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RESEARCH ARTICLE

The pulmonary deposition and retention of indium-111 labeled ultrafine carbon particles in healthy individuals

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Abstract

Context: Particulate air pollution, for example, from ultrafine (UF) particles, has negative health effects. However, there is still limited knowledge regarding the fate of inhaled particles in the human body.

Objectives: To describe the normal lung deposition and 1 week particle retention of indium-111 labeled UF carbon particles in healthy subjects. Additionally, the possibility to extend the follow-up period to 4 weeks was also investigated for one of the subjects.

Results: The cumulative pulmonary particle clearance 1 week post-administration, corrected for activity leaching and mucocilliary transport of activity deposited in the central airways, was 4.3 ± 8.5% (average ± standard deviation at group level), with marginal translocation of particles from lungs to blood, 0.3%. There was no observable elimination of particles from the body via urine. Seven days after exposure, the cumulated activity leaching was 3% (group level), which indicates a stable bonding between the particles and Indium-111. The volunteer followed for a total of 4 weeks, showed a cumulative decrease of activity retention in the lungs of 10.5%. After correction for activity leaching and clearance from central airway deposition, the estimated particle clearance was about 2%.

Conclusions: No evidence for particle translocation from the lungs could be proven 7 days after exposure. It is possible to follow-up Indium-111 labeled UF carbon particles at least 1 month post-administration without increasing the administered activity.

Keywords: Air pollution, ultrafine particles, pulmonary deposition and pulmonary retention

Introduction

Epidemiological studies show that the risk for developing respiratory and cardiovascular diseases or even cancer is connected to exposure to combustion products which contain ultrafine (UF) particles (Boffetta et al., 1997; Pope et al., 2002). However, the mechanisms by which UF particles trigger pathological processes in the body are not fully understood. It has been proposed that UF particles can negatively influence the heart, induce inflammation and accelerate atherosclerotic processes (Pope et al., 2004). Particle surface area seems to be crucial in the onset of inflammation. Ultrafine particles have a higher surface area per unit mass compared to larger size particles, thereby probably being more prompt to start an inflammatory process (Li et al., 1997; Renwick et al., 2004; Monteiller et al., 2007). Radiolabeled particles have been used for human clearance studies for some time (Camner & Philipson 1978; Burch et al., 1986; Philipson K et al., 1996), but there are limited human studies on translocation using radiolabeled UF carbon particles. Previous human studies with UF carbon particles labeled with Technetium-99m (^{99m}Tc) showed slow clearance rates with most particles retained in the lung region after 2 days (Wiebert et al., 2006a; Mills et al., 2006). Furthermore, no significant difference in UF carbon particle clearance from the lungs to the blood was observed between asthmatics, smokers and healthy individuals 24 h post-inhalation (Wiebert et al., 2006b). However, these results are in contradiction

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with the interpretations from another study performed with UF carbon particles labeled with ^{99m}Tc (Nemmar et al., 2002). These discrepancies among published data could be a consequence of the short physical half-life of ^{99m}Tc (6 h), the high chemical instability of ^{99m}Tc labeled UF carbon particles, 2.6% activity leaching 48 h after generation (Wiebert et al., 2006a) and the presence of free pertechnetate in the initially generated aerosol. All these factors in combination with the limited gamma camera sensitivity increase the uncertainties in the measurements and severely limit the follow-up period. A new method for labeling UF carbon particles with indium-111 (^{111}In) has been recently suggested (Sanchez-Crespo et al., 2011). ^{111}In labeled UF carbon particles present two major advantages compared to Technetium labeled UF carbon particles, their longer physical half-life (2.8 days) and their better chemical stability (about 97%, 7 days after generation). These two properties enable *in vivo* human studies for an extended period of time. In this study, we thereby apply this technique to investigate and describe the normal lung deposition and 1-week retention of UF carbon particles labeled with ^{111}In in a group of 10 healthy volunteers. Additionally, the possibility to extend the follow up period to 4 weeks post-inhalation for further studies with patients was investigated in one of the subjects.

Methods

Volunteers

Ten nonsmoking healthy volunteers (five men and five women with mean age of 29 years; range: 20–54) participated in the study. All volunteers underwent a routine physical examination including lung function test performed with a spirometer (Spirolab II, Medical International Research, Rome, Italy). Forced vital capacity and forced expiratory volume during the first second were measured and compared to European Community of Coal and Steel reference values (Quanjer et al., 1993). The subjects were also screened for the presence of specific IgE antibodies against common inhaled allergens (S-Phadiatop®, Pharmacia-Upjohn, Uppsala, Sweden). All participants had given their informed written consent to participate in the study. The Swedish regional ethical and radiation protection boards approved the study.

^{111}In labeled aerosol generation, administration and follow-up

The technique and equipment described by Sanchez-Crespo and colleagues for generation, administration and follow up of ^{111}In labeled UF carbon aerosol (Sanchez-Crespo et al., 2011), was used in this work. Each volunteer was exposed to between 0.5 and 1 MBq of ^{111}In. To convert total lung activity retention, as measured by gamma camera, to lung particle retention, correction for activity leaching and mucocilliary clearance from central airway deposition were carried out. Activity leaching tests from a sample of the inhaled aerosol and daily samples of blood and urine were performed using a membrane diffusion technique as previously described (Sanchez-Crespo et al., 2011). The clearance rate (%/h) due to mucocilliary transport from activity deposition in the central airway can be approximated by the difference of the slopes (Diff) of the least-squares linear fitting of respectively the central (C) and the peripheral (P) lung regions. The contribution of this clearance to the total lung clearance can then be simply obtained as the percentage activity deposited in C that is accessible for mucocilliary transport according to:

$$Central\ airway\ clearance\ (\%,t) = \frac{Diff(\%\cdot h^{-1})\cdot A_{C,0}}{A_{C,0}+A_{P,0}}\cdot t$$

where t is the time after administration and $A_{C,0}$ and $A_{P,0}$ are the activity deposited in the central and peripheral lung regions after administration, respectively.

Following up lung activity retention beyond 1 week

Lung clearance measurements were conducted for one of the subjects at the Swedish radiation safety authority laboratory for another 3 more weeks. The background radiation level in this lab is nearly negligible, enabling detection of activity levels in the body beyond the detection limits of a commercial hospital gamma camera. A whole body scanner with un-collimated sodium iodide (NaI) detectors (Harshaw, Paris, France) was used. The retention at day 7 after exposure was measured at both, the hospital and radiation safety authority laboratory, in order to normalize the data between sites.

Results

Subjects

Table 1 shows the basal characteristics of the study population. All subjects were of normal BMI and showed lung

Table 1. Basal characteristics of the group of volunteers and physical characteristics of the ^{111}In labeled aerosol during exposure.

Basal characteristics	Mean (range) ± SD
n	10
Age (years)	29 (20–54) ± 12
BMI (kg/m^2)	23.7 ± 3.5
FVC (% of predicted)	97 ± 8
FEV1 (% of predicted)	100 ± 6
Estimated inhaled median particle size (nm)	84 ± 20
Estimated GSD for median particle size	1.95 ± 0.18
Inhaled particle number concentration ($10^3 \times cm^{-3}$)	460 ± 150
Deposition fraction (%)	31.0 ± 11.0
Labeling efficiency (%)	99.7 ± 0.4

BMI, body mass index; FEV, force expiratory volume; FVC, force vital capacity; GSD, geometric standard deviation; n, number of subjects; SD, standard deviation.

function with predicted limits of normality. Two of the volunteers showed IgE antibodies for some common radioallergosorbents (cladosporium, birch, cat, horse, etc).

Aerosol deposition and retention measurements

Table 1 shows the physical characteristics of the ^{111}In labeled UF carbon particles during administration. Notice the high-labeling efficiency after production, 99.7 ± 0.4%, as well as the relatively low deposition fraction 31 ± 11% (averages at group level). Estimated average value for the median particle size at group level was 84 nm (range: 58–124), with a geometric standard deviation of 1.95 (1.6–2.2). Altogether, these results demonstrate the good reproducibility of the physical characteristics of the ^{111}In labeled UF carbon particles between different aerosol generations and subject exposures. Figure 1 shows the aerosol deposition and retention, measured with the gamma camera at three different occasions post-administration, for one of the subjects. This figure clearly shows no activity outside of the lungs at any time. Particularly, no activity accumulation in the liver or thyroid can be observed within the sensitivity limitations of the used gamma camera. This image also shows the homogenous distribution of the aerosol within the entire lung, with absence of hot spots or accumulation of activity in the central parts of the lungs, suggesting low airway impaction. Equal results were observed in all subjects.

Figure 2 reveals a significant difference in activity clearance rate between the central and the peripheral lung regions. Most likely, the faster central lung clearance reflects the mucocilliary escalator in the conducting airways. Quantitatively, this difference was found to be 0.06%/h, mean at group level.

The measured lung activity clearance as a function of time (mean ± standard deviation of the group) is shown in Table 2. Seven days after exposure, the measured intersubject lung activity clearance was 9.0 ± 8.3% (range: 0–17%). Table 2 also reveals that the total free activity measured outside of the lungs with the well chamber from blood and urine samples 1-week post-administration, accounted for 2.4% of the total activity retained in the lungs after administration (mean at group level).

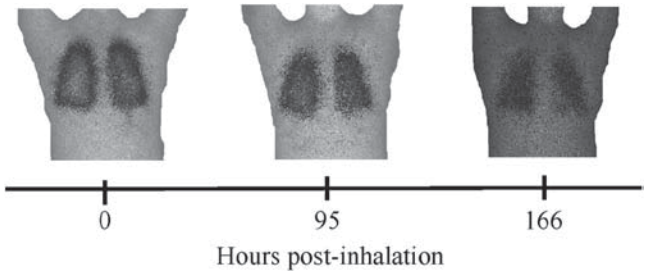

Figure 1. Gamma camera images of the pulmonary deposition and retention at different times to about 1MBq of ^{111}In labeled ultra fine carbon particles in one healthy subject. The ^{111}In photon emission images were fused with the corresponding anatomical transmission scan of the subject's chest obtained with a ^{57}Co flood source.

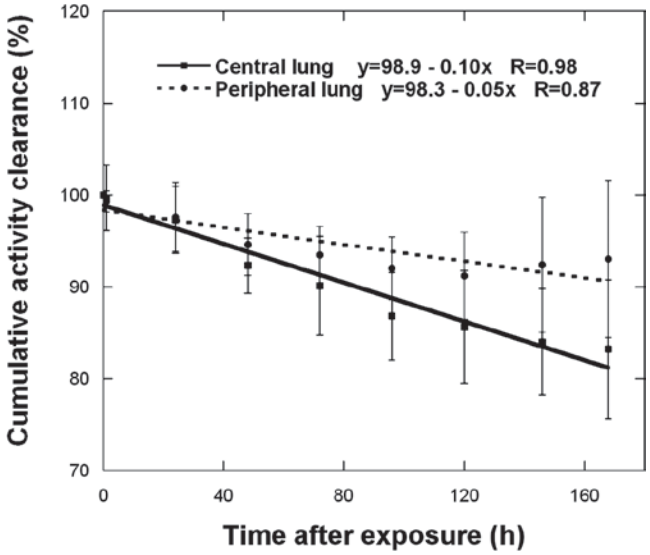

Figure 2. Activity retention in the central and peripheral lung regions as a function of time after administration of the ^{111}In labeled ultrafine carbon particles. Values are mean at group level. The error bars represent the standard deviation.

Table 2. Complete follow-up of the activity clearance from the lungs during 1-week post-administration.

Time after exposure (h)	Measured lung activity clearance (%)	Cumulative activity in urine (%)		Cumulative activity in blood (%)		Activity cleared by mucociliar transport from airway deposition (%)	Estimated ultrafine particle clearance from the lungs (%)
		Bond to carbon particle	Free	Bond to carbon particle	Free		
0	0	–	–	–	–	0	0
1	0.7 ± 0.8	0	0.1 ± 0.2	0	0.7 ± 1.1	0.0 ± 0.0	0.0 ± 1.8
24	3.9 ± 2.6	0	0.2 ± 0.2	0.1 ± 0.2	1.0 ± 1.1	0.3 ± 0.2	1.0 ± 4.1
48	6.1 ± 2.9	0	0.3 ± 0.2	0.1 ± 0.2	1.1 ± 1.0	0.6 ± 0.4	4.1 ± 3.7
72	7.4 ± 2.8	0	0.3 ± 0.2	0.2 ± 0.2	1.3 ± 1.4	0.7 ± 0.6	4.7 ± 3.8
96	8.4 ± 2.5	0	0.4 ± 0.2	0.2 ± 0.2	1.3 ± 1.4	1.1 ± 0.8	6.1 ± 4.1
120	9.9 ± 4.4	0	0.5 ± 0.3	0.3 ± 0.2	1.4 ± 1.5	1.3 ± 2.0	6.7 ± 5.0
146	9.5 ± 6.4	0	0.5 ± 0.4	0.3 ± 0.2	1.4 ± 1.5	1.4 ± 1.1	5.5 ± 6.7
168	9.0 ± 8.3	0	0.6 ± 0.4	0.3 ± 0.2	1.5 ± 1.5	1.7 ± 1.3	4.3 ± 8.5

The corresponding estimate for ultrafine particle clearance was obtained by subtracting free activity leaching and activity cleared from airway deposition from the measured lung activity clearance at individual level.
Values are mean ± SD at group level.

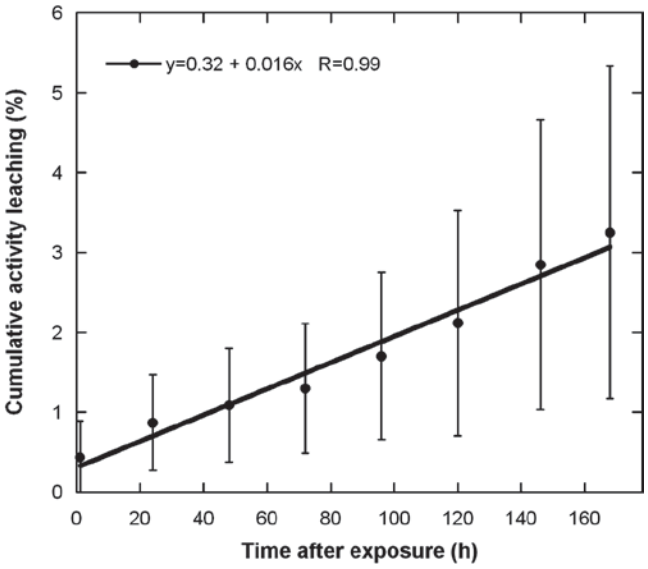

Figure 3. The cumulative ^{111}In activity leaching from ultrafine carbon particles as a function of time post-generation. Activity leaching was measured *in vitro* from a sample of the aerosol using the membrane diffusion technique. Values are mean at group level. The error bars represent the standard deviation.

Additionally, the Table 2 also shows the relative amount of activity cleared by mucocilliary transport. With these two corrections, Table 2 shows the best estimate for UF carbon particle clearance from the lungs. One week after exposure, about 4.3% of the inhaled particles were cleared (mean at group level). However, the uncertainties of this estimate are of the same order of magnitude, 8.5%. This result, together with the 0.3% bounded activity to particles found in blood samples, demonstrate a marginal translocation of particles from lungs during 1 week after inhalation.

Figure 3 shows the cumulative ^{111}In leaching from UF carbon particles with time measured *in vitro* from a sample of the generated aerosol (average at group level). Seven days after generation, the cumulative amount of free activity is about 3%. This value is comparable to the sum of the cumulative free activity encounter in blood and urine 7 days after exposure, 2.1% (Table 2). The linear regression has slope 0.016 %/h and zero intercept 0.3% (corresponding to a 99.7% initial labelling efficiency).

Extended activity retention follow up for one volunteer (Table 3) shows the complete follow-up of ^{111}In labeled UF carbon particles in the lungs during 4 weeks for a subject exposed to about 700 kBq of ^{111}In. The fluctuations in lung retention around 100% observed in Table 3 merely represent the measurement uncertainties as the initial activity decays in the body. No evidence of ultrafine particle translocation from the lungs, 4 weeks after exposure, could be proven for this subject.

Discussion

In this study, 10 healthy volunteers were exposed to ^{111}In labeled UF carbon particles. One week after exposure, the estimated cumulative pulmonary particle retention corrected for activity leaching and central airway clearance by mucocilliary transport was 95.7 ± 8.5% at group level, with marginal translocation of particles from the lungs to the blood (0.3%) and no observable particle elimination via urine. This small difference between measured particle clearance from the lungs (4.3%) and the amount of UF particles found in blood (0.3%) may be explained by the limited detection sensitivity of the equipment used and the possibility of particle translocation from the lung parenchyma into the lymph nodes (Philipson et al., 1985).

This study also demonstrates the possibility to follow up lung retention of ^{111}In labeled UF carbon particles during at least 4 weeks post-inhalation. The results for a single subject revealed a decrease of activity retention in the lungs of 10.5%, 29 days after administration. However, after correction for activity leaching and mucocilliary transport from central airway deposition, the estimated UF particle clearance was about 2% of the initially inhaled UF particles, 29 days post-inhalation (Table 3). Altogether, these results are coherent with previously performed short-term follow-up studies using ^{99m}Tc labeled UF carbon particles (Wiebert el al., 2006a and 2006b). However, notice that while the "clean" UF carbon particles in our study and in the studies by Wiebert et al. are insoluble in body fluids, the type of nanoparticles in the environment can be of many different chemical forms and hence partly soluble in body fluids. Furthermore, they can carry organic molecules of different sort, possibly correlated to the onset of inflammatory processes in the body in relation to their accumulation. Already, in 1973, it was stated that solubility of particles may affect measurements of clearance and deposition (Morrow, 1973). Furthermore, it has also been noticed that if insoluble particles reach the alveoli, they will mainly be cleared by macrophagial phagocytosis and be transported to the ciliated airways where they are cleared out, a process that may take several years (Philipson et al., 1985).

In this work, correction for particle clearance by mucocilliary transport from activity deposited in the central airways was estimated as the difference in clearance rate between the C and the P lung regions. Since the elimination rate is much faster in C as compared to P regions (Figure 2), it is most likely that mucocilliary transport is the dominant process since the rate of clearance of free activity or particle bound activity from lung parenchyma should theoretically be independent of the lung region. From Table 2, this correction was found to be approximately 1.7% of the total administered activity, assuming that mucocilliary clearance is linear over time. This activity was not found in the systemic circulation or in urine. This is consistent with the theoretical pathways for excretion of this activity by nose/mouth or via the liver and feces. It is generally assumed that mucocilliary transport is a process that terminates within a few days; this can be clearly seen in Figure 2. During the first 4 days after exposure, there is a fast clearance rate. Thereafter, the clearance rate significantly decreases from –0.14 to

Table 3. Follow-up of the lung retention of ^{111}In labeled ultrafine carbon particles for one healthy subject during 4 weeks post-administration.

Days after exposure	Measured cumulative pulmonary activity retention (%)	Cumulative activity leaching (%)	Cumulative mucocilliary transport from the central airways (%)	Estimated cumulative pulmonary ultrafine carbon particle retention (%)
0	100[a]	0	0	100.0
0.042	99.9[a]	0.16	0.03	100.1
1	98.4[a]	2.33	0.59	101.3
2	99.0[a]	2.44	1.31	102.7
3	95.2[a]	4.38	2.11	101.7
4	94.1[a]	4.44	2.72	101.3
5	95.7[a]	4.79	3.19	103.7
6	99.5[a]	4.83	3.60	107.9
7	100.7[ab]	4.83	4.12	109.6
14	93.0[b]	4.83	4.12	101.9
22	85.9[b]	4.83	4.12	94.8
29	89.5[b]	4.83	4.12	98.0

The total activity deposited in the subject lungs after exposure was approximately 700 kBq.
[a]Measured at the hospital with a collimated gamma camera.
[b]Measured at the low background radiation level laboratory with an uncollimated whole body counter.

–0.05%/h in the central lung region and from –0.09 to 0%/h in the peripheral lung region. However, since there is also mucocilliary transport even in the P regions, the obtained 1.7% correction (Table 2), 1 week after administration, certainly underestimates the overall lung particle clearance through mucocilliary transport.

Measurements of retention and clearance are very much dependent on the quality of the aerosol labelling; hence, activity leakage studies are mandatory in lung retention studies based on radiolabeled particles (Ghio and Bennett, 2007). In our study, the bonding between particle and radionuclide was very stable. Seven days after exposure, the *in vitro* activity leaching test showed a 3.2% free activity in the aerosol sample (at group level). This is in good correlation with the corresponding *in vivo* values of free activity measured in blood and urine (2.1%, 7 days after exposure, Table 2).

Uncertainties in gamma camera image quantification due to the low amount of activity initially administered to the subjects (between 0.5 and 1 MBq) and the limited detection sensitivity should be carefully considered. This uncertainty can be clearly seen in Figure 1, as the image noise increases with time after exposure and in Table 2, in the fluctuations of the estimated cumulative pulmonary carbon particle clearance. Hence, for an extended follow-up period, the initial administered activity is the critical factor. Notice that, in this study, 4 weeks after exposure, the subjects would have less than 1 kBq of ^{111}In activity left in their lungs (considering 100% particle retention and 0% activity leaching). For an extended follow-up period, activity detection uncertainty could be considerably decreased by increasing the amount of inhaled activity. As an example, 10 MBq inhaled aerosol would allow to accurately follow up the ^{111}In labeled UF carbon particles for about 40 days. The associated radiation burden to the subject for this exposure would be about 5 mSv (Sanchez-Crespo et al., 2011), which is comparable to the yearly background radiation dose in Sweden.

There are a limited number of human studies on translocation using UF carbon particles. Due to safety aspects, they are all short term and showed either none or limited translocation (Brown et al., 2002; Mills et al., 2006; Wiebert et al. 2006a, 2006b; Möller et al., 2008; Sanchez-Crespo et al., 2011). A translocation of 1% may be interpreted as limited translocation, while another author refers to it as major translocation. Even small translocation from lungs into the circulation may have harmful effects on the human health. Compared to these results, Nemmar et al. (2002) reported high elimination rate, a finding that most likely can be explained by activity leaching. Animal studies have also reported different degrees of particle translocation from the lungs (Takenaka et al., 2001, 2006; Kreyling et al., 2002, 2009; Oberdörster et al., 2004; Semmler et al., 2004; Elder et al., 2006). However, caution must be taken when extrapolating those results to humans, as high particle concentration levels may affect the permeability of lungs (Donaldson et al., 2001). Additionally, the chemical and surface properties of the UF particles differ among these studies, which make the results not directly comparable.

Little is known on how underlying inflammation or lung diseases may affect the deposition and retention of UF carbon particle. A study with chronic obstructive pulmonary disease (COPD) patients exposed to ^{99m}Tc labeled carbon particles showed a slightly increased deposition and retention as compared to a healthy subgroup (Brown et al., 2002). Patients with damaged lungs, like patients with chronic bronchitis, have been shown to have higher retention of Teflon particles labeled with ^{111}In as compared to healthy individuals, due to impaired mucocilliary function (Svartengren et al., 1996; Svartengren et al., 2004). In previous studies comparing ^{99m}Tc labeled UF carbon particle clearance in healthy and in mild asthmatics, there was no significant difference in translocation after 24 h (Wiebert et al., 2006b), but the disease was mild and the number

of subjects limited. Even a limited fraction of translocation might create great differences. A 5% translocation compared to 1% can represent a fivefold increases in systemic exposure. Subjects with lung injury might have increased permeability and warrant more detailed studies. This study can serve as a data base of the "normal" distribution of ^{111}In labeled UF carbon particles for future comparisons with a patient group.

Conclusions

In this study, we have not found evidence for a significant translocation of UF carbon particles from the lungs into the systemic circulation in healthy individuals 7 days after exposure (95.7 ± 8.0% cumulative UF particle lung retention and 0.3% cumulative particle bound activity in blood). It is possible to follow up ^{111}In labeled UF carbon particles at least 4 weeks after exposure without increasing the administered activity. The good stability of the ^{111}In labeling, the reproducibility of the physical and chemical characteristics of the generated aerosol and the low radiation doses enable administration of higher amounts of activity to extend the follow-up period. This will allow us to investigate in detail the subtle differences in lung retention between the healthy and the sick lung.

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Declaration of interest

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References

Boffetta P, Jourenkova N, Gustavsson P. 1997. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control 8:444–472.

Brown JS, Zeman KL, Bennett WD. 2002. Ultrafine particle deposition and clearance in the healthy and obstructed lung. Am J Respir Crit Care Med 166:1240–1247.

Burch WM, Sullivan PJ, McLaren CJ. 1986. Technegas–a new ventilation agent for lung scanning. Nucl Med Commun 7:865–871.

Camner P, Philipson K. 1978. Human alveolar deposition of 4 micron teflon particles. Arch Environ Health 33:181–185.

Donaldson K, Stone V, Clouter A, Renwick L, MacNee W. 2001. Ultrafine particles. Occup Environ Med 58:211–6, 199.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdörster G. 2006. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114:1172–1178.

Ghio AJ, Bennett WD. 2007. Metal particles are inappropriate for testing a postulate of extrapulmonary transport. Environ Health Perspect 115:A70; author reply A70–A70; author reply A71.

Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdörster G, 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 Part A 65:1513–1530.

Kreyling WG, Semmler-Behnke M, Seitz J, Scymczak W, Wenk A, Mayer P, Takenaka S, Oberdörster G. 2009. Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. Inhal Toxicol 21 Suppl 1:55–60.

Li XY, Gilmour PS, Donaldson K, MacNee W. 1997. *In vivo* and *in vitro* proinflammatory effects of particulate air pollution (PM10). Environ Health Perspect 105 Suppl 5:1279–1283.

Mills NL, Amin N, Robinson SD, Anand A, Davies J, Patel D, de la Fuente JM, Cassee FR, Boon NA, Macnee W, Millar AM, Donaldson K, Newby DE. 2006. Do inhaled carbon nanoparticles translocate directly into the circulation in humans? Am J Respir Crit Care Med 173:426–431.

Möller W, Felten K, Sommerer K, Scheuch G, Meyer G, Meyer P, Häussinger K, Kreyling WG. 2008. Deposition, retention, and translocation of ultrafine particles from the central airways and lung periphery. Am J Respir Crit Care Med 177:426–432.

Monteiller C, Tran L, MacNee W, Faux S, Jones A, Miller B, Donaldson K. 2007. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells *in vitro*: the role of surface area. Occup Environ Med 64:609–615.

Morrow PE. 1973. Alveolar clearance of aerosols. Arch Intern Med 131:101–108.

Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B. 2002. Passage of inhaled particles into the blood circulation in humans. Circulation 105:411–414.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C. 2004. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 16:437–445.

Philipson K, Falk R, Camner P. 1985. Long-term lung clearance in humans studied with Teflon particles labeled with chromium-51. Exp Lung Res 9:31–42.

Philipson K, Falk R, Gustafsson J, Camner P. 1996. Long-term lung clearance of 195Au-labeled teflon particles in humans. Exp Lung Res 22:65–83.

Pope CA 3rd, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston GD. 2002. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287:1132–1141.

Pope CA 3rd, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, Godleski JJ. 2004. Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. Circulation 109:71–77.

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. 1993. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 16:5–40.

Renwick LC, Brown D, Clouter A, Donaldson K. 2004. Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. Occup Environ Med 61:442–447.z

Sanchez-Crespo A, Klepczynska-Nyström A, Lundin A, Larsson BM, Svartengren M. 2011. ^{111}Indium-labeled ultrafine carbon particles; a novel aerosol for pulmonary deposition and retention studies. Inhal Toxicol 23:121–128.

Semmler M, Seitz J, Erbe F, Mayer P, Heyder J, Oberdörster G, Kreyling WG. 2004. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. Inhal Toxicol 16:453–459.

Svartengren K, Ericsson CH, Svartengren M, Mossberg B, Philipson K, Camner P. 1996. Deposition and clearance in large and small airways in chronic bronchitis. Exp Lung Res 22:555–576.

Svartengren M, Svartengren K, Europe E, Falk R, Hofmann W, Sturm R, Philipson K, Camner P. 2004. Long-term clearance from small airways in patients with chronic bronchitis: experimental and theoretical data. Exp Lung Res 30:333–353.

Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J. 2001. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect 109 Suppl 4:547–551.

Takenaka S, Karg E, Kreyling WG, Lentner B, Möller W, Behnke-Semmler M, Jennen L, Walch A, Michalke B, Schramel P, Heyder J, Schulz H. 2006. Distribution pattern of inhaled ultrafine gold particles in the rat lung. Inhal Toxicol 18:733–740.

Wiebert P, Sanchez-Crespo A, Falk R, Philipson K, Lundin A, Larsson S, Möller W, Kreyling WG, Svartengren M. 2006a. No significant translocation of inhaled 35-nm carbon particles to the circulation in humans. Inhal Toxicol 18:741–747.

Wiebert P, Sanchez-Crespo A, Seitz J, Falk R, Philipson K, Kreyling WG, Möller W, Sommerer K, Larsson S, Svartengren M. 2006b. Negligible clearance of ultrafine particles retained in healthy and affected human lungs. Eur Respir J 28:286–290.