}; the aerosol was neutralized by a radioactive [85]Kr source. The aerosol then was diluted with nitrogen and adjusted to 20% oxygen, 50–60% relative humidity, and 37°C. This created an aerosol with a number concentration of 3×10^7 cm^{-3} (continuously monitored by a condensation particle counter; CPC 3022A, TSI, Aachen, Germany) and a count median diameter (CMD) varying between 15 and 20 nm (continuously determined with a differential particle mobility analyzer; Classifier 3071 + CPC 3010, TSI, Aachen, Germany). Specific iridium activity was 10 GBq g^{-1} at reference date. Mass concentration was calculated to be 0.2 mg m^{-3} from the radioactivity measurement of an integral filter sample and the sampled aerosol volume.

Inhalation

Young adult healthy male WKY/Kyo rats (∼250 g body weight) were anesthetized by an intramuscular injection of a mixture of Medetomidin (15 μg/100 g body weight), Midazolam (0.2 mg/100 g body weight), and Fentanyl (0.5 μg/100 g body weight). For endotracheal intubation a flexible cannula (16 G, 2 in) was placed in the upper trachea under visual control and sealed against outside air with a modified pipette tip wedged gently into the laryngeal opening (Kreyling et al., 1993, 2002; Osier & Oberdörster, 1997). The animals were placed in a plethysmograph and connected with the endotracheal cannula to the aerosol line outside of the plethysmograph. Ventilation was computer controlled with a negative pressure of −1.5 kPa applied to the plethysmograph for 1 s of inspiration followed by 0.5 s expiration at ambient air pressure, resulting in a breathing frequency of 45 min^{-1}. This ventilation pattern caused inspiration of 75–80% of total lung capacity of the rat, so animals were slightly hyperventilated and did not breathe spontaneously but followed the computer-controlled breathing pattern. For radiation protection the entire aerosol system was maintained at −30 Pa low pressure and additionally installed in a glove box, which was highly ventilated through an absolute particle filter.

After 1 to 1.5 h of inhalation, depending on the anticipated deposited UFP dose, anesthesia of the animals was antagonized by a subcutaneous injection of a mixture of Atipamezol (0.075 mg/100 g body weight), Flumazenil (20 μg/100 g body weight), and Naloxon (10 μg/100 g body weight).

Retention and Clearance

UFP clearance. After exposure, rats were maintained in metabolic cages for the first 7 days, and afterward they were singly housed in individually ventilated cages (VentiRack, cage type CU-31). Excreta were collected quantitatively throughout the study; during wk 1 daily urine and feces samples were collected separately and stored frozen until radioanalysis; later on, urine and feces samples were collected together with the cage bedding for periods of 3 days and up to 7 days.

Terminal retention and distribution in organs. At time points of 3 wk and 2 and 6 mo after inhalation, rats were killed by exsanguination after an intraperitoneal anesthesia with ketamine (100 mg/100 g body weight) and xylazine (0.5 mg/100 g body weight). Then all organs, samples of skin, muscle, and bones, and the remaining carcass were taken separately and stored frozen until radioanalysis. A complete balance of ^{192}Ir activity retained in the body and cleared by excretion out of the body was quantified by gamma spectroscopy in a 1-L well-type scintillation detector (Kreyling et al., 1988, 1993). Hence, total activity represents the deposited activity.

Bronchoalveolar lavage. A bronchoalveolar lavage (BAL) was performed applying 6 times 5 ml phosphate-buffered saline without Ca^{2+} or Mg^{2+} under gentle massage of the thorax. The recovered BAL fluid (about 90% of instilled saline) was centrifuged at $500 \times g$, 20 min and 20°C, to divide the fluid into lavaged cells and supernatant. The total number of lavaged cells were acquired with a hemocytometer by a dilution of the spun-down cells and trypan-blue staining. Cytocentrifuged slide preparations of the lavaged cells were done for each sacrificed animal and stained with Diff-Quik.

Whole-body retention. Starting 3 days after exposure, whole-body retention was detected in a rodent whole-body gamma-spectroscopy counter for animals allocated to 2 and 6 mo of observation time. Intervals between measuring time points increased from 2 days to 2 wk with increasing retention time. Data were decay and background corrected and normalized to particle retention 3 days after inhalation.

Comparison With Micrometer-Sized Particles

Normalized to day 3 after ^{192}Ir UFP inhalation, measured whole-body retention was basically equal with lung retention as shown previously by Kreyling et al. (2002). Data from each animal were fitted with a function of two exponential terms. ^{192}Ir lung retention data were compared with two data sets for lung retention of micrometer-sized particles: (1) long-term lung retention data observed in Fischer 344 and Wistar rats after inhalation of ^{85}Sr-labeled polystyrene particles of about 3.5 μm size (Bellmann et al., 1994), and (2) lung retention data observed in Fischer 344 rats intratracheally instilled with 2.1-μm fluorescent, carboxylated, polystyrene microspheres (Lehnert et al., 1989). In addition, the kinetics involving long-term fractional excretion rates (FER) were determined for each animal by daily excreted fractions normalized to the actual lung retention, estimated by lung retention fit function as already described. For all examined animals these fractional rate data were also fitted with a function of two exponential terms and were compared with that of long-term fractional excretion rates obtained from studies using monodisperse micron-sized radio-labeled fused aluminosilicate particles (FAP) by Bailey et al. (1985) (1.2 μm) and calculated by Kreyling (1990) and using radio-labeled FAP of three different sizes: 0.5, 1.0, and 1.9 μm by Snipes et al. (1983).

RESULTS

Retention and Clearance

Deposition. Due to endotracheal intubation, there was neither extrathoracic particle deposition nor pelt contamination. Short-term retention/clearance data until 7 days after inhalation have been presented previously (Kreyling et al., 2002). For rats exposed to 15-nm particles and long-term studied, deposited ^{192}Ir doses at the reference time point are shown in Table 1. The specific activity of the particles was 10 GBq g^{-1} mass. Depending on the planned observation time, animals were loaded with different lung burden (time of inhalation 1–1.5 h), to get optimal samples with sufficient count rates in the 1-L well-type scintillation detector for every checked time point. Previously, we have shown that the ultrafine ^{192}Ir-labeled iridium particles were virtually insoluble; therefore, radioactivity measurement is proportional to the mass of the ultrafine iridium particles.

Lung retention and clearance. Even 6 h after inhalation, a mean fraction ($\pm$ standard deviation, SD) of 0.18 ± 0.05 of the deposited 15-nm particles was already cleared into the gastrointestinal tract. After 3 wk, 0.31 ± 0.05 of the deposited UFP is retained in the lungs; 2 and 6 mo after inhalation the retained fractions were 0.17 ± 0.03 and 0.07 ± 0.01, respectively. These lung data were the sum of measured lung and BAL fractions from each sacrificed animal (Figure 1).

At any time, ultrafine particles were cleared predominantly from the lungs via the gastrointestinal tract into feces, as shown by the cumulative daily excretion in Figure 1. After 6 mo, 92% of the deposited fraction was cleared out of the lung and excreted (Figure 1).

Bronchoalveolar lavage. A substantial part of the deposited iridium particles is likely to be lavaged from the peripheral lungs (Figure 1). Immediately after inhalation the mean lavagable particle fraction ($\pm$SD) was 0.46 ± 0.05 of the deposited material, but decreased with time to 0.05 ± 0.003 after 3 wk, 0.008 ± 0.002 after 2 mo, and 0.005 ± 0.002 after 6 mo (Figure 1).

TABLE 1
Deposited lung burden in kBq and the corresponding deposited Ir mass

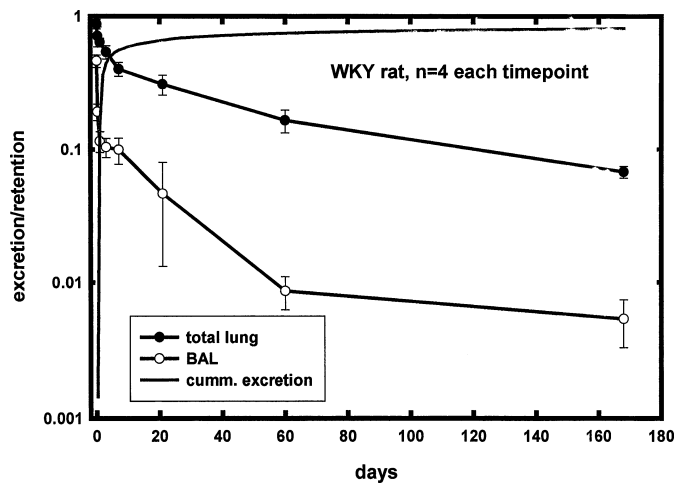| Parameter	Observation time		
	21 days	60 days	168 days
Deposited ^{192}Ir activity in the lungs (kBq), mean $\pm$ SD; $n = 4$	3.5 ± 0.6	58.7 ± 7.6	112.1 ± 17.1
Deposited ^{192}Ir mass in the lungs (μg), mean $\pm$ SD; $n = 4$	0.35 ± 0.06	5.88 ± 0.76	11.21 ± 1.71

FIG. 1. Retention and excretion during 6 mo after a single 1- to 1.5-h inhalation of 15-nm ^{192}Ir UFP, count median diameter. Cumulative excretion averaged over all rats as described in the Methods section. Note lung retention data are the sum of measured lung and BAL fraction from each sacrificed animal. At each time point, data obtained from four rats are averaged and error bars are standard deviation.

Long-Term Translocation to Secondary Target Organ

As previously described (Kreyling et al., 2002), there was a very small but detectable iridium UFP fraction translocated to secondary target organs like the liver, spleen, brain, and kidneys of about 0.002 each. For these secondary extrapulmonary organs, a peak was found at day 7 after inhalation. Long-term
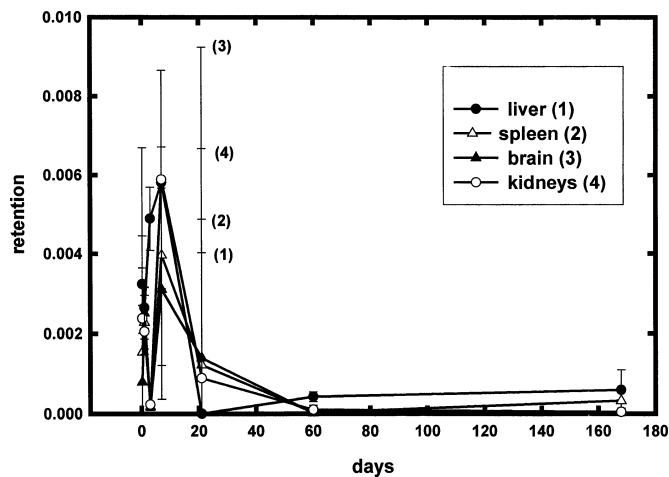

FIG. 2. Translocated fractions into secondary target organs during 6 mo after inhalation of 15-nm ^{192}Ir UFP. Large error bars resulted from low count rates obtained from studies performed several months after receipt of the radioactive iridium rods when the actual ^{192}Ir radioactivity had decayed 4–6 decay half-times. At each time point, data obtained from four rats are averaged and error bars are standard deviation.

retention data in this article show that no further accumulation occurred, but a net clearance from these target organs was found with time and decreased close to the level of detection limit (Figure 2). As shown in the previous investigation (Kreyling et al., 2002), the iridium UFP were virtually insoluble. To distinguish between the soluble and particulate fraction, we performed the same analysis as previously and confirmed that we always were able to detect a particulate fraction even though it decreased with time.

Lung Retention of UFP Versus Micrometer-Sized Particles

Whole-body retention of ^{192}Ir of the animals sacrificed 2 and 6 mo after inhalation was assessed using a rodent whole-body counter. The measured activity corrected for radioactive decay was set as iridium retention in the lungs, because it was known from earlier studies by Kreyling et al. (2002) that virtually all radioactivity in the body was retained as UFP in the lungs (Figure 3). In addition, organ and tissue analyses at each time point confirmed predominant lung retention.

In order to prove whether ultrafine particles follow different kinetics of lung retention, we compared our data with rat lung retention data obtained from insoluble ^{85}Sr-labeled micrometer-sized 3.5-μm polystyrene particles (^{85}Sr-PSL) published previously (Bellmann et al., 1994). In a series of several studies, these authors had found a range of long-term retention rates in lungs of Fischer 344 rats to be between 0.009 day^{-1} and 0.012 day^{-1}. Our measured lung retention data of the four rats living during the whole observation period were fitted and the
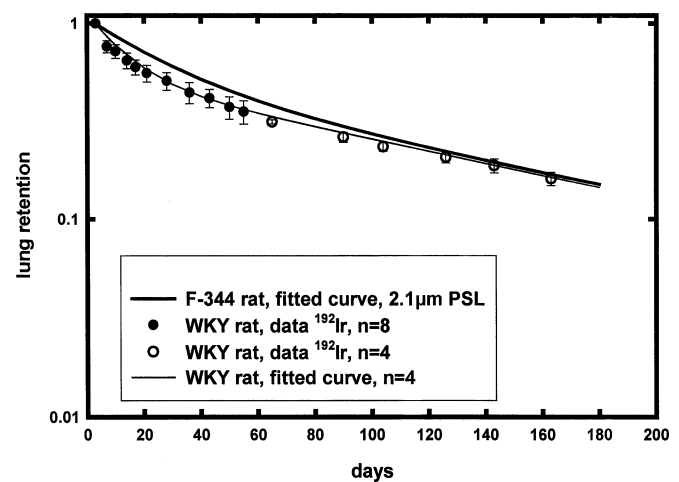

FIG. 3. Lung retention data of inhaled 15-nm ^{192}Ir UFP and the fitted curve of this ^{192}Ir UFP data from the 4 rats studied during the whole observation period are compared with the fitted curve of lung retention of instilled fluorescent, carboxylated polystyrene microspheres (2.1 μm) according to Lehnert et al. (1989) data. Data are averages obtained from all rats analyzed at this time point, and error bars are standard deviation. Measured data and fitted curves were normalized to the retained particle burden at day 3 after inhalation.

average long-term lung retention rate (±SD) for ^{192}Ir UFP was estimated to be 0.007 ± 0.0005 day⁻¹, representing a half-life of 98 ± 6.7 days; short-term lung retention 3 days after inhalation was determined to be 0.07 ± 0.02 day⁻¹, for a half-life of 9.3 ± 2.4 days. Therefore, analysis of the kinetics of long-term iridium UFP retention in the WKY rat lungs beginning with day 40 revealed no difference to that of micrometer-sized particles. Since no short-term retention kinetics was reported for the micrometer-sized [85]Sr-PSL, no comparison was performed for the initial retention time. Since one cannot tell from the literature at which initial retention day the long-term rates were determined for the micrometer-sized particles, we did not compare short-term clearance rates (i.e., clearance for about the first 40 days), but the measured data were in the same range as for micrometer-sized 3.5-μm particles. Lehnert et al. (1989) determined short-term (days 7–57 after instillation) and long-term (days 57–176 after instillation) lung retention after intratracheal instillation of fluorescent, carboxylated polystyrene microspheres (2.1 μm) and reported a lung retention function LR(Ir UFP) of:

$$LR(\text{Ir UFP}) = 0.52 \exp(0.037t) + 0.48 \exp(0.0068t) \quad [1]$$

This function also agrees very well with the lung retention of ultrafine iridium particles (Figure 3).

Daily Excretion Relative to Contemporary Lung Retention of UFP and Micrometer-Sized Particles

Since daily excretion provides differential data on particle clearance from the lungs, we also compared our data to those obtained from micrometer-sized particles. The long-term kinetics of fractional excretion rates (FER) followed a single exponential function: $0.017 \exp(-0.009t)$. Similar functions but with a mid-term and a long-term component were determined by Bailey et al. (1985) studying 1.2-μm radiolabeled fused aluminosilicate particles ([57]Co-FAP) in HMT rats:

$$FER(^{57}\text{Co-FAP}) = 0.018 \exp(-0.019t) \quad [2]$$
$$+ [0.0045 \exp(-0.0039t)]$$

and by Snipes et al. (1983) studying 0.5-, 1.0-, and 1.9-μm [134]Cs-FAP in F-344 rats:

$$FER(^{134}\text{Cs-FAP}) = 0.02 \exp(-0.007t) \quad [3]$$
$$+ [0.001 \exp(-0.0001t)]$$

At 20 days postinhalation the kinetics of fractional excretion rates of insoluble ^{192}Ir UFP indicated no differences to the FER functions obtained from micrometer-sized particles (Figure 4).

DISCUSSION

Ultrafine aerosol production using the spark generator with neutron-activated pure iridium rods and continuous monitoring of the size distribution and number concentration proved to
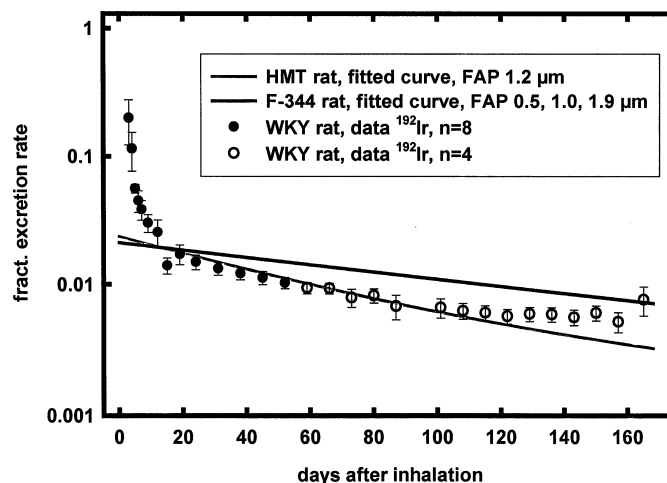

FIG. 4. Daily fractional excretion rates of insoluble ^{192}Ir UFP beginning 3 days after inhalation compared with fitted curves of those obtained from different micrometer-sized fused aluminosilicate particles (FAP) according to Bailey et al. (1985) and Snipes et al. (1983). Data are averages obtained from all rats analyzed at this time point, and error bars are standard deviation.

be a reliable technology for rat exposures. The ^{192}Ir particles appeared to be suitable for these studies because of their very low in vitro solubility and their simple biokinetics shown in the prior investigation (Kreyling et al., 2002). Note, however, that iridium particles are of low relevance for ambient air particles. Instead, they may be considered a rather precise tool to study the biokinetics of basic particle-associated clearance pathways of ultrafine particles. Administering the aerosol via an endotracheal tube allowed thoracic particle deposition and quantitatively balanced clearance measurements starting immediately after inhalation, since no particles deposited in extrathoracic airways or contaminated the pelt.

Ultrafine iridium particles of 15 nm size were retained mainly in the lungs. At any time, UFP were cleared predominantly from the peripheral lung via the mucociliary escalator into the GIT and were found in feces. Lavageable particle fractions throughout the whole study of retention confirmed the predominance of this clearance pathway.

Referring to the earlier investigation of Kreyling et al. (2002), there was a trend of time-dependent increase of ultrafine particle burden in secondary target organs. These investigators discussed various pathways and concluded that the only plausible remaining pathway is particle transport into the pulmonary vasculature and systemic circulation at a very marginal fraction by eventual binding to proteins to penetrate the air-blood barrier in the lung. However, the current investigation showed no further accumulation but rather clearance out of the secondary target organs after 1 wk (Figure 2). Since we cannot exclude continuous long-term translocation from the lungs to extrapulmonary organs, the observed kinetics may indicate an overall

more effective clearance from those secondary target organs than long-term translocation toward them. Taking the previously proven negligible solubility into account, this means that there is further particle transport out of these organs that may have resulted in further redistribution within the body. Besides particle clearance from the liver via biliary secretion into the small intestine with fecal excretion, ultrafine particle clearance via the kidneys and bladder into urinary excretion cannot be excluded. Such a clearance pathway had been shown earlier by Cooper et al. (1979) and Stradling et al. (1980) using 1- to 4-nm actinide oxide particles.

Particle uptake and potential health effects may also depend on genetic susceptibility and the acquired health status of the exposed organism, as well as on particle properties like physical structure and chemical composition of the surface and the matrix of the particle.

In contrast to these findings using ultrafine iridium particles, studies by Oberdörster et al. (2002) using ultrafine 25-nm ^{13}C carbonaceous particles suggest a major translocation pathway from the respiratory tract to the liver within the first 24 h.

The two hypotheses formulated by Kreyling et al. (2002) still endure:

- Aggregated ^{13}C particles may disaggregate much more completely and rapidly on the lung epithelium than ^{192}Ir particles such that very small units of <5 nm translocate into systemic circulation and accumulate in extrapulmonary organs like the liver.
- Some ultrafine particles or their disaggregated subunits may bind to high-molecular-weight proteins, depending on their surface properties. These proteins covering the UFP then may determine the subsequent metabolic fate of the complexes. For instance, ^{192}Ir particles or their disaggregates form complexes with proteins on the lung epithelium other than carbonaceous particles, such that ^{192}Ir particle–protein complexes are retained in the lungs while ^{13}C particle–protein complexes translocate systemically and accumulate specifically in extrapulmonary organs like the liver.

In order to determine long-term lung retention without contributions from fast lung clearance and significant ^{192}Ir-UFP burdens in the GIT, we started whole-body retention measurements 3 days after inhalation and normalized the measured data to this time point. Cessation of fast clearance was confirmed by the daily separately determined urinary and fecal excretion measurements during the first 7 days after inhalation.

As mentioned in the introduction in contrast to micrometer-sized particles, there is evidence that UFP are not predominantly retained on the epithelium but penetrate into the interstitium. Therefore, it is indeed surprising that lung retention and differential clearance kinetics evaluated by excretional rates did not significantly differ between Ir-UFP and various micrometer-sized particles. The similarity of clearance kinetics of ultrafine and micrometer-sized particles suggests that UFP clearance pathways from the lung periphery to the larynx is more complex than simple UFP transport on the lung epithelium toward the distal end of the mucociliary escalator and mucociliary action up the airways to the larynx. This clearly requires further investigation.

CONCLUSION

Long-term translocation of ultrafine ^{192}Ir particles from lungs to secondary target organs does not steadily increase the particle burden in those organs but is superimposed by clearance from these organs, yielding a constant but very slow fraction of about 0.001 of the deposited amount of particles. Long-term clearance of ultrafine ^{192}Ir particles from the lungs is dominated by macrophage-mediated transport toward the larynx followed by passage through the GI tract and subsequent fecal excretion. The kinetics of lung retention and fecal excretion are not different between ultrafine ^{192}Ir particles and micrometer-sized particles.

REFERENCES

Bailey, M. R., Hodgson, A., and Smith, H. 1985. Respiratory retention of relatively insoluble particles in rodents. *J. Aerosol Sci.* 16:279–293.

Bellmann, B., Creutzenberg, O., Ale, H., and Mermelstein, R. 1994. Models for deposition, retention clearance of particles after dust overloading of lungs in rats. *Ann. Occup. Hyg.* 38(suppl. 1):303–311.

Brown, J. S., Zeman, K. L., and Bennett, W. D. 2002. Ultrafine particle deposition and clearance in the healthy and obstructed lung. *Am. J. Respir. Crit. Care Med.* 166(9):1240–1247.

Cooper, J. R., Stradling, G. N., Smith, H., and Breadmore, S. E. 1979. The reactions of 1.0 nanometre diameter plutonium-238 dioxide particles with rat lung fluid. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 36(5):453–466.

Donaldson, K., Stone, V., Clouter, A., Renwick, L., and MacNee, W. 2001. Ultrafine particles. *Occup. Environ. Med.* 58(3):211–216, 199.

Ferin, J., Oberdorster, G., and Penney, D. P. 1992. Pulmonary retention of ultrafine and fine particles in rats. *Am. J. Respir. Cell Mol. Biol.* 6(5):535–542.

Ibald-Mulli, A., Wichmann, H. E., Kreyling, W., and Peters, A. 2002. Epidemiological evidence on health effects of ultrafine particles. *J. Aerosol Med.* 15(2):189–201.

Kreyling, W. G. 1990. Interspecies comparison of lung clearance of "insoluble" particles. *J. Aerosol Med.* 3:S93–S110.

Kreyling, W. G., and Scheuch, G. 2000. Clearance of particles deposited in the lungs. In *Particle–lung interactions*, eds. P. Gehr and J. Heyder, pp. New York: Marcel Dekker, pp. 323–375.

Kreyling, W. G., Schumann, G., Ortmaier, A., Ferron, G. A., and Karg, E. 1988. Particle transport from the lower respiratory tract. *J. Aerosol Med.* 1(4):351–369.

Kreyling, W. G., Cox, C., Ferron, G. A., and Oberdorster, G. 1993. Lung clearance in Long-Evans rats after inhalation of porous, monodisperse cobalt oxide particles. *Exp. Lung Res.* 19(4):445–467.

Kreyling, W. G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., Oberdorster, G., and Ziesenis, A. 2002. Translocation of ultrafine insoluble iridium particles from lung epithelium to

extrapulmonary organs is size dependent but very low. *J. Toxicol. Environ. Health A.* 65(20):1513–1530.

Lehnert, B. E., Valdez, Y. E., and Tietjen, G. L. 1989. Alveolar macrophage-particle relationships during lung clearance. *Am. J. Respir. Cell Mol. Biol.* 1(2):145–154.

Nemmar, A., Vanbilloen, H., Hoylaerts, M. F., Hoet, P. H., Verbruggen, A., and Nemery, B. 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am. J. Respir. Crit. Care Med.* 164(9):1665–1668.

Oberdorster, G., Ferin, J., and Lehnert, B. E. 1994. Correlation between particle size, in vivo particle persistence, and lung injury. *Environ. Health Perspect.* 102(suppl. 5):173–179.

Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., and Cox, C. 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J. Toxicol. Environ. Health A* 65(20):1531–1543.

Osier, M., and Oberdoerster, G. 1997. Intratracheal inhalation vs intratracheal instillation: Differences in particle effects. *Fundam. Appl. Toxicol.* 40(2):220–227.

Snipes, M. B., Boecker, B. B., and McClellan, R. O. 1983. Retention of monodisperse or polydisperse aluminosilicate particles inhaled by dogs, rats, and mice. *Toxicol. Appl. Pharmacol.* 69(3):345–362.

Stradling, G. N., Smith, H., and Cooper, J. R. 1980. *Factors affecting the mobility of actinide oxides and their influence on radiological protection.* In Pulmonary toxicology of respirable particles. 19th Annual Hanford Life Sciences Symposium, eds. C. L. Sanders, F. T. Cross, G. E. Dagle, and J. A. Mahaffey, pp. 209–223. Washington, DC: Technical Information Center US DOE CONF-791002.

Takenaka, S., Karg, E., Kreyling, W., Lentner, B., Schulz, H., Ziesenis, A., Becke, H., Heinzmann, U., Schramel, P., and Heyder, J. 2003. Distribution patterns of inhaled ultrafine silver particles in the rat lung. AAAR PM Meeting Particulate Matter 2003: Atmospheric Sciences, Exposure and the Fourth Colloquium on PM and Human Health. Pittsburgh, PA: American Association for Aerosol Research http://www.abstracts2view.com/aaarpm/P06–09.

U.S. Environmental Protection Agency 1996. In air quality criteria for particulate matter, Chapter 12, Epidemiology studies of health associated with exposure to airborne particles/acid particles. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/P-95/001bF.

Wichmann, H.-E., and Peters, A. 2000. Epidemiological evidence of the effects of ultrafine particle exposure. *Philos. Trans. R. Soc. Lond. A* 358:2751–2769.

Wichmann, H.-E., Spix, C., Tuch, T., Wölke, G., Peters, A., Heinrich, J., Kreyling, W. G., and Heyder, J. 2000. *Daily mortality and fine and ultrafine particles in Erfurt, Germany, part I: Role of particle number and particle mass.* 98, 1–95. Health Effects Institute, Boston, MA.