Eur Respir J 2006; 28: 286–290
DOI: 10.1183/09031936.06.00103805

Negligible clearance of ultrafine particles retained in healthy and affected human lungs

P. Wiebert*,#, A. Sanchez-Crespo¶, J. Seitz+, R. Falk+, K. Philipson§, W.G. Kreyling, W. Möller**, K. Sommerer+, S. Larsson¶ and M. Svartengren*,#**

ABSTRACT: Ambient particles are believed to be a specific health hazard, although the underlying mechanisms are not fully understood. There are data in the literature indicating fast and substantial systemic uptake of particles from the lung.

The present authors have developed an improved method to produce ultrafine particles with more stable radiolabelling and defined particle size range. Fifteen subjects inhaled technetium 99m (^{99m}Tc)-labelled carbonaceous particles of 100 nm in size. Radioactivity over the lung was followed for 70 h. The clearance of these ultrafine particles from the lungs and specifically translocation to the circulation was tested.

Lung retention for all subjects at 46 h was mean±SD 99±4.6%. Cumulative leaching of ^{99m}Tc activity from the particles was 2.6±0.96% at 70 h. The 24-h activity leaching in urine was 1.0±0.55%.

No evidence of a quantitatively important translocation of 100-nm particles to the systemic circulation from the lungs was found. More research is needed to establish if the ~1% cleared activity originates from leached activity or insoluble translocated particles, and whether a few per cent of translocated particles is sufficient to cause harmful effects.

KEYWORDS: Air pollution, circulation, clearance, ultrafine particles

Epidemiological studies provide evidence that air pollution contributes to systemic as well as pulmonary diseases and reactive airway effects [1–6]. These findings pertain to elderly people and susceptible persons with underlying diseases of various origins [7]. Ultrafine particles (<100 nm diameter) represent a substantial component of the particulate matter in ambient air. Ultrafine particles are more toxic and induce more severe inflammation than larger particles [8]. The mechanisms underlying the effects are largely unknown, but autonomic regulation of the heartbeat, inflammation and systemic coagulation effects, and direct metal toxicity to the heart muscle are proposed mechanisms [7, 9, 10]. It is essential to establish if insoluble particles can enter the systemic circulation, or if particles' specific effects are initiated in the lung.

Recently, NEMMAR *et al.* [11] exposed five human subjects by inhalation to technetium 99m (^{99m}Tc)-labelled ultrafine particles and observed translocation of ^{99m}Tc into the blood compartment. Leaching of the label affected the result due to the difficulty in differentiating between the solute label and labelled ultrafine particles in blood and extrapulmonary organs.

The aim of the study was to examine whether there is a significant uptake of ultrafine particles (~100 nm) into the systemic circulation, by measuring pulmonary retention and activity over the liver. The test particles were generated with a new modified method developed by the research groups involved.

MATERIALS AND METHODS

Generation of ultrafine particles

A Technegas Generator™ (Tetley Manufacturing Ltd, Sydney, Australia) was used to generate the ''Technegas'' aerosol, which is an aerosol of ^{99m}Tc-labelled ultrafine carbonaceous particles (6-h half-life) in a pure argon atmosphere. The production technique needed modification, as the aerosol normally contains a portion of soluble pertechnetate ($^{99m}TcO_4^-$; isotope loosely bound to the particles). In the current study, size distribution and number concentration of the aerosol were monitored before and after inhalation by a differential mobility particle sizer (DMPS; classifier 3071 and condensation particle counter (CPC)

AFFILIATIONS
*Dept of Public Health Sciences, Karolinska Institute,
#Dept of Occupational and Environmental Health, Stockholm County Council,
¶Dept of Nuclear Medicine, Institution of Oncology-Pathology, Karolinska Hospital,
+The Swedish Radiation Protection Authority,
§The Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden.
fIntelligent Aerosol Medicine, InAMed GmbH, and
**GSF National Research Centre for Environment and Health, Institute for Inhalation Biology, Gauting and Munich, Germany.

CORRESPONDENCE
P. Wiebert
Dept of Occupational and Environmental Health
Norrbacka
Karolinska Hospital
SE-171 76 Stockholm
Sweden
Fax: 46 8334333
E-mail: pernilla.wiebert@ki.se

Received:
September 05 2005
Accepted after revision:
April 13 2006

SUPPORT STATEMENT
The current work was supported by grants from the Swedish National Air Pollution and Health Effects Program, the European Commission (FIGD-CT-2000-00053) and the Center for Allergy Research, Stockholm.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

3010; TSI Incorporated Particle Instruments, Shoreview, MN, USA) and a CPC 3020 (TSI; detection limit 10 and 7 nm). Inhalation started 4 min after aerosol generation.

Modifications

Leaching-free particles of a controlled size were required, and to produce these, the following were carried out: 1) sodium was eliminated from the ^{99m}Tc elute; 2) the argon gas was purified; 3) the particle aerosol was diluted to reduce coagulation; and, for modulation of particle size: 4) evaporation time was modified; and 5) temperature was modified. The method was developed for the current study and is described by MÖLLER *et al.* [12].

Exposure

A fresh aerosol of radiolabelled carbonaceous particles of 100-nm diameter was produced for every subject using a Technegas Generator™ (fig. 1).

The subjects were instructed to inhale aerosol in deep and slow breaths with a breath-hold. Mean±SD deposited activity was 26±11 MBq. The specific activity of each aerosol was calculated as activity deposited on teflon filters (0.2-μm pore, PTFE; Pall Corporation, East Hills, NY, USA) divided by the aerosol volume sampled through the filter.

Measurement of retention and clearance

Activity in the chest region was measured with the subject in a supine position, immediately after aerosol inhalation and after 2, 24, 46 and 70 h (fig. 2). In the first three measurements, planar images were performed with a gamma camera (TRIAD XLT 20; Trionix, Twinsburg, OH, USA), and the subsequent measurements were made with a whole-body scanner with sodium iodide (NaI) detectors (Harshaw, Paris, France) [13].

Deposition fraction is defined as deposited activity divided by inhaled activity. The deposited activity is the inhaled activity minus exhaled activity.

A whole-lung region of interest (ROI) was placed around the borders of each lung. Retention was computed from combined counts of left and right whole-lung ROIs.

Estimates of leaching

Activity leached from the particles was estimated by the following methods: 1) estimating the number of aerosol particles collected on a filter; and 2) measurement of activity in urine.

Method 1

After exposure, the particles of the remaining aerosol in the flexible bag were collected on a 0.2-μm pore-size membrane filter TF (PTFE; Pall Corporation). Leaching studies of each aerosol were performed using the membrane filter with the collected particles, mounted in an open filter holder between two 0.025-μm pore-size nitrate cellulose filters (Schleicher & Schuell, Dassel, Germany) forming a sandwich tightly closed at its perimeter by the filter holder. The filter sandwich was submersed in 1 L of 0.9% NaCl solution. At 0.3, 1.5, 21, 45 and 70 h, the filter sandwich was temporarily removed for activity measurement in the solution.

Method 2

The second estimate of leaching was performed by measuring activity in urine [14, 15] from the subjects sampled during the first 24 h after exposure.

Subjects

Fifteen subjects, including six healthy nonsmokers, five subjects with asthma symptoms and four asymptomatic

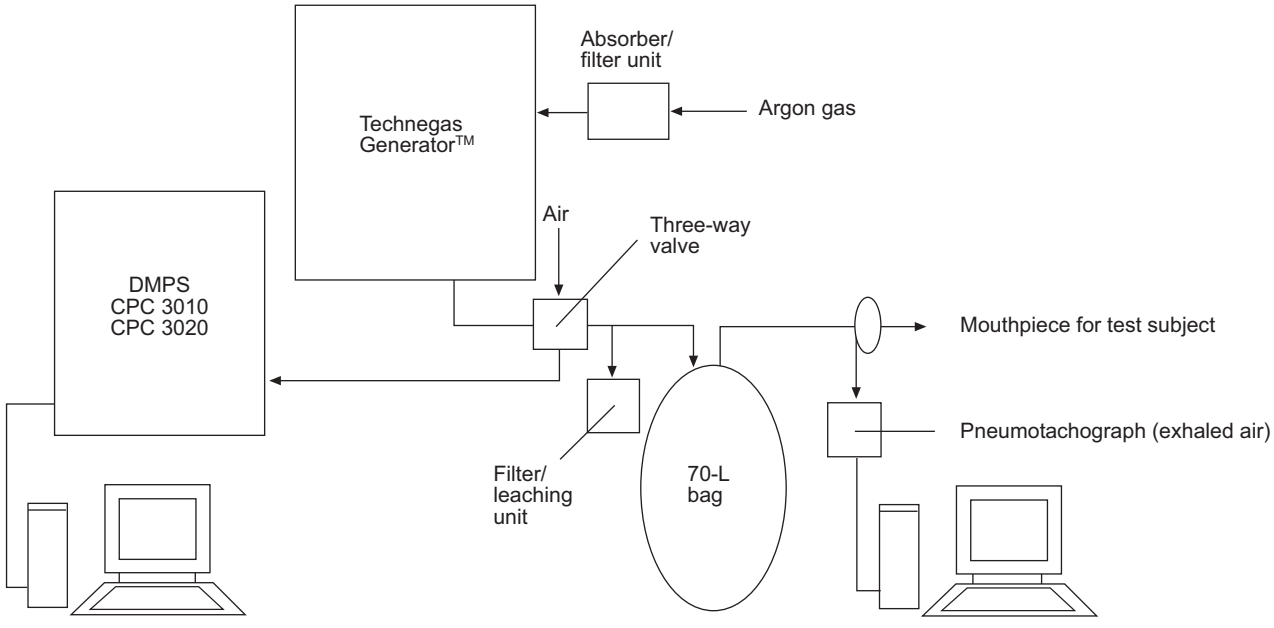

FIGURE 1. Experimental set-up. The concentrated aerosol in the Technegas Generator™ (Tetley Manufacturing Ltd, Sydney, Australia) is diluted into the flexible conductive bag (70 L). Size distribution and number concentration of the diluted aerosol is monitored before and after inhalation (differential mobility particle sizer (DMPS) classifier model 3071, condensation particle counter (CPC) models 3010 and 3020). The test subject inhales aerosol *via* a mouthpiece connected to the flexible bag.

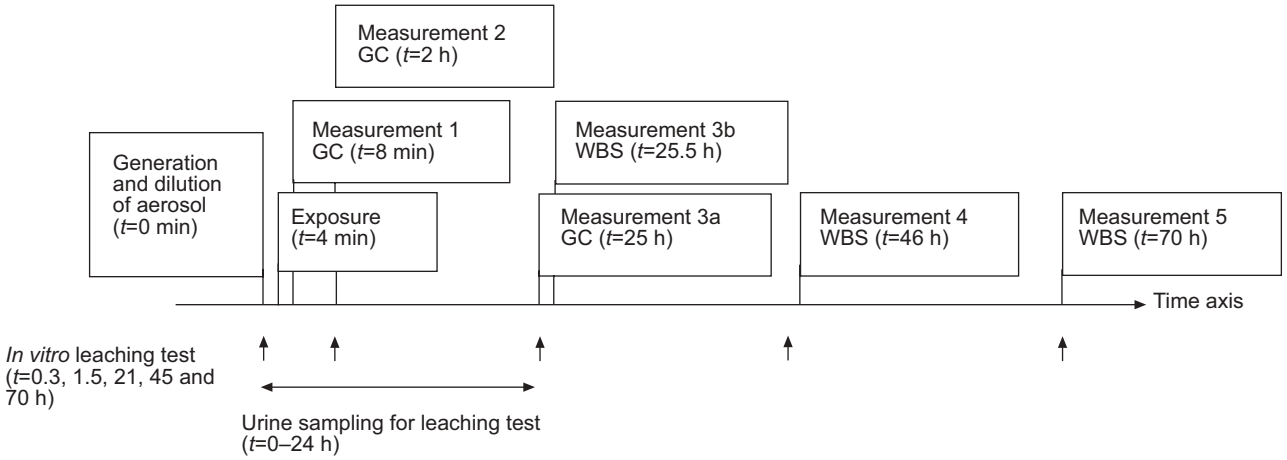

FIGURE 2. Schematic diagram showing study design. Exposure started ~4 min after aerosol generation. Activity in the lungs, thyroid and liver was measured initially by gamma camera (GC; 5+5 min planar scan of the anterior and posterior lung day 1, and 10+10 min day 2) and then by whole body scanner (WBS). The first measurement with the WBS (3b) was normalised to the last measurement with the GC (3a). *In vitro* leaching tests (↑) were performed on the remaining aerosol particles from each exposure occasion. The cumulative amount of activity leached from the particles was measured at approximately the same time interval as lung measurements were taken. In an additional leaching test, activity was measured in urine samples provided by the subjects during the first 24 h following exposure.

smokers (nine males and six females; 46–74 yrs old) participated in the current study. Median (range) pulmonary function values (forced expiratory volume in one second/forced vital capacity) were as follows: for healthy subjects, 83 (74–86); for asthmatics, 78 (56–82); and for smokers, 78 (52–85). There were no significant differences in pulmonary function between healthy and affected lungs ($p=0.166$).

Statistical analysis

Values are presented as mean±SD, unless otherwise stated.

RESULTS

Particles

The aerosols had a count median diameter of 98 nm (87 nm before inhalation; 110 nm after inhalation), a geometric SD of 1.7 (1.7–1.7), and the number concentration was 1.5×10^6 cm^{-3} (2.2×10^6-0.9×10^6; fig. 3).

Retention, clearance and pulmonary deposition

Lung retention at 24, 46 and 70 h was 99±3.0%, 99±4.6% and 99±10.4%, respectively (fig. 4). No activity could be detected in the liver or thyroid during the first and second days. The deposition fraction was 41±10%. The median value for tidal volume during exposure was 1.8 L (0.8–3.3).

Leaching

Leaching data are presented in figure 5. Cumulative leaching from particles at 70 h was 2.6±0.96%. Activity leaching in urine was 1.0±0.55% during the first 24 h. In contrast, leaching from particles produced with the standard Technegas Generator™ method was 11% within 24 h (fig. 5). Individual leaching did not correlate to individual retention.

DISCUSSION

The current authors have shown that inhaled 100-nm carbonaceous particles largely remain inside the lungs for >3 days. Except for radiotracer leaching (1%), the particles do not show significant clearance from the lung during the 70-h observation period. If translocation occurred, it was below the detection limit of the experimental approach of the current study. There is a possibility that inhaled particles may translocate into the pulmonary interstitium without reaching the circulation [16]. Other methods are required to make this distinction in particle location.

In the current study, the authors were able to produce aerosols of ultrafine carbonaceous particles labelled with ^{99m}Tc isotopes. Particle coagulation was greatly reduced, which is clear from the results of size monitoring before and after particle inhalation. Leaching estimates were assessed for all exposures.

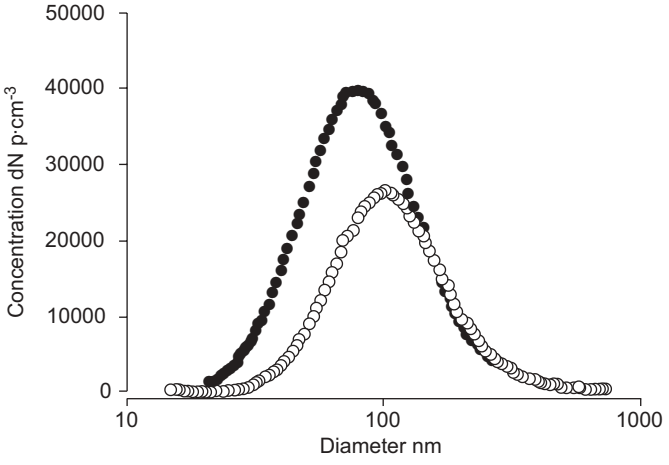

FIGURE 3. Size distribution of technetium 99m particle aerosol. Size distribution was measured by a differential mobility particle analyser before inhalation (●) and after inhalation (○). The decrease in particle number concentration and the slight shift of the spectrum to larger sizes reflects the dynamic changes of coagulation and wall losses of the aerosol within the two time-points of measurement.

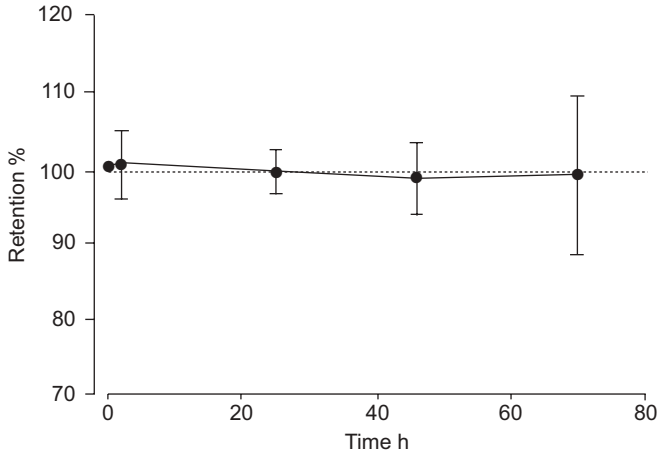

FIGURE 4. Relative retained fraction of radioactivity as a percentage of the initial lung deposition. Mean retention values of all subjects (n=15) during 70 h of observation are shown. Data from each subject were normalised to the initial measurement of the gamma camera. Error bars indicate SD.

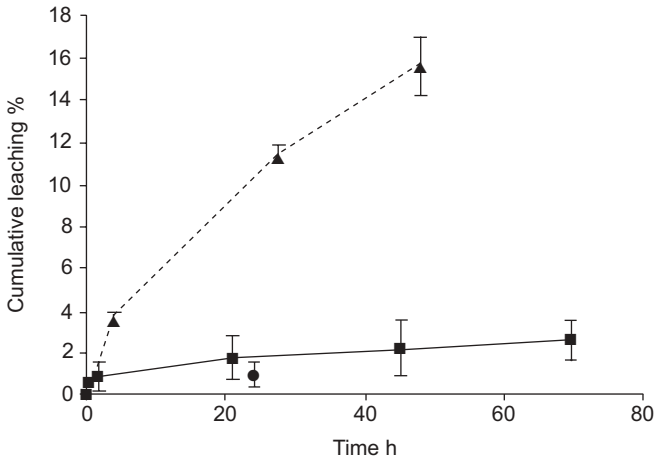

FIGURE 5. Cumulative technetium 99m leaching from particles as a percentage of initial activity. Leaching from particles generated by the modified method (■; n=15, except at 21 h (n=14) and 70 h (n=7)) is compared with the standard method (▲; n=2). Twenty-four hour cumulative urine excretion after particle exposure (●; n=15) is also shown for particles generated with the modified method (n=2). Error bars indicate SD.

Results on retention, activity in urine and leaching from particles indicate limited leaching of activity.

Lung retention was measured for 70 h with very sensitive detectors, which corresponds to a 3,300-fold natural decay in ^{99m}Tc activity. The present authors think this can explain the uncertainty seen in the measurement at 70 h.

The current study shows that a convenient way to study ultrafine particles in the human lungs is to use radiolabelled particles, such as particles generated with the Technegas Generator^{TM}. The standard use, however, has limitations. Presence of oxygen during particle generation produces pertechnetate ($^{99m}TcO_4^-$), which instantaneously leaches off the labelled particles in body fluids [17–19]. Pertechnetate assembles in the thyroid and is excreted with urine within days [14, 15]. This may contribute to the results interpreted as translocation in the study by NEMMAR *et al.* [11], in which thyroid glands and bladder were visible in the whole body scan. Particle-bound ^{99m}Tc, on the contrary, accumulates in the liver and only a limited amount is found in the bladder [20]. Another problem with the standard method is that particle size grows rapidly due to large coagulation rates. Primary Technegas particles are 5–20 nm, but coagulate to a median diameter of >100 nm within minutes if not diluted into filtered air [21–23].

No retention results are reported in the study by NEMMAR *et al.* [11], but they found 8% of the inhaled activity in the liver almost immediately after inhalation when using particles comparable to those produced with a Technegas Generator^{TM} under standard conditions. In the current study, no activity was found in the liver immediately after exposure or on day 2. This can be due to sensitivity problems, as only a small percentage of the activity escaped from the lung in the current study. The results of NEMMAR *et al.* [11], however, present evidence of leaching activity from the particles, and it is not clear whether the activity in the liver is bound to primary carbon particles or plasma proteins.

Another human study reporting declining retention values was performed by BROWN *et al.* [24], who assessed lung retention in healthy and chronic obstructive pulmonary disease patients. The ultrafine carbon particles were generated using a commercially available generator, arcing between graphite electrodes under a high-purity argon atmosphere. Lung retention was 86% 1 day after deposition, but because leaching from the particles was substantial (23%), 24-h retentions were corrected based on measured *in vitro* leaching of label from the particles. Another difference from the current study is that the subjects inhaled smaller particles (60 nm) under realistic resting breathing conditions (*i.e.* not deep breathing with breath-holding), which may have resulted in a greater fraction of particles depositing on bronchial airways, *i.e.* particles that might clear by mucociliary clearance.

Particle-bound and free activity in blood samples was not examined in the current study; this would have added valuable information about possible translocation. However, it is uncertain if the low levels of translocated activity seen in the current study were sufficient to be detected in the blood. In a recent study by MILLS *et al.* [25], humans were exposed to 108-nm Technegas particles produced by the standard method. Retention was measured for a shorter time period than in the current study; at the last measurement at 6 h, retention was 95.6%. *In vitro* leaching from the particles was slightly higher: 5% after 6 h *versus* 2.6% after 70 h in the current study. Besides retention, and similar to the approach of NEMMAR *et al.* [11], MILLS *et al.* [25] also measured blood activity and performed thin layer chromatography; however, in contrast to NEMMAR *et al.* [11], MILLS *et al.* [25] found the small amount of blood-borne ^{99m}Tc to be unbound and thus concluded that translocation of particles from the lungs to the circulation is negligible, a result confirmed by the current study.

In an animal study by KREYLING *et al.* [16] using ^{192}Ir particles, the fraction of translocated particles from the lung to

extrapulmonary organs was only <0.002 and 0.001 for deposited 15- and 80-nm particles, respectively. The ^{192}Ir particles did not leach and the results of KREYLING *et al.* [16] are in agreement with the current findings.

Conclusions

There is no evidence of a quantitatively important (mass-based) translocation of 100-nm particles to the systemic circulation from either healthy or affected lungs. The present authors therefore challenge earlier studies stipulating a rapid and substantial uptake of ultrafine particles; results of earlier studies may be a consequence of technical shortcomings.

More research is needed to establish if the ~1% cleared activity originates from leached activity or insoluble translocated particles, and whether a few per cent of translocated particles is sufficient to cause harmful effects. 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 disease, requires further detailed and differentiated consideration.

ACKNOWLEDGEMENTS

The authors would like to thank I. Östergren at the Swedish Radiation Protection Authority, and J. Hallberg and M. Alderling at the Dept of Occupational and Environmental Health, Stockholm County Council.

REFERENCES

1 Areskoug H, Camner P, Dahlén SE, *et al.* Particles in ambient air – a health risk assessment. *Scand J Work Environ Health* 2000; 26: Suppl. 1, 90–94.
2 Atkinson RW, Anderson HR, Sunyer J, *et al.* Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project. Air Pollution and Health: a European Approach. *Am J Respir Crit Care Med* 2001; 15: 1860–1866.
3 Dockery DW, Pope CA, Xu X, *et al.* An association between air pollution and mortality in six US cities. *N Engl J Med* 1993; 329: 1753–1759.
4 Goodman PG, Dockery DW, Clancy L. Cause-specific mortality and the extended effects of particulate pollution and temperature exposure. *Environ Health Perspect* 2004; 112: 179–185.
5 Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. *Circulation* 2001; 103: 2810–2815.
6 Svartengren M, Strand V, Bylin G, Järup L, Pershagen G. Short-term exposure to air pollution in a road tunnel enhances the asthmatic response to allergen. *Eur Respir J* 2000; 15: 716–724.
7 Seaton A, MacNee W, Donaldson K, Godden D. Particulate air pollution and acute health effects. *Lancet* 1995; 345: 176–178.
8 Oberdorster G, Ferin J, Gelein R, Soderholm SC, Finkelstein J. Role of the alveolar macrophage in lung injury: studies with ultrafine particles. *Environ Health Perspect* 1992; 97: 193–199.
9 Dockery DW. Epidemiologic evidence of cardiovascular effects of particulate air pollution. *Environ Health Perspect* 2001; 109: 483–486.
10 Pekkanen J, Brunner EJ, Anderson HR, Tiittanen P, Atkinson RW. Daily concentrations of air pollution and plasma fibrinogen in London. *Occup Environ Med* 2000; 57: 818–822.
11 Nemmar A, Hoet PHM, Vanquickenborne B, *et al.* Passage of inhaled particles into the blood circulation in humans. *Circulation* 2002; 105: 411–414.
12 Möller W, Felten K, Seitz J, *et al.* A generator for the production of radiolabelled ultrafine carbonaceous particles for deposition and clearance studies in the respiratory tract. *J Aerosol Sci* 2006; 37: 631–644.
13 Falk R, Philipson K, Svartengren M, Jarvis N, Bailey M, Camner P. Clearance of particles from small ciliated airways. *Exp Lung Res* 1997; 23: 495–515.
14 Beasley TM, Palmer HE, Nelp WB. Distribution and excretion of technetium in humans. *Health Physics* 1966; 12: 1425–1435.
15 Gerber GB, van Hees M, Garten CT Jr, *et al.* Technetium absorption and turnover in monogastric and polygastric animals. *Health Physics* 1989; 57: 315–319.
16 Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J Toxicol Environ Health* 2002; 65: 1513–1530.
17 Scalzetti EM, Gagne GM. The transition from technegas to pertechnegas. *J Nucl Med* 1995; 36: 267–269.
18 Xu JH, Moonen M, Johansson Å, Bake B. Dynamics of ''Technegas'' deposited in the lung. *Nucl Med Commun* 2001; 22: 383–387.
19 Isawa T, Anazawa Y, Miki M, Mahmud AM. Inhalation of pertechnegas: similar clearance from the lungs to that of inhaled pertechnetate aerosol. *Nucl Med Commun* 1995; 16: 741–746.
20 Oberdorster G, Sharp Z, Atudorei V, *et al.* Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Health* 2002; 65: 1531–1543.
21 Lemb M, Oei TH, Eifert H, Günther B. Technegas: a study of particle structure, size and distribution. *Eur J Nucl Med* 1993; 20: 576–579.
22 Lloyd JJ, Shields RA, Taylor CJ, Lawson RS, James JM, Testra HJ. Technegas and pertechnegas particle size distribution. *Eur J Nucl Med* 1995; 22: 473–476.
23 Senden TJ, Moock KH, Gerald JF, *et al.* The physical and chemical nature of technegas. *J Nucl Med* 1997; 38: 1327–1333.
24 Brown JS, Zeman KL, Bennett WD. Ultrafine particle deposition and clearance in the healthy and obstructed lung. *Am J Respir Crit Care Med* 2002; 166: 1240–1247.
25 Mills NL, Amin N, Robinson SD, *et al.* Do inhaled carbon nanoparticles translocate directly into the circulation in humans? *Am J Respir Crit Care Med* 2006; 173: 426–431.