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### TRANSLOCATION OF ULTRAFINE INSOLUBLE IRIDIUM PARTICLES FROM LUNG EPITHELIUM TO EXTRAPULMONARY ORGANS IS SIZE DEPENDENT BUT VERY LOW

W. G. Kreyling<sup>a</sup>, M. Semmler<sup>a</sup>, F. Erbe<sup>a</sup>,  
P. Mayer<sup>a</sup>, S. Takenaka<sup>a</sup>, H. Schulz<sup>a</sup>, G.  
Oberdörster<sup>b</sup> & A. Ziesenis<sup>a</sup>

<sup>a</sup> GSF-National Research Center for Environment  
and Health, Institute for Inhalation Biology,  
Neuherberg/Munich, Germany

<sup>b</sup> University of Rochester, Medical Center,  
Rochester, New York, USA

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## TRANSLOCATION OF ULTRAFINE INSOLUBLE IRIIDIUM PARTICLES FROM LUNG EPITHELIUM TO EXTRAPULMONARY ORGANS IS SIZE DEPENDENT BUT VERY LOW

W. G. Kreyling, M. Semmler, F. Erbe, P. Mayer, S. Takenaka, H. Schulz

GSF-National Research Center for Environment and Health, Institute for Inhalation Biology, Neuherberg/Munich, Germany

G. Oberdörster

University of Rochester, Medical Center, Rochester, New York, USA

A. Ziesenis

GSF-National Research Center for Environment and Health, Institute for Inhalation Biology, Neuherberg/Munich, Germany

*Recently it was speculated that ultrafine particles may translocate from deposition sites in the lungs to systemic circulation. This could lead to accumulation and potentially adverse reactions in critical organs such as liver, heart, and even brain, consistent with the hypothesis that ultrafine insoluble particles may play a role in the onset of cardiovascular diseases, as growing evidence from epidemiological studies suggests. Ultrafine <sup>192</sup>Ir radio-labeled iridium particles (15 and 80 nm count median diameter) generated by spark discharging were inhaled by young adult, healthy, male WKY rats ventilated for 1 h via an endotracheal tube. After exposure, excreta were collected quantitatively. At time points ranging from 6 h to 7 d, rats were sacrificed, and a complete balance of <sup>192</sup>Ir activity retained in the body and cleared by excretion was determined gamma spectroscopically. Thoracic deposition fractions of inhaled 15- and 80-nm <sup>192</sup>Ir particles were 0.49 and 0.28, respectively. Both batches of ultrafine iridium particles proved to be insoluble (<1% in 7 d). During wk 1 after inhalation particles were predominantly cleared via airways into the gastrointestinal tract and feces. This cleared fraction includes particles deposited in the alveolar region. Additionally, minute particle translocation of <1% of the deposited particles into secondary organs such as liver, spleen, heart, and brain was measured after systemic uptake from the lungs. The translocated fraction of the 80-nm particles was about an order of magnitude less than that of 15-nm particles. In additional studies, the biokinetics of ultrafine particles and soluble <sup>192</sup>Ir was studied after administration by either gavage or intratracheal instillation or intravenous injection. They confirmed the low solubility of the particles and proved that (1) particles were neither dissolved nor absorbed from the gut, (2) systemically circulating particles were rapidly and quantitatively accumulated in the liver and spleen and retained there, and (3) soluble <sup>192</sup>Ir instilled in the lungs was rapidly excreted via urine with little retention in the lungs and*

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Address correspondence to Dr. Wolfgang G. Kreyling, GSF-National Research Center for Environment and Health, Institute for Inhalation Biology, PO Box 1129, D-85758 Neuherberg/Munich, Germany. E-mail: Kreyling@gsf.de

*other organs. This study indicates that only a rather small fraction of ultrafine iridium particles has access from peripheral lungs to systemic circulation and extrapulmonary organs. Therefore, the hypothesis that systemic access of ultrafine insoluble particles may generally induce adverse reactions in the cardiovascular system and liver leading to the onset of cardiovascular diseases needs additional detailed and differentiated consideration.*

Recent epidemiological studies provide evidence that an increase in ultrafine particles (UP) is associated with adverse cardiovascular effects (Wichmann et al., 2000; Wichmann & Peters, 2000). These findings pertain not only to elderly people but also to susceptible persons with underlying diseases of various origins (U.S. EPA, 1996). Animal studies indicate enhanced access of UP in the lung interstitium compared to larger particles (Ferin et al., 1991, 1992), and even significant accumulation and retention in liver and heart (Stone & Godleski, 1999). Translocation pathways of UP are different from those of larger particles (>100 nm diameter); for example, lung retention and clearance of UP are less alveolar macrophage-mediated than are those of larger particles (Kreyling & Scheuch, 2000). UP interstitialization gives rise to prolonged particle contact and reactivity of interstitial cells—which usually encounter increased numbers of particles only under overload conditions—and the release of proinflammatory mediators (Donaldson et al., 2001). Furthermore, increased systemic translocation of UP may result in the initiation of adverse alterations of the cardiovascular system (Stone & Godleski, 1999; U.S. EPA, 1996; Oberdörster et al., 2002). Although alveolar macrophages do not phagocytize a prominent fraction of UP—as they do in the case of particles larger than 100 nm—they are still involved in phagocytic and pinocytic processes of UP 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). This applies also for UP. Since in vivo dissolution of UP superimposes upon and may even dominate particle clearance from the lungs and subsequent uptake in other organs of the body, either UP must be insoluble or the dissolved compounds of UP must be excreted completely without any accumulation in other organs. Considering the large specific surface area (SSA) of UP and the fact that particle dissolution is SSA dependent, great emphasis is needed to ensure the already mentioned requirements of UP. Such studies of use of UP so far stressed the importance of the insolubility of the test particles but did not satisfactorily quantify this parameter. Therefore, some open questions remain on the kinetics of UP lung retention, clearance pathways out of the body, and systemic uptake and translocation pathways toward secondary organs (Finch et al., 1999; Oberdörster et al., 2000; Takenaka et al., 2001; Nemmar et al., 2002). The aim of this investigation was to assess translocation of inhaled ultrafine insoluble particles from lungs to extrapulmonary organs. Therefore, we searched for insoluble UP and then chose to use radio-

labeled iridium particles, although we are aware that iridium is not an important contaminant of the ambient aerosol. However, we like to emphasize that not only the chemical composition of the particle surface but also its physical structure are prominent parameters of the interface dominating the interaction between the particle and biological fluids, cells, and tissues. These studies need to be complemented by succeeding studies using other insoluble UP with more relevant chemical composition and physical structure of the particle surface.

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  $^{192}\text{Ir}$  were inhaled by rats via intratracheal intubation. To further test the postulated inverse particle size dependence of translocation, studies were performed using two ultrafine particle sizes.

## METHODS

### Animals

Young, adult, healthy, male WKY/NCrl BR rats (Charles River, Sulzfeld, Germany; body weight  $170 \pm 10$  g) were housed in a humidity- (55% relative humidity) and temperature- ( $22^\circ\text{C}$ ) controlled room. They were maintained on a 12-h day/night cycle. Rats were allowed to acclimate to the facility for a minimum of 10 d prior to use. 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 No. 211-2531-108/99) and by the GSF Institutional Animal Care and Use Committee.

### Aerosol Production and Characterization

Aerosols of ultrafine iridium particles radiolabeled with  $^{192}\text{Ir}$  were generated with a spark generator (GFG 1000, Palas). The radio isotope  $^{192}\text{Ir}$  is a beta and gamma emitter with a half-life of 74 d and gamma energies of 296, 308, 316, 468, and 588 keV (26, 29, 73, 47, and 5% efficiency, respectively); there are also some higher and some lower energies of low efficiency; gamma spectroscopy was performed using the photo peaks of 296, 308, and 316 keV. Sparks of constant energy were ignited between neutron-activated pure iridium electrodes at a frequency of either 3 or 15 Hz in an argon stream of 3.5 L/min. At the exit of the spark generator, the aerosol was quasi-neutralized by a radioactive  $^{85}\text{Kr}$  source (40 MBq). The aerosol was diluted with nitrogen and—for rat exposures—with oxygen adjusted to obtain 20% oxygen and was air conditioned at 50–60% relative humidity and  $37^\circ\text{C}$  for rat exposure. Size distribution and number concentration were monitored continuously by a differential mobility particle sizer (DMPS; classifier model 3070 and CPC model 7610, TSI) and a condensation par-

ticle counter (CPC3022A, TSI), respectively. The lower particle size detection limit of the former was 10 nm.  $^{192}\text{Ir}$  radioactivity of the aerosol was determined by continuous aerosol sampling at a flow rate of 0.2 L/min, volume measurement, and gamma counting in a well-type scintillation detector. Aerosol mass concentration was derived from the given specific activity of the electrodes.

### **In Vitro Dissolution Tests**

In a first attempt in vitro tests on the solubility of ultrafine  $^{192}\text{Ir}$  particles were performed. Ultrafine iridium particles were collected on a 0.2- $\mu\text{m}$  pore size filter, sandwiched and tightly mounted in between 0.02- $\mu\text{m}$  pore size Teflon filters (50 mm diameter). The filter sandwich was placed in a 1-L volume of either (1) saline buffered at pH 7.2 by either hydrochloric acid or sodium hydroxide, or (2) 1 N hydrochloric acid continuously stirred. To determine the in vitro dissolution kinetics, the  $^{192}\text{Ir}$  activity retained in the filter sandwich was measured (Kreyling & Ferron, 1984).

### **Particle Collection by Liquid Impingement**

To obtain suspensions containing ultrafine  $^{192}\text{Ir}$  particles, the freshly generated aerosol was bubbled through a fine frit at a flow rate of 2 L/min into a washing flask containing 50 ml saline. A backup filter downstream from the flask was used to determine the efficiency of the impingement in the suspension. The usual sampling time was 20 min. The freshly produced suspension was ultimately ultrasonicated (3 min) immediately prior to use for auxiliary in vivo studies.

### **Inhalation**

For inhalation, rats were ventilated for 1 h via an endotracheal tube. The animals were anesthetized by intramuscular injection of a mixture of medetomidine (15  $\mu\text{g}/100$  g body weight), midazolam (0.2 mg/100 g body weight), and fentanyl (0.5  $\mu\text{g}/100$   $\mu\text{g}$  body weight). For intubation a flexible cannula (16G, 2 in) was placed in the upper trachea under direct visualization. The endotracheal tube was sealed against outside air with a modified pipette tip wedged gently into the laryngeal opening (Kreyling et al., 1993; Osier & Oberdörster, 1997). Rats were placed into an airtight plethysmograph box, with the endotracheal tube connected to the aerosol line outside the box. Inhalation and exhalation was maintained by computer controlled switching of valves either applying  $-1$  kPa low pressure or ambient pressure to the box, respectively. Inhalation interval was set to be 1 s, while spontaneous exhalation to functional residual capacity was allowed for 0.5 s. This ventilation pattern with an inhaled volume of 75% of total lung capacity caused slight hyperventilation such that rats did not breath spontaneously but followed the computer-controlled breathing pattern. For radiation protection the entire aerosol system was maintained at  $-30$  Pa low pressure, and the entire apparatus is built in a containment like a glove box. This containment is

highly ventilated through an absolute particle filter, which was periodically controlled for radioactive contamination.

After exposure the anesthesia of the rats was antagonized by subcutaneous injection of a mixture of atipamezole (0.0075 mg/100 g body weight), flumazenil (20  $\mu$ g/100 g body weight), and naloxone (12  $\mu$ g/100 g body weight). Thereafter the rats were maintained in metabolic cages and excreta were collected separately and quantitatively. At time points ranging from 6 h to 7 d, rats were sacrificed by exsanguination after intraperitoneal anesthesia with ketamine (100 mg/100 g body weight) and xylazine (0.5 mg/100 g body weight). All organs, samples of skin, muscle, bone, the remaining carcass, and a blood sample of about 1 ml (obtained by heart puncture) were taken. Prior to obduction, a bronchoalveolar lavage (BAL) was performed applying 6 times 5 ml phosphate-buffered saline under gentle massage of the thorax.  $^{192}\text{Ir}$  activity of lavaged cells and supernatant and total and differential cell counts were determined. A complete balance of  $^{192}\text{Ir}$  activity retained in the body and cleared by excretion was measured gamma spectroscopically in a 1-L well-type scintillation detector (Kreyling et al., 1988, 1993).

### Auxiliary Studies

In additional studies the biokinetics of ultrafine  $^{192}\text{Ir}$  particles and soluble  $^{192}\text{IrCl}_3$  was studied after administration by either lung instillation or gavage or intravenous injection. In all of the subsequent biokinetic studies, rats were maintained in metabolic cages. Complete fecal and urinary excretion was collected separately. At given time intervals rats were sacrificed, dissected, and a complete balance of  $^{192}\text{Ir}$  activity in all organs, tissues, blood samples, and excreta was measured gamma spectroscopically, as already described.

*Biokinetics of dissolved  $^{192}\text{Ir}^{3+}$  administered to the rat lungs* A solution of soluble  $^{192}\text{IrCl}_3$  (10 kBq, 25  $\mu$ g, in 20  $\mu$ l 0.1 N hydrochloric acid) was diluted and buffered in 0.2 ml phosphate-buffered saline. This volume was instilled into the lungs of 18 rats by tracheal intubation under visual control. Prior to instillation, rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed.

*Biokinetics after particle gavage* Phosphate-buffered saline suspensions of ultrafine  $^{192}\text{Ir}$  particles (5 kBq, 0.2 ml) were administered into the esophagus of 8 rats. Rats were anesthetized with isoflurane as already described. At 1 d and 6 d after administration, 4 rats each were sacrificed and analyzed.

*Particle distribution after systemic application* Phosphate-buffered saline suspensions of ultrafine  $^{192}\text{Ir}$  particles (2.5 kBq, 0.1 ml) were injected into the jugular vein of 8 rats. For this purpose a small incision was made just above the right clavicle and the jugular vein was exposed. Anesthesia was performed by use of the mixture of medetomidine, midazolam, and fentanyl as already described. At 1 d and 5 d after administration 4 rats each were sacrificed and analyzed.

*Biokinetics after intratracheal instillation of a particle suspension* Phosphate-buffered saline suspensions of ultrafine  $^{192}\text{Ir}$  particles (5 kBq, 0.2 ml) were intratracheally instilled into 8 rats under direct visualization and isoflurane anesthesia. The particle suspension was ultrasonicated for 3 min immediately prior to use. At 1, 3, and 7 d after administration 2 rats each were sacrificed and analyzed.

## RESULTS

### Aerosol Characterization

Two different sizes of ultrafine  $^{192}\text{Ir}$  aerosols—15 nm and 80 nm count median diameter (CMD)—were produced with the spark generator. Particle size distributions are shown in Figure 1 and particle parameters are listed in Table 1. Because of the lower detection limit of the DMPS, particles smaller than 10 nm could not be analyzed. Therefore, the left side of the distribution in the left panel of Figure 1 is cut at 10 nm. Additional studies with a CMD of 20 and 25 nm confirmed a lognormal distribution as shown for the 80 nm particles in the right panel of Figure 1. The transmission electron micrograph clearly shows nonisometric aggregated particles of a CMD of 15 nm with substructures below 5 nm (see Figure 2).

### Suspensions of Ultrafine $^{192}\text{Ir}$ Particles

Phosphate-buffered saline suspensions of 25-nm  $^{192}\text{Ir}$  particles showed an  $^{192}\text{Ir}$  concentration of  $2.5 \mu\text{g}/\text{cm}^3$  with  $25 \text{ kBq}/\text{cm}^3$ , corresponding to a particle number concentration of about  $5 \times 10^9 \text{ cm}^{-3}$ . Sampling efficiency during impingement was about 50%.

### In Vitro Dissolution Studies

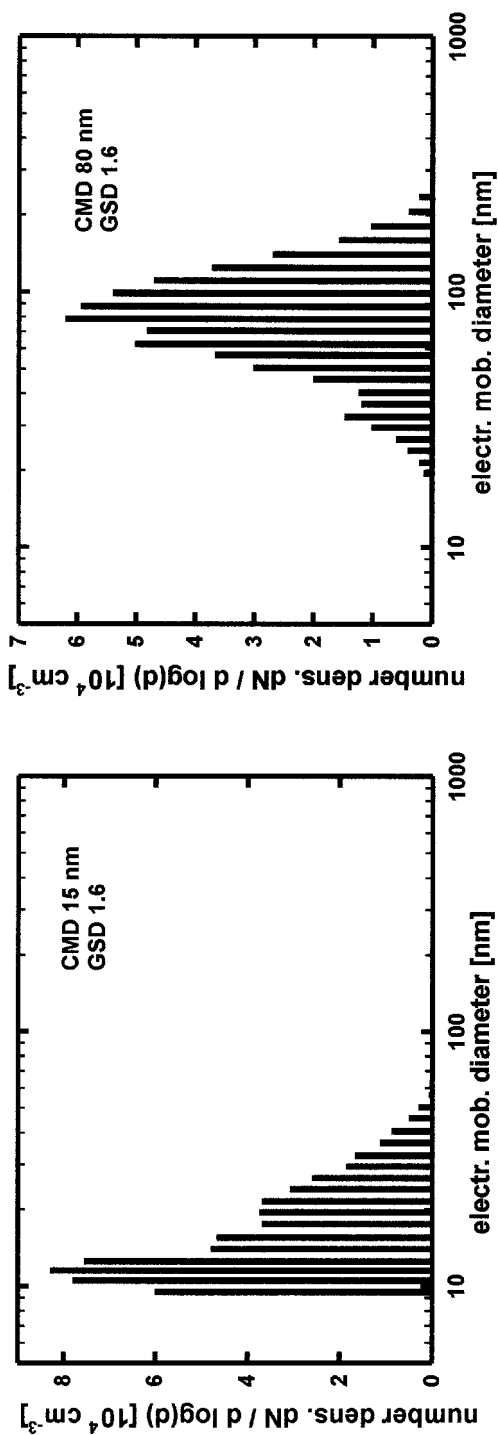
Ultrafine  $^{192}\text{Ir}$  particles showed a very low solubility in buffered saline at pH 7.2 and in 1 N hydrochloric acid. After 7 d, less than 1% of the particles were dissolved in buffered saline, while in 1 N hydrochloric acid the dissolved fraction was 6% after 24 h.

### Inhalation Studies

*Deposition* Due to endotracheal intubation, there was neither extra-thoracic particle deposition nor pelt contamination. Rats exposed to 15- and 80-nm particles deposited  $^{192}\text{Ir}$  doses of  $6.1 \pm 2.5$  and  $90 \pm 57$  kBq, corresponding to  $0.6 \pm 0.3$  and  $9.0 \pm 6.0$   $\mu\text{g}$  iridium mass in the lungs, respectively. Deposited fraction ( $\pm$  standard deviation) of inhaled 15- and 80-nm  $^{192}\text{Ir}$  particles was  $0.49 \pm 0.12$  and  $0.28 \pm 0.10$ , respectively. The fast-cleared fraction of inhaled particles eliminated from the lungs within 24 h was  $0.15 \pm 0.03$  and  $0.03 \pm 0.01$ , respectively.

*Lung Retention* Even at the earliest time point of sacrifice, 6 h after inhalation, a fraction of  $0.18 \pm 0.05$  and  $0.10 \pm 0.02$  of the deposited 15- and 80-nm particles, respectively, was already cleared into the gastrointesti-





**FIGURE 1.** Particle size distributions of 15 nm (left) and 80 nm CMD (right) <sup>192</sup>Ir aerosols measured by the differential mobility particle analyzer (DMPS) during rat exposures. Lower particle size limit of the DMPS is 10 nm. Because of the lower detection limit of the DMPS, the left side of the small-particle distribution in the left panel of Figure 1 could not be analyzed.

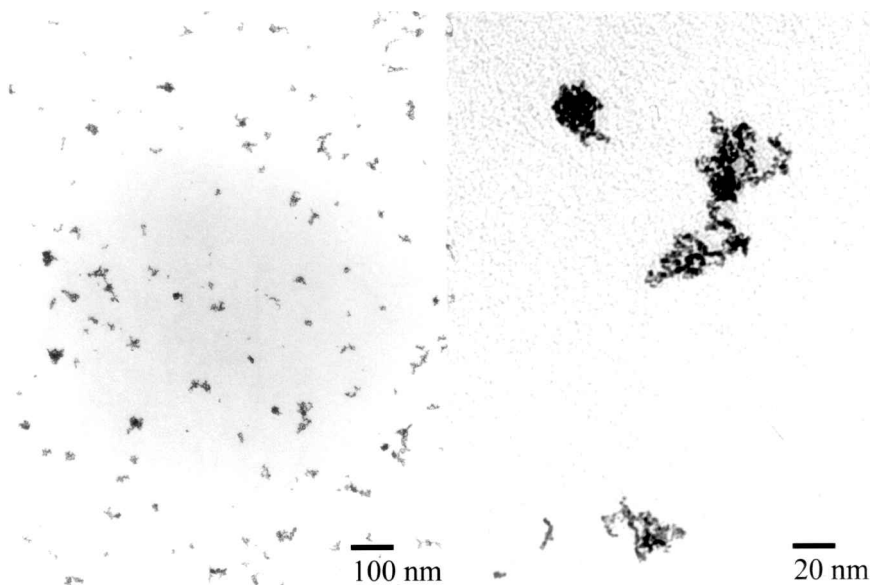
**TABLE 1.** Parameters of the Two Batches of Ultrafine  $^{192}\text{Ir}$  Aerosols Used for Rat Exposures

	Small UP	Large UP
Generator, spark frequencies (Hz)	3	15
Count median diameter (CMD) (nm)	15	80
Geometric standard deviation (GSD)	1.6	1.6
Number concentration ( $\text{cm}^{-3}$ )	$3 \times 10^7$	$0.5 \times 10^7$
Mass concentration ( $\text{mg m}^{-3}$ )	0.2	6.0
Specific $^{192}\text{Ir}$ activity ( $10 \text{ GBq g}^{-1}$ iridium) <sup>a</sup>	10	10

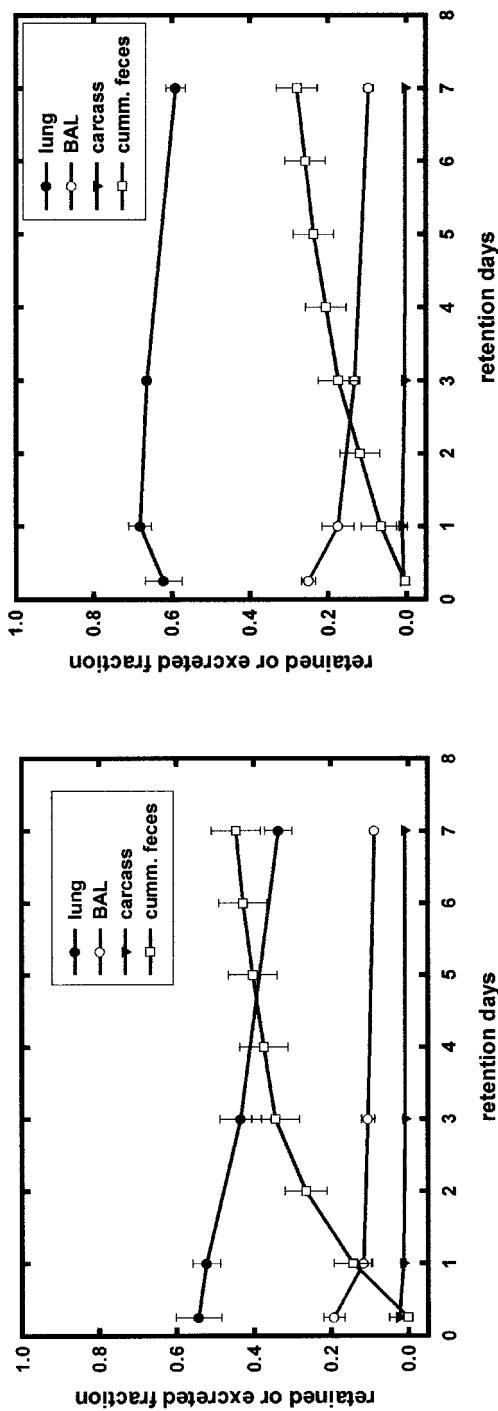
<sup>a</sup>At 1 July 2000.

nal (GI) tract. Note that this holds for the lavaged lungs. A substantial part of the total  $^{192}\text{Ir}$  content in the BAL of  $0.16 \pm 0.06$  and  $0.25 \pm 0.02$  of the deposited 15- and 80-nm particles, respectively, is likely to be lavaged from the peripheral lungs (see Figure 3). Thereafter, lung retention decreased very slowly for both ultrafine particle sizes (see Figure 3). At each time point of sacrifice a low but significant fraction of retained particles was obtained in the BAL for both particles (see Figure 3). Even 6 h after inhalation more than half of  $^{192}\text{Ir}$  particles in BAL was found in the cell pellet, and at time points 3 days and later  $^{192}\text{Ir}$  particle fractions were larger than 0.9 in the cell pellet.

*Clearance from the lungs* Both ultrafine particles were cleared predominantly from the lungs via the GI tract into feces as shown by the cumulative daily excretion in Figure 3. Seven days after inhalation, a fraction of



**FIGURE 2.** Transmission electron micrograph of 15-nm  $^{192}\text{Ir}$  particles. Note the nonisometric structure of the aggregated particles with substructures smaller than 5 nm.



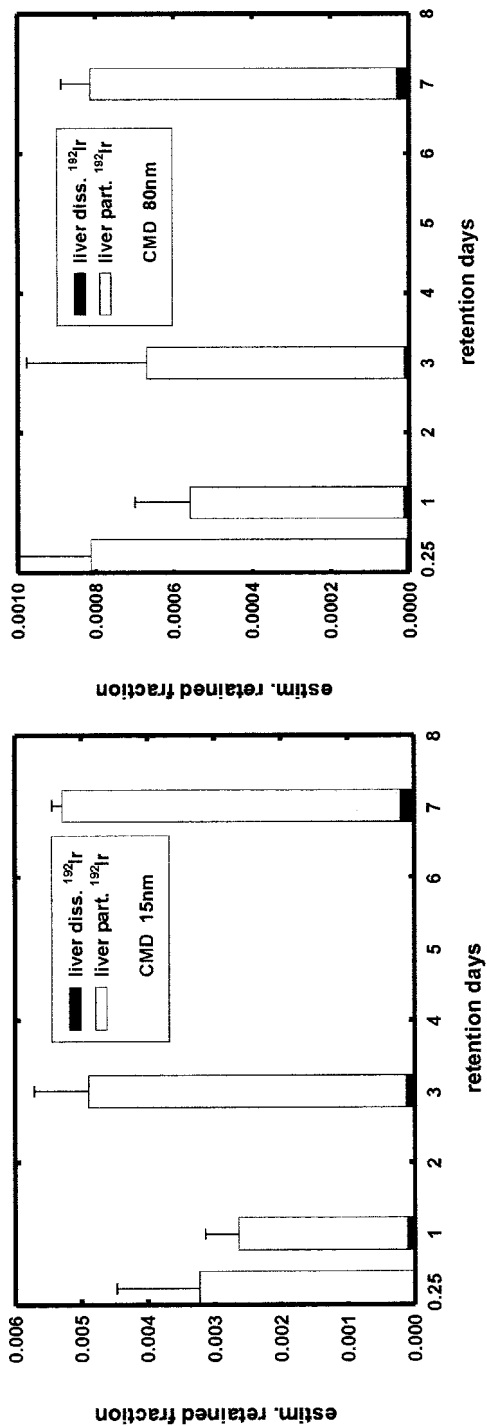
**FIGURE 3.** Iridium retention in lavaged lungs, bronchoalveolar lavage, GI tract, and remaining carcass after organ dissection during 7 d after inhalation of 15-nm (left) and 80-nm (right) count median diameter (CMD). Fractions of initially deposited particles are means (bars are standard deviation) for lungs, carcass, GI tract, and bronchoalveolar lavage (BAL) obtained from four rats at each time point. Fractions of BAL are sum of cell pellet (dominant fraction) and supernatant. Mean daily fecal excretion is plotted cumulatively using all data of each rat in this biokinetic study.

$0.47 \pm 0.06$  and  $0.36 \pm 0.07$  of the deposited 15- and 80-nm particles were cumulatively cleared by this pathway, respectively. Only a fraction of  $0.02 \pm 0.003$  was cleared via urine 7 d after inhalation; there was no difference within the results of both particle sizes. At each time point of sacrifice a blood sample obtained from heart puncture revealed  $^{192}\text{Ir}$  activity at the limits of detection.

*Translocation* At each time point of sacrifice, fractions of translocated  $^{192}\text{Ir}$  remained very low in any of the organs: liver, spleen, kidneys, heart, and brain,  $<0.002$  and  $0.001$  of deposited 15- and 80-nm particles. However, in the remaining carcass maximally a fraction of  $0.035$  and  $0.02$  was found. This is in agreement with blood levels of  $^{192}\text{Ir}$  close to the detection limit starting from 6 h to 7 d after inhalation. For these minute translocated fractions an attempt was made to distinguish particulate from dissolved  $^{192}\text{Ir}$ : First, the ratio of  $^{192}\text{Ir}$  found in a given organ or the carcass to the cumulative  $^{192}\text{Ir}$  found in urine was determined from an auxiliary study after soluble  $^{192}\text{IrCl}_3$  instillation (see results given later); for example, this ratio was  $0.02$  for liver/urine. To estimate the dissolved  $^{192}\text{Ir}$ , the fraction in a given organ or the remaining carcass, this ratio was applied to the urine fractions found after particle inhalation (given earlier); for example, for dissolved  $^{192}\text{Ir}$ , the fraction in the liver was  $0.0004$ . When this dissolved  $^{192}\text{Ir}$  fraction was subtracted from the observed fraction in any of the organs or the carcass it appeared that almost the entire observed organ fraction was particulate (see Figure 4). Since this held for any time point of sacrifice until 7 d after inhalation, there was a very small but detectable fraction of  $^{192}\text{Ir}$  particles translocated from the lungs into liver, spleen, kidneys, heart, brain, and the carcass.

Although Figure 4 suggests a tendency of time-dependent increasing particle fractions in the liver, no such trends were seen in other organs or the carcass. The only remaining pathway is particle penetration through the lung epithelium into systemic circulation, since particles had exclusively been deposited in the lungs and since particles were not taken up from the gut after being cleared to the larynx and being swallowed as shown by the auxiliary gavage studies given later. Interestingly, although translocated fractions in extrapulmonary organs were below  $0.01$ , they were significantly larger by almost 1 order of magnitude for 15-nm particles when compared to 80-nm particles, as shown in Figure 4 for the liver. This suggests an inverse particle-size-dependent transport phenomenon into the circulation, as hypothesized. It also may be indicative that particles had not lost their originally aggregated structure and did not disaggregate to a great extent prior to systemic translocation and accumulation in the various organs and the carcass.

A similar observation was found for tracheobronchial lymph nodes. Fractions were below  $0.002$  for 15-nm particles, while they stayed an order of magnitude lower, below  $0.0003$ , for the 80-nm particles, relating to a similar inverse particle-size-dependent transport phenomenon.



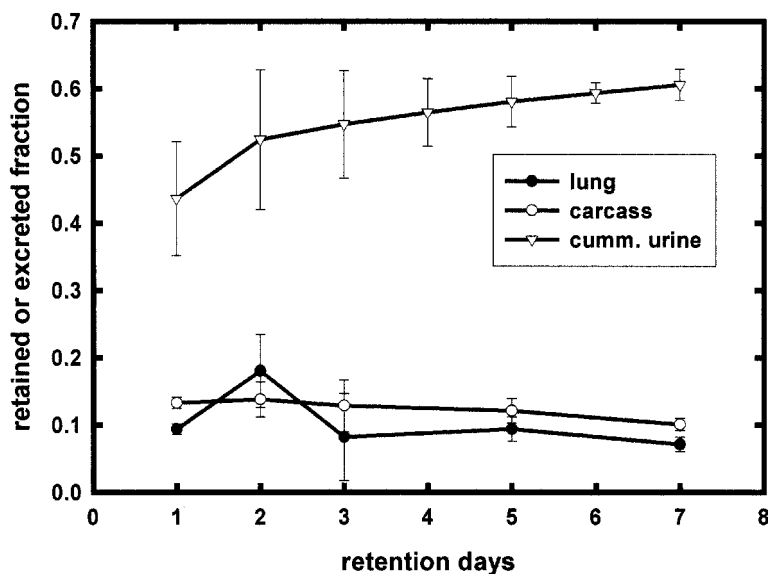
**FIGURE 4.** Iridium uptake and retention in liver during 7 d after inhalation of 15-nm (left) and 80-nm (right) count median diameter (CMD). The particulate <sup>192</sup>Ir fraction is distinguished from the <sup>192</sup>Ir fraction, which had been dissolved from the particles in the lungs and was taken up from circulation, based on the estimates described in the text. Fractions of initially deposited particles are means (bars are standard deviation) obtained from four rats at each time point.

## Auxiliary Studies

**Biokinetics of dissolved  $^{192}\text{Ir}^{3+}$  administered to the rat lungs** Lung retention and daily urinary excretion after the instillation of soluble  $^{192}\text{IrCl}_3$  in the lungs of the rat are shown in Figure 5. After 1 wk, a fraction of 0.60 of the administered  $^{192}\text{Ir}$  was excreted via urine, 0.08 stayed in the lungs, and fractions of 0.10 were retained in soft tissue and bone each; a small fraction of activity was retained in other organs such as liver, kidneys, or spleen. About 0.10 of the instilled activity was excreted via feces within the first 3 d; afterward, there was no further fecal activity measurable.

**Biokinetics after particle gavage** After esophageal administration of ultrafine  $^{192}\text{Ir}$  particle suspension, virtually the whole amount of  $^{192}\text{Ir}$  was found in fecal excretion within 2–3 d. During the 6-d observation period no detectable  $^{192}\text{Ir}$  in urine was observed at any day. Six days after administration there was no detectable  $^{192}\text{Ir}$  in any organ or tissue of the body. Hence, there also was no uptake and/or absorption from the GI tract.

**Particle distribution after systemic application** During 7 d after intravenous injection of an ultrafine particle suspension the main  $^{192}\text{Ir}$  fraction of  $0.83 \pm 0.02$  remained in the liver and  $0.079 \pm 0.015$  in the spleen. Interestingly, a fraction of  $0.034 \pm 0.006$  of the administered  $^{192}\text{Ir}$  was found in the lungs and nearly the same amount in the carcass,  $0.037 \pm 0.004$ . Negligible



**FIGURE 5.** Iridium retention in lavaged lungs, bronchoalveolar lavage, and remaining carcass after organ dissection during 7 d after intratracheal instillation of soluble  $^{192}\text{Ir}^{3+}$  in saline. Fractions of administered dose for lungs, carcass, and bronchoalveolar lavage are means obtained from the examined rats at each time point. On d 1 and 2 data are obtained from 4 rats, on d 3 and 5 data resulted from 2 rats each, and on d 7 data were obtained from 6 rats. Mean daily urinary excretion is plotted cumulatively using all data of each rat in this biokinetic study.

fractions were found in other organs. After 7 d only a small fraction of 0.006 was excreted in urine. Nothing was found in feces.

*Biokinetics after intratracheal instillation of a particle suspension*  
During 7 d after intratracheal instillation of ultrafine  $^{192}\text{Ir}$  particles, lung retention was very similar to particle inhalation. Interestingly, the rapidly cleared fraction into the GI tract and feces was only 0.1 of the initial deposit 1 d after instillation, indicating less deposition in the airways after instillation than during inhalation. After 7 d a fraction of 0.07 of the initial deposit was lavageable from the lungs, 0.52 was retained in the lavaged lungs, and 0.35 had been fecally excreted, while only 0.01 was found in the carcass and  $<0.002$  in extrapulmonary organs. Cumulative urinary excretion was 0.001. These results are consistent with the inhalation data, indicating almost exclusive lung retention minus particle transport to the larynx into the GI tract and feces, minute particle translocation into extrapulmonary organs, and very low particle solubility.

## 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 be a reliable technology for rat exposures. The  $^{192}\text{Ir}$  particles appeared to be suitable for these studies because of their very low in vitro solubility. The latter was confirmed by auxiliary studies administering ultrafine  $^{192}\text{Ir}$  particles either into the systemic circulation, or to the GI tract or to the lungs. In addition, the gavage study showed there was no particle uptake from the GI tract but particles just passed the gut and were excreted quantitatively in feces. On the other hand, soluble  $^{192}\text{Ir}^{3+}$  administered to the lungs was mainly excreted via urine, while there is little retention in the lungs and other organs and tissues. Hence the overall biokinetics of the ultrafine  $^{192}\text{Ir}$  particles and soluble iridium compounds is rather easy and straightforward, underlining their suitability for these kinds of biokinetic studies. This is in contrast to ultrafine cobalt oxide and zinc oxide particles, produced under similar conditions in previous experiments in our laboratory, which proved to be too soluble (Kreyling, private communication). 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 was advantageous since particles did not deposit in extrathoracic airways and did not contaminate the pelt. Therefore, the deposited fraction of the inhaled particles in the thorax and the fraction cleared within 24 h could be evaluated directly from activity measurements. As expected from diffusion behavior, the deposited fraction increased from 0.28 to 0.49 for 80- and 15-nm particles, respectively. This is in good agreement with theoretical predictions by a multiple-

path particle deposition model for rats, MPPDep (Anjilvel & Asgharian, 1995), which predicts thoracic deposition excluding head deposition of 0.19 and 0.49 for 80- and 15-nm particles and GSD of 1.6, respectively. Similarly, the rapidly cleared fraction increased drastically from 0.03 to 0.15, indicating an increased deposited fraction of the 15-nm versus the 80-nm particles in the ciliated bronchial airways. This is in excellent agreement with theoretical data of 0.04 and 0.14 for the 80- and 15-nm particles.

The exclusive particle deposition in the thorax allowed distinct determination of retention and clearance kinetics unbiased by extrathoracic and pelt contamination. Ultrafine iridium particles of both 15- and 80-nm size were retained predominantly in the lungs. Earlier studies (Ferin et al., 1991, 1992) had shown that ultrafine 20-nm titanium dioxide ( $\text{TiO}_2$ ) particles have greater access to interstitial spaces than larger 250-nm  $\text{TiO}_2$  particles. This was also shown by larger yields (78%) of 250-nm particles in bronchoalveolar lavages as compared to 66% for 20-nm particles 1 d after particle instillation (Ferin et al., 1994). Lavageable fractions of both iridium particles were consistently lower than even that of 20-nm  $\text{TiO}_2$  particles, which probably was caused by the different modes of application— $\text{TiO}_2$  instillation versus Ir inhalation. Even 6 h after inhalation only a fraction of 0.2 of the 15-nm  $^{192}\text{Ir}$  particles was lavageable. Thereafter this fraction declined to 0.1 at d 7. Interestingly, at all time points the lavageable fraction of 80-nm particles was slightly larger than that of the 15-nm particles as found for the  $\text{TiO}_2$  particles (see Figure 3). Each of these observations is in agreement with an increasingly rapid transport into interstitial spaces the smaller the particles are.

One day after inhalation, both particles were cleared predominantly from the peripheral lung via thoracic airways to the larynx into the GI tract and feces. All lavaged particle fractions confirmed this clearance pathway throughout wk 1 after inhalation. Particle dissolution estimated from urinary excretion appeared to be very low.

Comparison of clearance patterns after soluble  $^{192}\text{IrCl}_3$  instillation and after ultrafine particle inhalation allowed us to determine particle solubility and the biokinetic fate of dissolved  $^{192}\text{Ir}$  within and out of the body. No detectable particle uptake or dissolution/absorption occurred in the GI tract. Taking the results from the auxiliary and the inhalation studies together, they clearly showed that a very small fraction of particles deposited in the lower respiratory tract had been translocated from the lungs into liver, spleen, heart, brain, and the carcass. The trend of time-dependent increase of particle burden in liver was not observed in other organs. The only plausible pathway remains particle transport through the lung epithelium into the pulmonary vasculature and systemic circulation. Translocated fractions of 15-nm Ir particles were consistently larger by a factor of 5–10 when compared to the 80-nm particles. The rate of systemically translocated iridium UP was found to be rather small but size dependent in healthy adult WKY rats. However, this short-term exposure may not allow for extrapolation after



chronic exposure. Therefore, one cannot exclude adverse health effects in secondary target tissues that are not evolved to deal with particles compared to tissues of the respiratory tract. Furthermore, particle uptake and potential health effects may 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. Future biokinetic studies over extended retention periods of half a year will provide insight on long-term systemic translocation and accumulation in extrapulmonary organs.

Interestingly, although translocated fractions in extrapulmonary organs and tracheobronchial lymph nodes were below 0.01, they were significantly larger by almost 1 order of magnitude for 15-nm particles when compared to 80-nm particles, as shown in Figure 4 for the liver. This suggests an inverse particle-size-dependent transport phenomenon into blood circulation, as hypothesized. It also may be indicative that particles did not disaggregate to a great extent prior to systemic uptake. Ongoing studies will evaluate whether this translocation pathway continues with time, resulting in increasing fractions in the various organs and tissues, or whether this phenomenon occurs initially without continuation over retention time.

Studies after systemic injection of ultrafine iridium particle suggest that obviously negligible fractions passed through glomerular filters of the kidneys into urine. This may indicate that injected particles either were rapidly and tightly bound to proteins or other structures in blood or they were agglomerated to larger aggregates, which were not able to penetrate the kidneys toward the bladder.

In contrast to these findings using ultrafine iridium particles, studies by Oberdörster et al. (2002) using ultrafine 25-nm  $^{13}\text{C}$  carbonaceous particles suggest a major translocation pathway from the respiratory tract to the liver within the first 24 h. In  $^{13}\text{C}$  studies, rats were whole-body exposed for 6 h at an aerosol concentration similar to that in our  $^{192}\text{Ir}$  studies. Both the differences in exposure conditions and the composition of the particle material with clearly different surface properties in terms of physical structure and chemical composition may account for the differences observed. Exposure to  $^{13}\text{C}$  particles led to a significantly higher particle burden than in our  $^{192}\text{Ir}$  studies because of the six times longer exposure, with major fractions being deposited in the extrathoracic airways and the pelt. The latter will have caused a much higher amount of particles passing through the GI tract, which is likely to be in the range of 2 orders of magnitude more than after our 1-h inhalation protocol using endotracheal intubation. In the case of  $^{13}\text{C}$ , neither ultrafine particle uptake from extrathoracic airways nor systemic uptake from the GI tract is known, nor is there a direct proof of the insolubility or solubility of the ultrafine  $^{13}\text{C}$  particles used. Additionally, determination of  $^{13}\text{C}$  resulting from particle exposure in biological samples is affected by the natural biological abundance of  $^{13}\text{C}$  in those samples. Taking the differences observed between systemic translocation of ultrafine  $^{13}\text{C}$  and

$^{192}\text{Ir}$  particles into account, two hypotheses can be formulated that should be addressed in follow-up studies:

- Aggregated  $^{13}\text{C}$  particles disaggregate much more completely and rapidly on the lung epithelium than  $^{192}\text{Ir}$  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 then may determine the subsequent metabolic fate of the complexes. For instance,  $^{192}\text{Ir}$  particles or their disaggregates form complexes with proteins on the lung epithelium other than particles such that  $^{192}\text{Ir}$  particle–protein complexes are retained in the lungs while  $^{13}\text{C}$  particle–protein complexes translocate systemically and accumulate specifically in extrapulmonary organs like the liver.

Regarding the latter hypothesis, intensive studies had been performed on the systemic translocation of 1- to 4-nm actinide oxide particles from the rat lungs after instillation (Cooper et al., 1979; Stradling et al., 1980). Rapid systemic 24-h translocation of particles bound to either a complex pattern of proteins and/or components of surfactant was observed depending on parameters of particle material and surface properties like the net charge of the particles. The authors clearly emphasize the need to distinguish between absorption of dissolved particle material chelated by citrate anions and proteins like transferrin and particles bound to surfactant components and a protein pattern which they were unable to resolve. They discuss epithelial pores in the size range of 1 nm as a potential pathway. Besides those pathways, active transport of particle–protein complexes also needs to be considered. In any case they provide evidence that ultrafine particles of 1–4 nm are translocated systemically, which could also apply to disaggregated subunits of the  $^{13}\text{C}$  particles according to the first hypothesis just stated. However,  $^{13}\text{C}$  accumulated in the liver, while no uptake in the liver was reported for the actinide oxide particles but rather rapid passage through the glomerular filters of the kidneys. This difference may shed light on the importance of different particle surface properties and/or formation of different complexes. Although the primary structures of the iridium particles used in the present study were below 5 nm, the very small fraction of  $^{192}\text{Ir}$  activity in urine either after inhalation or after systemic injection suggests that the administered particles did not disaggregate and/or form protein complexes that were subject to either systemic translocation or passage to urine, according to the second hypothesis.

## CONCLUSION

Radiolabeled, almost insoluble ultrafine iridium particles proved to be a suitable tool to perform totally balanced biokinetic studies in rats after the unique and controlled exposure via an endotracheal tube. The clearance

pattern of soluble radiolabeled iridium—not hampered by a complex pattern of uptake in secondary tissues like bone and soft tissue—allowed us to determine not only major clearance pathways after particle inhalation but also minute particle translocation pathways into secondary organs and tissues after systemic uptake. This study indicates that within wk 1 after inhalation only very small fractions of ultrafine iridium particles have access to systemic circulation and extrapulmonary organs. Chemical composition and physical structure of the particle surface may be an important determinant influencing systemic translocation of UFP.

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