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## Do Inhaled Carbon Nanoparticles Translocate Directly into the Circulation in Humans?

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### **ABSTRACT**

*Rationale:* Increased exposure to particulate air pollution (PM<sub>10</sub>) is a risk factor for death and hospitalization with cardiovascular disease. It has been suggested that the nanoparticulate component of PM<sub>10</sub> is capable of translocating into the circulation with the potential for direct effects on the vasculature.

*Objective:* The study's aim was to determine the extent to which inhaled technetium-99m (<sup>99m</sup>Tc)-labeled carbon nanoparticles (Technegas) were able to access the systemic circulation.

*Methods and Main Results:* Ten healthy volunteers inhaled Technegas and blood samples were taken sequentially over the following 6 h. Technegas particles were 4–20 nm in diameter and aggregated to a median particle diameter of approximately 100 nm. Radioactivity was immediately detected in blood, with levels increasing over 60 min. Thin-layer chromatography of whole blood identified a species that moved with the solvent front, corresponding to unbound <sup>99m</sup>Tc-pertechnetate, which was excreted in urine. There was no evidence of particle-bound <sup>99m</sup>Tc at the origin. <sup>99m</sup>Tc-camera images demonstrated high levels of Technegas retention (95.6 ± 1.7% at 6 h) in the lungs, with no accumulation of radioactivity detected over the liver or spleen.

*Conclusions:* The majority of <sup>99m</sup>Tc-labeled carbon nanoparticles remain within the lung up to 6 h after inhalation. In contrast to previous published studies, thin-layer chromatography did not support the hypothesis that inhaled Technegas carbon nanoparticles pass directly from the lungs into the systemic circulation.

Epidemiologic studies have demonstrated that exposure to air pollution is an important risk factor in the development of cardiovascular disease (1–3). The World Health Organization identifies particulate matter with an aerodynamic diameter of less than 10  $\mu\text{m}$  ( $\text{PM}_{10}$ ) as the most hazardous component of air pollution. However, only fine particles with a diameter less than 2.5  $\mu\text{m}$  ( $\text{PM}_{2.5}$ ) are able to penetrate to the terminal bronchioles and proximal alveoli to any great extent. Nanoparticles have a diameter of less than 0.1  $\mu\text{m}$ , and while constituting a small fraction of the total mass of ambient particulate matter, they represent a substantial proportion in terms of particle number. Toxicologists suggest that the nanoparticulate component of ambient particulate matter is the most potent and likely to be responsible for adverse cardiovascular health effects (4).

The precise mechanism by which air pollution influences cardiovascular risk has not been determined. However, a number of interesting hypotheses have been proposed to explain how inhaled particles could interact with the cardiovascular system (1, 5). The traditional view is that inhaled particles provoke an inflammatory response in the lungs, with consequent release of prothrombotic and inflammatory cytokines into the circulation (6). However, a recent publication by Nemmar and colleagues suggests that inhaled insoluble nanoparticles may be capable of rapid translocation into the circulation (7), with the potential for direct effects on hemostasis or cardiovascular integrity. They used an aerosol of technetium-99m ( $^{99\text{m}}\text{Tc}$ )-labeled carbon nanoparticles, Technegas (Vita Medical Ltd., Sydney, Australia), which is used routinely in clinical practice for radionuclide lung ventilation imaging. Primary Technegas particles are 7 to 23 nm in diameter and aggregate to a mean particle diameter of approximately 100 nm (8). Thin-layer chromatography of whole blood identified the presence of  $^{99\text{m}}\text{Tc}$ -labeled carbon nanoparticles in the bloodstream as early as 1 min after Technegas inhalation.

Although a number of relevant studies have been conducted in animals (9, 10), the work by Nemmar and colleagues is the only study to date that suggests translocation of insoluble inhaled nanoparticulate to the circulation in humans. Alternative explanations for Nemmar and colleagues' findings have been proposed, which focus on the method of Technegas generation (11, 12). In particular, the rapid clearance of  $^{99\text{m}}\text{Tc}$  from the lungs in this study suggests the presence of large quantities of soluble unbound  $^{99\text{m}}\text{Tc}$  in both the aerosol and bloodstream. The original study did not report the proportion of  $^{99\text{m}}\text{Tc}$  remaining in the lung, preventing comment on the importance of this clearance mechanism. As part of a study into the vascular effects of combustion-derived nanoparticulate, we reinvestigate whether inhaled Technegas nanoparticles enter the circulation in humans. Some of the results of this study have been previously reported in the form of an abstract (13).

## **METHODS**

Ten healthy nonsmoking volunteers (five male, five female; aged 21–24 yr) participated in the study, which was undertaken with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki, and with the written, informed consent of each subject. Volunteers were lifelong nonsmokers with normal lung function and no regular exposure to dust or passive smoke in the workplace. None of the volunteers had infective illnesses or received medication other than oral contraception in the week before the study.

Immediately after aerosol generation, subjects inhaled approximately 100 MBq of Technegas in three breaths via a mouthpiece according to standard clinical procedure (14). Subjects rinsed their mouths with water after inhalation to

minimize delivery to the gastrointestinal tract. Blood samples for thin-layer chromatography and measurement of activity were collected via a 17-gauge intravenous cannula into 2.9-ml citrated blood tubes (Sarstedt-Monovette, Nümbrecht, Germany) at 1, 10, 30, 60, and 360 min after Technegas inhalation. A urine sample was collected 60 min after inhalation. The activity of each sample was measured with a  $\gamma$  counter (Cobra II Auto-Gamma; Packard Bioscience Co., Frankfurt, Germany), decay corrected to the time of Technegas inhalation, and expressed as counts per minute per gram of whole blood or urine.

### **Technegas Generation**

Technegas was generated using a commercially available generator (Technegas generator; Vita Medical Ltd., Sydney, Australia) and following the manufacturer's recommended protocol (15). Briefly, 0.14 ml of sodium  $^{99m}\text{Tc}$ -pertechnetate ( $\text{Na}^{99m}\text{TcO}_4$ ) solution (2 GBq/ml) was added to a graphite crucible. The solution was evaporated to dryness during the 10-min simmer phase. The Technegas was then generated during the burn phase in which the crucible was resistively heated at 2,550°C for 15 s in an atmosphere of 99.998% argon.

To obtain samples for *in vitro* studies, Technegas was drawn through 10 ml of liquid in a conical flask; distilled water was used for electron microscopy and 0.9% sodium chloride in water for thin-layer chromatography.

### **Particle Sizing**

Samples for particle sizing by transmission electron microscopy were prepared by placing one drop of the Technegas suspension on a copper grid coated with a carbon film. The grid was left to dry in air at room temperature. Particles were sized using a transmission electron microscope (JEM-1200EX; JEOL, [UK] Ltd., Herts, UK) working at 80 kV. Particle size distribution was evaluated using an automatic image analyzer and Origin software (OriginLab Corp., Northampton, MA). The size distribution of aggregates in the aerosol was measured at the outlet of the Technegas generator by a scanning mobility particle sizer (TSI, Inc., Shoreview, MN), which consists of a condensation particle counter and a differential mobility analyzer, and can detect aerosol particles in the size range from 7 to 487 nm.

### **Thin-Layer Chromatography**

Thin-layer chromatography was performed on 20- x 2.5-cm silica gel-impregnated glass fiber plates (Pall Corporation, New York, NY) with a mobile phase of 0.9% sodium chloride in water. A 50- $\mu\text{l}$  blood sample was applied 2.5 cm from the bottom of the plate. The plate was placed immediately in the mobile phase and the solvent front was allowed to rise 12 cm. The plate was cut into 1-cm-high transverse strips starting 0.5 cm below the origin. This resulted in 13 strips. These were placed into tubes in pairs with only a single strip in the final tube. The count rate from each tube was measured using an automatic  $\gamma$  counter (Cobra II Auto-Gamma; Packard Bioscience Co.). Each strip was counted for 10 min. The chromatogram was constructed by expressing the count rate from each tube as a percentage of the total of the count rates from all seven tubes. A blood sample from one subject was analyzed in triplicate to assess repeatability of the thin-layer chromatographic technique.

Control samples consisting of Technegas in 0.9% sodium chloride solution, sodium  $^{99m}\text{Tc}$ -pertechnetate solution, whole blood + Technegas solution, and whole blood + sodium  $^{99m}\text{Tc}$ -pertechnetate solution were prepared and analyzed by thin-layer chromatography. The potential for dissociation of the particle-bound  $^{99m}\text{Tc}$  in Technegas to unbound  $^{99m}\text{Tc}$ -pertechnetate *in vitro* was assessed by thin-

layer chromatography of the Technegas sample in 0.9% sodium chloride solution at 1, 60, and 360 min after aerosol generation.

### Radionuclide Imaging

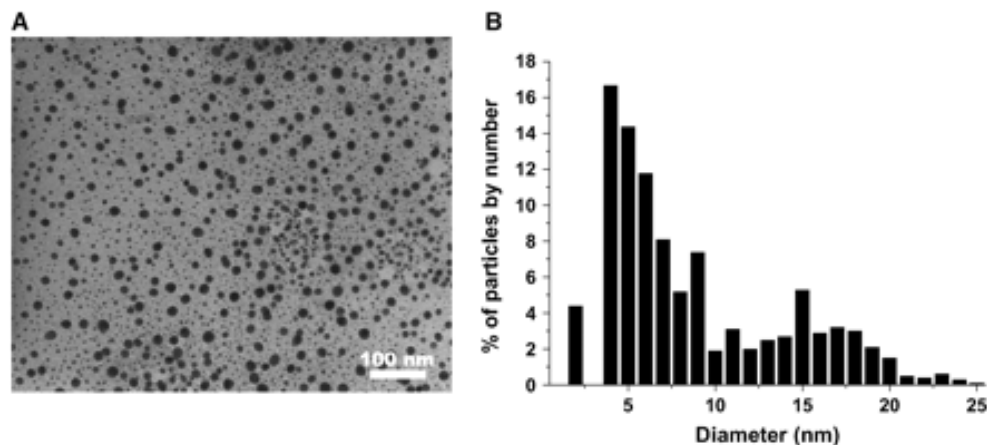
Between 0 and 5 min after inhalation of the Technegas, a static image of the thorax was acquired with a GE Millennium Tcamera (General Electric, Giles, UK). This was followed by the acquisition of 10 dynamic abdominal images between 5 and 55 min. Further 5-min static thoracic and abdominal images were acquired at 60 and 360 min to assess pulmonary retention of  $^{99m}\text{Tc}$ .

### Data Analysis

All results included a correction for background radioactivity and the decay of  $^{99m}\text{Tc}$  (half-life = 6.02 h), with values presented as mean  $\pm$  SEM. The total amount of radioactivity in the lung was recorded as total counts from each 5-min static image taken at 0, 60, and 360 min. The bladder and left and right upper quadrants of the abdomen were selected as regions of interest on the dynamic scans. Radioactivity within each region of interest was recorded as counts per pixel from each 5-min abdominal image and expressed as a percentage of initial lung radioactivity.

## RESULTS

A size distribution study of Technegas particles ( $n = 1,500$ ) using transmission electron microscopy indicated that the majority of particles were 4 to 20 nm in diameter (Figure 1). There appeared to be a bimodal distribution, where more than two-thirds of all particles were less than 10 nm in diameter with a mode of 4 nm, one-third were larger with a mode of 15 nm, and rare aggregates were seen of up to 200 nm. Inhaled particles also had a bimodal distribution with the first peak, due to primary particles, increasing exponentially to the lower detection limit of the scanning mobility particle sizer at 7 nm. The size of aggregated particles showed a log-normal distribution and typically ranged from 50 to 150 nm, with a count median diameter of 108 nm (geometric SD = 2.2 nm).



**Figure 1.** (A) Transmission electron micrograph of Technegas particles and (B) a size distribution study of 1,500 Technegas particles

Radioactivity was detected in whole blood within the first minute, reaching a maximum at 60 min, and declining thereafter (Figure 2).

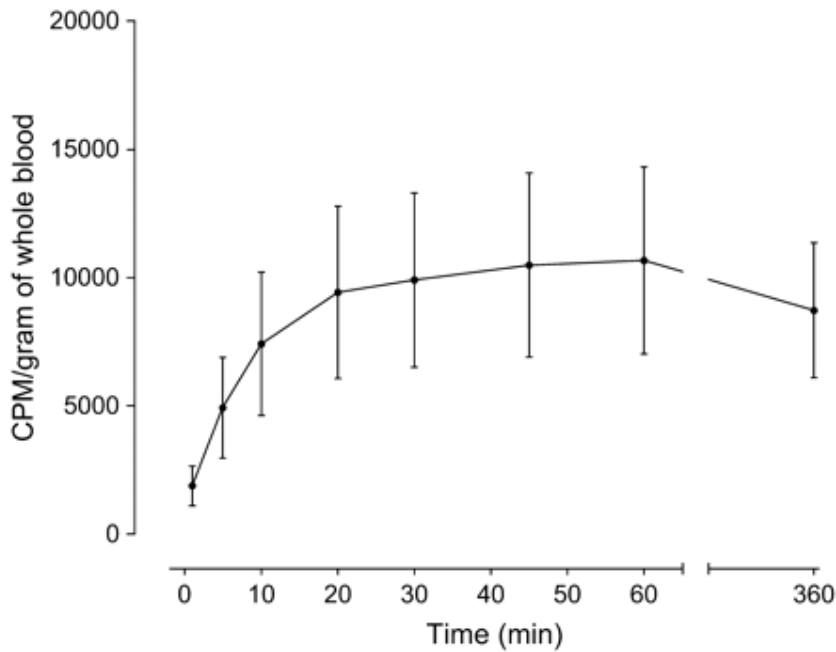


Figure 2. Radioactivity measured as counts per minute (CPM) per gram of whole blood at intervals after inhalation of Technegas. Values are mean  $\pm$  SEM.

### Thin-Layer Chromatography

In the chromatograms obtained from blood samples taken at 1, 10, 30, and 60 min after Technegas inhalation, there was little evidence of  $^{99m}\text{Tc}$  at the origin, but a consistent peak trailing the solvent front was seen at all time points (Figure 3). The large error bar at the origin, seen at 1 min, was due to a single outlier. A small peak was present at the origin at 360 min.

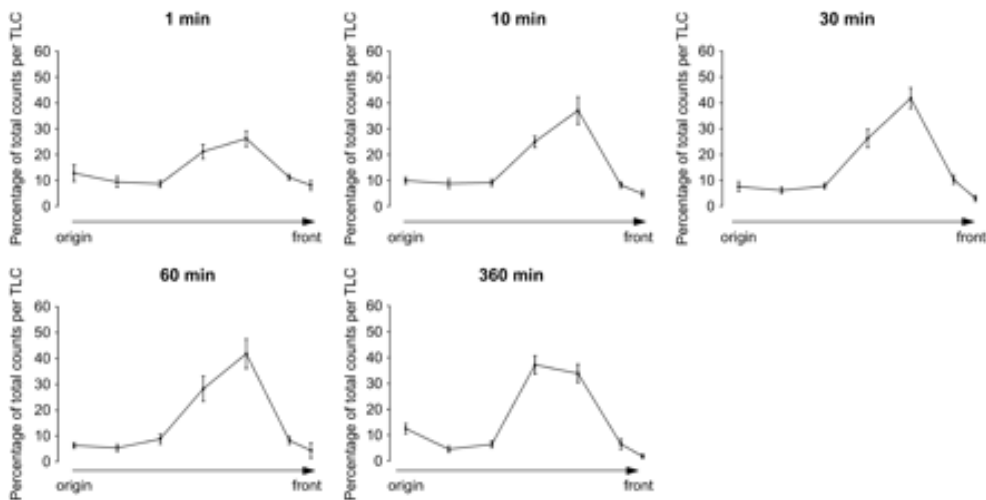


Figure 3. Thin-layer chromatograms from whole blood samples taken 1, 10, 30, 60, and 360 min after inhalation of Technegas. TLC = thin-layer chromatography. Values are mean  $\pm$  SEM

Chromatography of sodium  $^{99m}\text{Tc}$ -pertechnetate solution resulted in a single peak at the solvent front (Figure 4A). Chromatography of Technegas aerosol solution (Figure 4B) resulted in two peaks, one at the origin and one at the solvent front. This is consistent with the presence of both particle-bound  $^{99m}\text{Tc}$  and unbound  $^{99m}\text{Tc}$ -pertechnetate. The ratio of particle-bound  $^{99m}\text{Tc}$  to unbound  $^{99m}\text{Tc}$ -pertechnetate did not change over 360 min, suggesting that the Technegas aerosol contained both particle-bound and unbound  $^{99m}\text{Tc}$  at the onset, and that dissociation of  $^{99m}\text{Tc}$  from the particles does not occur *in vitro*. A single peak was seen at the solvent front in urine sampled 60 min after Technegas inhalation (Figure 4C), suggesting that unbound  $^{99m}\text{Tc}$ -pertechnetate or a similar species is excreted in the urine.

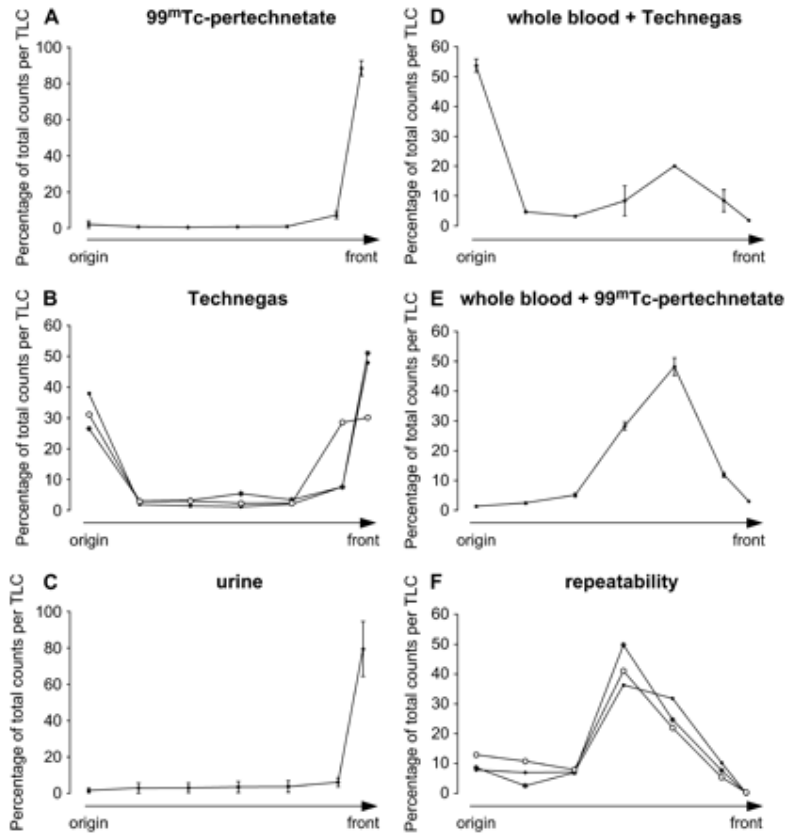


Figure 4. Thin-layer chromatography (TLC) controls: (A) Sodium  $^{99m}\text{Tc}$ -pertechnetate, showing a single peak at the solvent front; (B) Technegas aerosol showing two peaks, one at the origin and the other at the solvent front, immediately (solid circles), 60 (asterisks), and 360 (open circles) minutes after aerosol collection; (C) urine sample 60 min after inhalation showing a single peak at the solvent front; (D) Technegas aerosol added to whole blood showing peaks at the origin and trailing the solvent front; (E) sodium  $^{99m}\text{Tc}$ -pertechnetate added to whole blood showing a single peak trailing the solvent front; (F) replication of chromatography on a single blood sample. Values are mean  $\pm$  SEM.

Peaks were identified at the origin and trailing the solvent front when Technegas aerosol solution was added to whole blood (Figure 4D). The addition of sodium  $^{99m}\text{Tc}$ -pertechnetate solution to whole blood produced a single peak trailing the solvent front, with no peak at the origin or front (Figure 4E). It is probable that

this peak was due to  $^{99m}\text{Tc}$ -pertechnetate held up by an interaction with one of more components in blood. Replication of chromatography on a single blood sample confirmed good repeatability of our technique (Figure 4F).

### Radionuclide Imaging

The average decrease in lung radioactivity was  $3.2 \pm 0.7\%$  over the first hour, and  $1.2 \pm 1.7\%$  over the subsequent 5 h (Figure 5A). Radioactivity was present within the gastrointestinal tract and bladder, but no obvious uptake of  $^{99m}\text{Tc}$  by the liver or spleen was detected (Figure 5B). The level of radioactivity over the bladder region gradually increased with time, but did not accumulate in any other region (Figure 6).

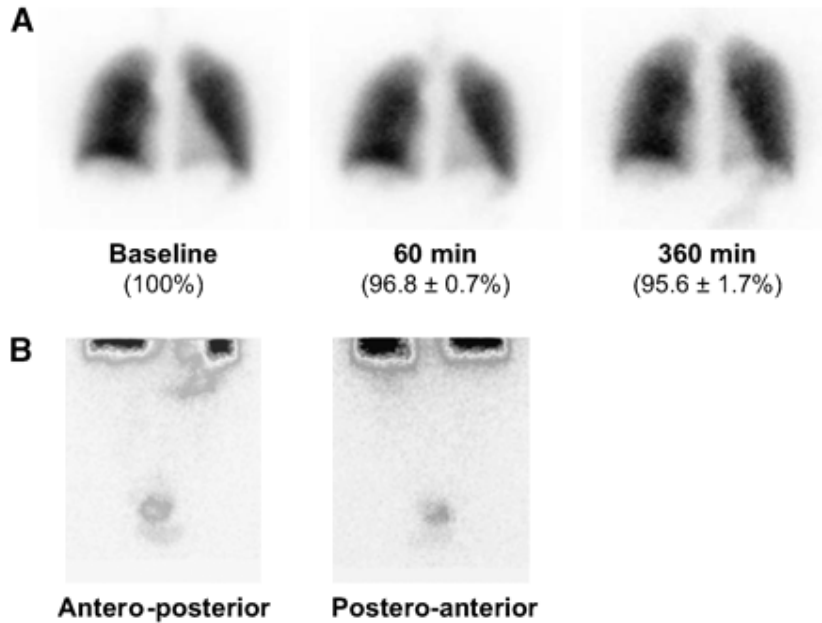


Figure 5. (A) Typical anteroposterior lung images at 1, 60, and 360 min after inhalation of Technegas. Values of retention are mean  $\pm$  SEM. (B) Typical anteroposterior and posteroanterior abdominal images recorded at 360 min postinhalation of Technegas.

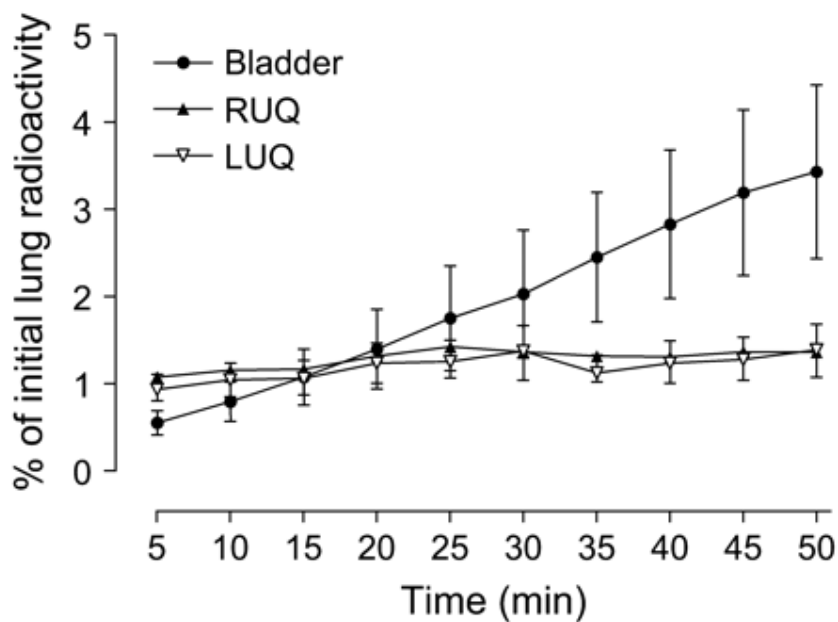


Figure 6. Time-activity curve over bladder and right and left upper quadrants (RUQ, LUQ) expressed as percentage of initial lung radioactivity. Values are mean  $\pm$  SEM

## DISCUSSION

Air pollution has been consistently linked to adverse cardiovascular events (1–3); however, the mechanism responsible for this association is not well understood. Rapid translocation of inhaled nanoparticles into the systemic circulation with direct effects on the vasculature could potentially explain this association with cardiovascular risk. However, using  $^{99m}\text{Tc}$ -radiolabeled carbon nanoparticles, we found no evidence to support this hypothesis.

After inhalation of Technegas,  $^{99m}\text{Tc}$  was immediately detected in blood, with levels increasing steadily over the first 60 min. Thin-layer chromatography of whole blood identified a species that moved with the solvent, corresponding to unbound  $^{99m}\text{Tc}$ -pertechnetate, but no evidence of significant particle-bound  $^{99m}\text{Tc}$  at the origin. The  $\gamma$  camera images demonstrated high levels of  $^{99m}\text{Tc}$  retention ( $96.8 \pm 0.7\%$  at 1 h) in the lungs. Losses were explained by the accumulation of  $^{99m}\text{Tc}$  in the bladder ( $3.4 \pm 1.0\%$ ), which was confirmed as  $^{99m}\text{Tc}$ -pertechnetate by chromatography of urine.

Nemmar and colleagues also detected  $^{99m}\text{Tc}$  in the bloodstream immediately after inhalation of Technegas (7). In contrast, the majority of  $^{99m}\text{Tc}$  remained at the origin of the chromatogram, suggesting the presence of circulating particle-bound  $^{99m}\text{Tc}$ . In addition, substantial quantities of radioactivity were measured over the liver and other extrathoracic organs. The authors concluded that inhaled particle-bound  $^{99m}\text{Tc}$  rapidly translocates into the circulation, with particulate accumulating in the endorecticular system. Differences in aerosol composition, radionuclide imaging, and chromatography between our study and that of Nemmar and coworkers merit further discussion.

In the presence of even minute quantities of oxygen, the Technegas generator produces a mixed aerosol of  $^{99m}\text{Tc}$ -labeled particles and soluble oxides of  $^{99m}\text{Tc}$ -pertechnetate (Pertechnegas). Therefore, the presence of  $^{99m}\text{Tc}$  activity in the bloodstream or over extrathoracic organs does not in itself provide evidence of



particle translocation. Per technegas is cleared from the lungs with a half-life of approximately 10 min (16), and has been used clinically to assess lung epithelial permeability (17). Per technegas and the free radiolabel  $^{99m}\text{Tc}$ -per technetate behave similarly on chromatography (18), have identical clearance kinetics after inhalation (19), and identical distributions after intravenous infusion (18). The 6-h retention of approximately 95% of inhaled  $^{99m}\text{Tc}$  in our study is consistent with rates quoted by the manufacturer (11) and previous independent studies (12). The loss of 5% of  $^{99m}\text{Tc}$  over and above decay occurred within the first hour, with little further loss over the remaining 5 h. The time course of this clearance pattern is highly suggestive of the diffusion of small quantities of Per technegas into the bloodstream.

Abdominal images demonstrate a clear increase in radioactivity in the bladder, which accounted for the loss of lung radioactivity after 60 min. Chromatography confirmed that the activity in the bladder was due to  $^{99m}\text{Tc}$ -per technetate in the urine. The presence of soluble  $^{99m}\text{Tc}$  in the Technegas aerosol, the bloodstream, and in urine strongly suggests that a small amount of  $^{99m}\text{Tc}$  in the aerosol produced by our generator was in the form of  $^{99m}\text{Tc}$ -per technetate or Per technegas, which diffused rapidly into the circulation and was eliminated via the renal tract.

In the study by Nemmar and colleagues, radioactivity over the bladder was present early and increased steadily with as much as 25% of total initial lung radioactivity present in the bladder at 45 min (7); chromatography confirmed that this activity was due to  $^{99m}\text{Tc}$ -per technetate in urine. In addition, 10% of the initial lung radioactivity was present in the liver after 5 min and there were large quantities of radioactivity visible and unaccounted for in the thyroid, salivary glands, and stomach. From their data, we can infer that pulmonary retention of  $^{99m}\text{Tc}$  after 45 min would be no more than 65%, although this value was not formally reported. This is in stark contrast to previous studies and the present study, and led to the highly plausible suggestion from one correspondent that large quantities of soluble  $^{99m}\text{Tc}$  had been produced by a generator contaminated with oxygen (11).

Given the very low pulmonary retention rates and the rapid accumulation of  $^{99m}\text{Tc}$ -per technetate in urine, it is surprising that chromatography of the aerosol in Nemmar and coworkers' study suggests it consisted only of  $^{99m}\text{Tc}$ -labeled particles with no  $^{99m}\text{Tc}$ -per technetate or Per technegas. Despite using the same generator and purity of argon, this was not the case in our study or in a previous study (20). Scalzetti and Gagne used thin-layer chromatography to investigate the effect of increasing the ratio of oxygen to argon in the generator on the transition of Technegas to Per technegas (20). When using the recommended highly purified argon (99.998% purity), as in our study, they still found approximately 20% of the  $^{99m}\text{Tc}$  migrated with the solvent front. In oxygen concentrations of 0.1% or more, an abrupt transition from Technegas to Per technegas occurred, with more than 50% of the  $^{99m}\text{Tc}$  moving with the solvent front (20).

Approximately 5% of inhaled  $^{99m}\text{Tc}$  in our study was in the form of unbound  $^{99m}\text{Tc}$ -per technetate and diffused rapidly into the circulation before elimination via the renal tract. Thin-layer chromatography identified this small quantity of  $^{99m}\text{Tc}$ -per technetate in the bloodstream as a consistent and reproducible peak trailing the solvent front. In the study by Nemmar and colleagues, 25% of  $^{99m}\text{Tc}$  was filtered by the kidneys within the first 45 min, yet chromatography did not reliably identify unbound  $^{99m}\text{Tc}$ -per technetate in the bloodstream; the largest peak occurred at the origin and was ascribed to particle-bound  $^{99m}\text{Tc}$ . To explain the lack of consistency between chromatograms of the aerosol, blood, and urine,

Nemmar and colleagues suggested that soluble oxides of  $^{99m}\text{Tc}$ -pertechnetate may be being produced *in vivo* (7). Although plausible, there was no evidence of *in vitro* oxidation in either Nemmar and coworkers' or our own control studies.

It is possible that differences in chromatography technique could explain this disparity. Nemmar and colleagues dried the blood sample at the origin of the plate before running the chromatogram (A. Nemmar, personal communication), whereas we placed it immediately into the mobile phase. It is possible that a period of drying would favor  $^{99m}\text{Tc}$  retention at the origin. Interestingly, in our study, there was a small but reproducible peak of activity at the origin in blood sampled 6 h after inhalation. This may reflect particle-bound activity due to translocation via a different mechanism involving a slower active transfer of particles from the lung into the circulation. Alternatively, the activity may be intracellular due to phagocytosis of particulate in the lung alveolar space or interstitium by cells of the monocyte/macrophage lineage, which then enter the circulation. Further studies are required to determine the significance of this finding. Thin-layer chromatography is an indirect method of particle detection, and ideally, a more direct strategy could be used to track particles in the bloodstream. Techniques such as electron microscopy would be limited by the small concentration of particles, difficulty resolving structures in the nanoparticulate range, and the low electron density of carbon.

In the study by Nemmar and colleagues (7), 10% of the initial lung radioactivity was present in the liver after 5 min, which the authors suggest is due to the accumulation of circulating particles in the Kupffer cells. If most of the circulating  $^{99m}\text{Tc}$  were particle bound and cleared in this way, then radioactivity should increase over time in the liver. However, it did not, but rather increased steadily in the bladder. It is possible that the large amount of activity measured over the liver was not associated with the liver and simply reflected scatter from the lung. An alternative explanation could relate to the vascularity of this organ. If there is a large quantity of circulating unbound  $^{99m}\text{Tc}$ , either as Pertechnegas from a generator contaminated with oxygen or from oxidation *in vivo*, then radioactivity over highly vascular organs might well be high. Furthermore, it would remain static, as hepatic blood flow is unlikely to change during the course of the study, or even decrease, because  $^{99m}\text{Tc}$ -pertechnetate is filtered by the kidneys.

In our study, the only discernable pattern of radioactivity across the abdomen, other than the bladder, was suggestive of gastrointestinal uptake, which is likely to be due to either mucociliary clearance of particles from the bronchial tree or swallowing of particles that had deposited in the mouth. The small quantities of radioactivity detected in the left and right upper quadrants likely reflect scatter from the lung or overlap of the lung and liver parenchyma. This absence of appreciable radioactivity in the liver or spleen is consistent with previous animal (21) and human studies (12). There were small amounts of activity in the thyroid and stomach at 60 and 360 min after inhalation, both glandular organs in which  $^{99m}\text{Tc}$ -pertechnetate is known to localize.

The ability of nanoparticles to cross the lung–blood barrier is likely to be influenced by particle size. Differences in size of the primary particle and in the degree of particle aggregation could potentially explain the differences between study results. In our aerosol, the majority of particles were singlet and less than 10 nm in diameter, but these rapidly formed aggregates of approximately 100 nm in diameter in the inhaled aerosol. Nemmar and colleagues identified 5-nm particles (7), but the average particle size and the size distribution of aggregated particles in the inhaled aerosol were not reported. During our study, a standard protocol for generating and administering Technegas was followed, making it

unlikely that particles would aggregate to a greater or lesser extent than in previous studies.

The escape of nanoparticulate from the lung into the bloodstream has been advanced as a mechanism for the adverse cardiovascular effects of air pollution, although "engineered" nanoparticles in the nanotechnology industry could pose the same risks (22). The fact that Technegas nanoparticles do not translocate directly into the blood in this study does not necessarily mean that other nanoparticles behave similarly, nor does it rule out an interaction between inhaled particulate and the vasculature. Environmental combustion-derived nanoparticulate, as a carrier of soluble organic compounds from unburned hydrocarbon fuels (23) and oxidized transition metals (24), may well exert an important influence on the cardiovascular system.

In conclusion, our results suggest that small quantities of soluble  $^{99m}\text{Tc}$  species, not  $^{99m}\text{Tc}$ -radiolabeled nanoparticles, are responsible for the increase in radioactivity found in the bloodstream after Technegas inhalation. Robust chromatography and clear radionuclear imaging provide support for this statement. The low pulmonary retention rates of  $^{99m}\text{Tc}$  and whole body images reported in Nemmar and colleagues are suggestive of a high degree of Pertechnegas contamination (7). However, thin-layer chromatographic analysis of their aerosol, blood, and urine are not entirely consistent with this, and in light of our findings are difficult to explain. A better knowledge of the toxicokinetics of nanoparticulate is required before we can make general conclusions about the behavior of other inhaled environmental and engineered nanoparticles.

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### **FOOTNOTES**

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