# Pulmonary and Systemic Distribution of Inhaled Ultrafine Silver Particles in Rats

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The cardiovascular system is currently considered a target for particulate matter, especially for ultrafine particles. In addition to autonomic or cytokine mediated effects, the direct interaction of inhaled materials with the target tissue must be examined to understand the underlying mechanisms. In the first approach, pulmonary and systemic distribution of inhaled ultrafine elemental silver (EAg) particles was investigated on the basis of morphology and inductively coupled plasma mass spectrometry (ICP–MS) analysis. Rats were exposed for 6 hr at a concentration of 133 µg EAg $m^3$ ($3 \times 10^6$ $cm^3$, 15 nm modal diameter) and were sacrificed on days 0, 1, 4, and 7. ICP–MS analysis showed that 1.7 µg Ag was found in the lungs immediately after the end of exposure. Amounts of Ag in the lungs decreased rapidly with time, and by day 7 only 4% of the initial burden remained. In the blood, significant amounts of Ag were detected on day 0 and thereafter decreased rapidly. In the liver, kidney, spleen, brain, and heart, low concentrations of Ag were observed. Nasal cavities, especially the posterior portion, and lung-associated lymph nodes showed relatively high concentrations of Ag. For comparison, rats received by intratracheal instillation either 150 µL aqueous solution of 7 µg silver nitrate ($AgNO_3$) (4.4 µg Ag) or 150 µL aqueous suspension of 50 µg agglomerated ultrafine EAg particles. A portion of the agglomerates remained undissolved in the alveolar macrophages and in the septum for at least 7 days. In contrast, rapid clearance of instilled water-soluble $AgNO_3$ from the lung was observed. These findings show that although instilled agglomerates of ultrafine EAg particles were retained in the lung, Ag was rapidly cleared from the lung after inhalation of ultrafine EAg particles, as well as after instillation of $AgNO_3$, and entered systemic pathways. *Key words*: distribution, ICP–MS, inhalation, instillation, morphology, silver, ultrafine. — *Environ Health Perspect* 109(suppl 4):547–551 (2001). *http://ehpnet1.niehs.nih.gov/docs/2001/suppl-4/547-551takenaka/abstract.html*

An association between inhaled particulate matter and increased mortality/morbidity has been well documented by epidemiologic studies (*1*,*2*), with the cardiovascular system as the main target (*3*,*4*). Recent experimental studies using concentrated ambient particles showed cardiac effects in dogs (*5*). Of the particulate matter in the ambient air, Seaton et al. (*6*) have suggested that ultrafine (< 100 nm) particles are the main cause for cardiovascular disorders. Materials such as an elemental carbon found in flames or metallic vapor formed during combustion are likely candidates for solid ultrafine particles (*7*).

Several mechanisms linking the cardiovascular disorders to inhaled particles have been hypothesized but remain to be verified. In addition to autonomic or cytokine-mediated effects, direct interaction of inhaled materials with the target tissue must be examined to understand the underlying mechanism (*5*,*8*,*9*). Pulmonary retention and systemic redistribution of inhaled particles may be important contributing factors. It has been reported that inhaled or intratracheally instilled ultrafine particles of iron oxide, India ink, or titanium dioxide were found mainly in alveolar macrophages (*10*–*14*). It is possible that the particles can also enter the alveolar wall and lung-associated lymph nodes (*10*–*14*). A potential artifact in these studies is that the ultrafine particles were either suspensions (intratracheal instillation study) or administered at high aerosol concentrations (8–200 $mg/m^3$), which promoted particle coagulation and resulted in larger agglomerated particles (*15*). Such agglomerated particles as well as particles > 100 nm will be readily phagocytized by alveolar macrophages (*16*). Consequently, alveolar macrophages play a key role in the fate of these larger particles. However, it is currently speculated that ultrafine particles may not be readily detected and phagocytized by alveolar macrophages in the alveolar region. Instead, these ultrafine particles may directly enter the alveolar wall and subsequently the systemic circulation.

Test particles made of elemental silver (EAg) may be suitable for investigating the systemic distribution of ultrafine particles, as EAg is considered inert and not rapidly dissolved (*17*–*19*). In other studies iron oxide particles were used for similar purposes (*10*); however, unlike iron oxide the natural content of Ag in laboratory animals is negligible. Because the detection limit for Ag is 10 ng/L by inductively coupled plasma mass spectrometry (ICP–MS) analysis (*20*,*21*), the Ag content in the lung and other organs such as the heart can be analyzed even after exposure to low concentrations relevant to the environment. Our previous study showed that agglomerated EAg particles were morphologically visible for at least 7 days in both macrophage-like J774 cells (*in vitro* study) and the rat lung after intratracheal instillation (*22*). Therefore, EAg remains essentially undissolved during this time, and EAg particles can be used to mimic the distribution pattern of ambient solid ultrafine particles.

In this article, we present results on the pulmonary and systemic distribution of EAg after inhalation at a relatively low concentration based on morphology and the ICP–MS analysis. In addition, instilled agglomerated EAg particles and water-soluble silver nitrate ($AgNO_3$) were also used for comparison purposes.

## Materials and Methods

### Generation of Ultrafine Particles

Ultrafine EAg particles were generated by spark discharging through an argon atmosphere (model GFG 1000, Palas, Karlsruhe, Germany) (*23*). The size distribution and the number concentration were measured with a differential mobility analyzer (model EMS 150, Hauke, Gmunden, Austria) and a condensation nucleus counter (model 3022A, TSI, St. Paul, USA). The particles were either used immediately for inhalation exposure or collected on polytetrafluroethylene filters (pore size 0.2 µm; Sartorius, Goettingen, Germany) for subsequent intratracheal instillation.

### Animals

Female Fischer 344 rats (body weight 150–200 g) were obtained from Charles River (Sulzfeld, Germany) and housed in an animal facility under filtered air (22 ± 2°C, 50 ± 5% relative humidity). They received a standard pellet diet and water *ad libitum*.

This study was conducted under federal guidelines for the use and care of laboratory

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animals and was approved by the Government of the District of Upper Bavaria and by the animal care and use committee of this research center.

### Inhalation Exposure

Sixteen animals were exposed to ultrafine EAg particles in whole body chambers (330 L in volume for 16 rats, laminar horizontal flow, ventilation exchange rate of 20 times/hr) for 6 hr at a mass concentration of 133 µg Ag $m^3$ and a particle number concentration of $3 \times 10^6$ $cm^3$ (*24*). The modal diameter of the number size distribution was 14.6 ± 1.0 nm, median 17.1 ± 1.2 nm, and geometric standard deviation 1.38. The estimated total inhaled cumulative dose was 7.2 µg according to the formula of delivered dose (*25*,*26*). Four rats each were sacrificed on days 0, 1, 4, and 7 after the exposure for morphology and elemental analysis. Eight animals were exposed to clean air in another whole body chamber and served as controls.

### Intratracheal Instillation

For comparison purposes, rats received either 150 µL aqueous solution of 7 µg $AgNO_3$ (4.4 µg Ag) (12 animals) or 150 µL aqueous suspension of 50 µg EAg (7 animals) by intratracheal instillation. Two to 4 rats each were sacrificed on days 1, 4, and 7 after instillation, and morphology and elemental analysis performed.

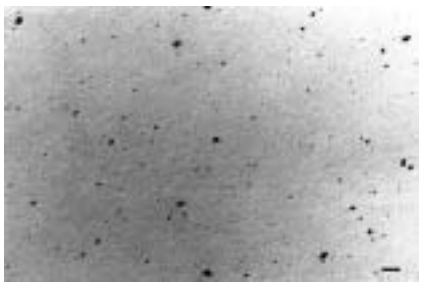

**Figure 1.** Ultrastructure of EAg-aerosols. Bar = 50 nm.

### Morphology

The ultrastructure of the Ag particles collected from the inhalation exposure air stream or the instillation aqueous suspension was examined by a transmission electron microscope. Left lobes of the lungs were fixed with 2.5% glutaraldehyde, pH 7.4, 340 mOsm in sodium potassium buffer under 25 cm pressure. Small samples were re-fixed with 1% osmium tetraoxide, dehydrated with serial alcohol and propylene oxide, and embedded in Epon. Semithin sections were stained with toluidine blue, ultrathin sections with uranyl acetate and with or without lead citrate.

### ICP–MS Analysis

Ag was quantified by ICP–MS (Element, Finnigan MAT, Germany) after pressurized digestion of tissue samples with nitric acid ($HNO_3$). The following tissues/organs were analyzed: blood from the abdominal aorta; heart, LALNs (lung associated-lymph nodes, i.e., tracheobronchial and mediastinal lymph nodes); lung (right caudal lobe); liver (middle lobe); kidney; nasal cavity (anterior portion, i.e., nasal- and maxilloturbinates plus epithelia of septum and lateral wall; posterior portion, i.e., ecto- and endoturbinates and epithelia of the septum); and brain (olfactory portion, i.e., olfactory bulb and surrounding tissues, and the rest).

### Statistical Analysis

Clearance rates of Ag in the EAg inhalation group and $AgNO_3$ instillation group were compared statistically using the Mann Whitney U-test.

## Results

### Particle Morphology

The ultrastructure of EAg particles collected from the air stream (aerosol) and the aqueous suspension is shown in Figures 1 and 2. Almost all EAg particles were compact, spherical, and electron-dense particles with diameters of 4–10 nm. Thus, the aerosol was composed entirely of ultrafine particles. In the aqueous EAg suspension, agglomerated particles larger than 100 nm were dominant, but ultrafine particles were also seen. High magnification revealed that the agglomerates were composed of ultrafine particles that were very similar in shape and size to those inhaled.

### Inhalation Studies

Rats were exposed for 6 hr at a concentration of 133 µg EAg $m^3$. Morphologic analysis showed no accumulation of particle-laden alveolar macrophages. Investigation of several tissue sections with or without contrast by lead citrate showed no EAg particles in the lung. The Ag concentrations and content in the main organs on days 0, 1, 4 and 7 are shown in Table 1. Ag in the lungs decreased rapidly with time, and by day 7 only 4% of the initial burden remained. In the blood, the Ag concentration was 8.9 ng/g on day 0, and thereafter decreased rapidly. At each time point 9–21% of lung content was observed in the liver. In the other organs, low concentrations of Ag were detected. Nasal cavities, especially the posterior portion, and the LALNs showed relatively high concentrations of Ag.

### Instillation Studies

In order to determine the persistence of particles in the lung, rats receiving aqueous suspensions of EAg or aqueous solutions of $AgNO_3$ by intratracheal instillation were examined. As shown in Figure 3, accumulation of particle-laden alveolar macrophages was observed even at 4 and 7 days after the instillation of EAg. In addition, the particles were seen within the alveolar walls. Ultrastructurally, EAg particles were located in the phagolysosomes of the alveolar macrophages (Figure 4A). At a high magnification both agglomerated and separate ultrafine particles were detectable in the

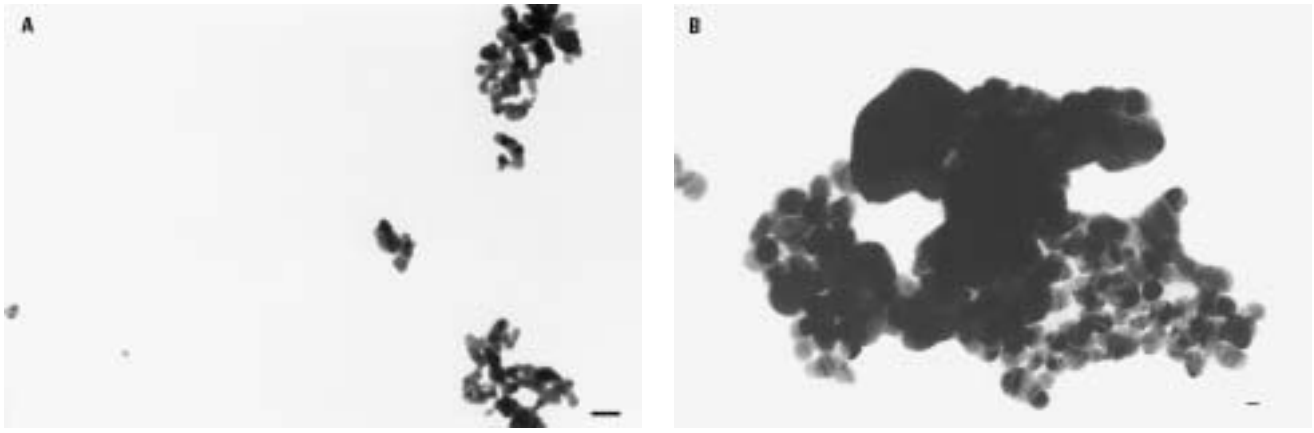


**Figure 2.** (*A*) Ultrastructure of EAg-suspension. Bar = 50 nm. (*B*) Higher magnification of *A*. Bar = 10 nm.

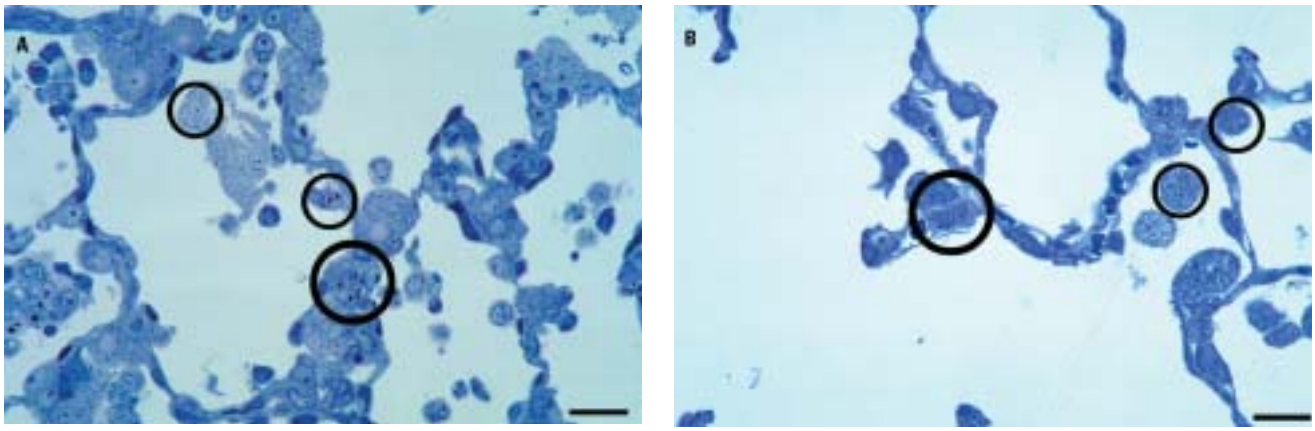


**Figure 3.** Lungs of a rat receiving 50 µg EAg particles by intratracheal instillation on (*A*) day 4 and (*B*) day 7. Circles are agglomerated particles in alveolar macrophages (small circles) and the alveolar wall (large circles). Photomicrographs are of semithin sections stained with toluidine blue. Bar = 19 µm.

phagolysosome (Figure 4B). Elemental analysis showed that 9–16 µg of the 50 µg EAg instilled was retained in the lung on day 1. By day 7, the content of Ag was almost unchanged (Table 2). A portion of the agglomerated EAg particles remained undissolved in the target tissue for at least 7 days.

In contrast, rapid clearance of instilled water-soluble $AgNO_3$ from the lung was observed (Table 3). Nevertheless, the clearance rate from days 1–7 in this group is significantly slower than that in the EAg inhalation group (Figure 5).

## Discussion

This study shows that particle size and the tendency of particles to form agglomerates affect the distribution pathway in the lungs. In the alveolar region, inhaled fine particles (> 100 nm) are readily phagocytized by alveolar macrophages. Consequently, alveolar macrophages play a key role in the fate of such particles. The total elimination of fine particles from the alveolar region may take place through three major routes: *a*) elimination of particles through the tracheobronchial tree, with subsequent ingestion into gastrointestinal tract and excretion with the feces; *b*) translocation of particles into lymph nodes; and *c*) dissolution of particles with subsequent transfer of the material into the blood (*16,27*). For relatively insoluble particles, the elimination is a slow process even in small rodents (*28*). Our results show that instilled agglomerated particles were morphologically detectable in the lung. Most instilled particles were phagocytized by alveolar macrophages. Particles were also frequently found in the alveolar walls. These findings are consistent with those of other studies involving exposure to agglomerated ultrafine particles by intratracheal instillation (*12,29*) and by inhalation of high concentrations (*11,13,14*). In a prolonged inhalation study, the disaggregation of phagocytized particles has been suggested as the cause for particles entering the alveolar wall (*13,30*). Although 50 µg Ag was instilled intratracheally, the Ag content in the lung decreased to approximately 10 µg by day 1. In addition to direct return from the trachea and mucociliary clearance of agglomerates deposited in the nonalveolar region, fast removal of ultrafine particles may be the reason for the observed rapid clearance of Ag. The remaining agglomerates were then phagocytized by alveolar macrophages and stayed in the lung for at least 7 days.

After inhalation of a low concentration of ultrafine EAg particles, we found a significant Ag content not only in the lung but also other organs such as the heart. A significant amount of Ag was also detected in the blood, which shows that systemic distribution occurred. After inhalation, rapid clearance of

**Table 1.** Ag concentrations and estimated content in tissues/organs of rats exposed to ultrafine EAg particles for 6 hr.[a]

| Tissues/organs | | Time after exposure: Immediately (30 min–2 hr) | Day 1 | Day 4 | Day 7 |
|---|---|---|---|---|---|
| Lung | Concentration[b] | 2,375(171) | 904(31) | 199(41) | 98(19) |
| | Content[c] | 1,716(169) | 656(31) | 152(35) | 75(14) |
| Liver | Concentration | 33(13) | 24(5.1) | 5.6(1.8) | 3.0(1.1) |
| | Content | 156(60) | 113(24) | 29(10) | 16(7) |
| Kidney | Concentration | ND | 39(8.1) | 4.7(4.4) | ND |
| | Content | ND | 45(10) | 5(5) | ND |
| Heart | Concentration | ND | 2.8(0.5) | 0.7(0.1) | ND |
| | Content | ND | 1.5(0.3) | 0.4(0.1) | ND |
| LALN (tracheobronchial lymph nodes) | Concentration | ND | 21(7.7) | 72(63) | ND |
| | Content | ND | 0.6(0.3) | 1.7(1.4) | ND |
| LALN (mediastinal tissues including mediastinal lymp nodes) | Concentration | ND | 6.8(0.7) | 1.6(0.1) | ND |
| | Content | ND | 2.1(0.4) | 0.5(0.1) | ND |
| Nasal cavity, anterior | Concentration | 59.2(22.6) | 13.9(2.2) | ND | ND |
| | Content | 13(6.5) | 1.8(0.5) | ND | ND |
| Nasal cavity, posterior | Concentration | 96(20.4) | 68.3(4.5) | ND | ND |
| | Content | 16.3(5.6) | 8.8(1.3) | ND | ND |
| Brain olfactory portion | Concentration | 1.9(1.1) | 3.1(1.3) | ND | ND |
| | Content | 0.3(0.02) | 0.4(0.2) | ND | ND |
| Brain rest | Concentration | 1.4(0.5) | 1.2(0.2) | ND | ND |
| | Content | 1.6(0.6) | 1.3(0.1) | ND | ND |
| Blood | Concentration | 8.9(6.2) | 6.2(0.8) | 2.9(1.5) | 1.0(0.2) |
| Unexposed | | | | | |
| Lung | Concentration | < 1.2(0.2) | | | |
| | Content | < 0.9(0.1) | | | |
| Blood | Concentration | < 0.6(0.1) | | | |

ND, not done.
[a] $n = 4$ for each examination (for unexposed lungs $n = 8$). [b] Concentration: ng/g wet weight, mean (SD). [c] Estimated content: concentration × organ weight; ng, mean (SD).

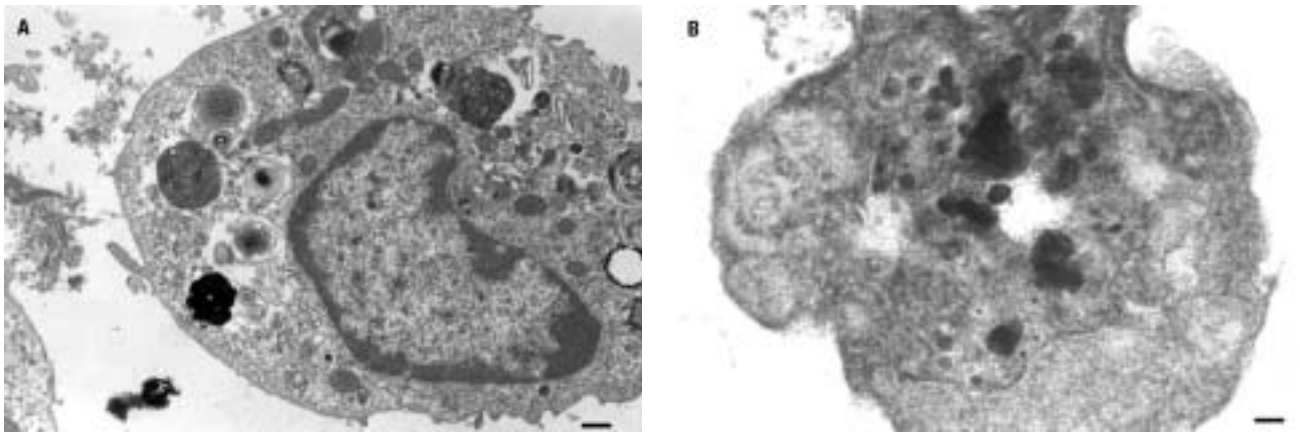


**Figure 4.** (*A*) Ultrastructure of EAg particles in an alveolar macrophage of a rat receiving 50 µg EAg particles by intratracheal instillation on day 4. Bar = 2.5 µm. (*B*) Higher magnification of *A*. Bar = 50 nm. Staining is lead citrate and uranyl acetate.

**Table 2.** Estimated Ag content in the lung and liver of rats receiving 50 µg EAg by intratracheal instillation.[a]

| | Time after instillation | | |
|---|---|---|---|
| | Day 1, µg (SD) | Day 4, µg (SD) | Day 7, µg (SD) |
| Lung | 12.9[a] (16.4, 9.4) | 16.3 (16.5, 16.3, 16.2) | 13.6 (15.7, 11.4) |
| Liver | 0.35 (0.55, 0.14) | 0.42 (0.58, 0.39, 0.28) | 0.41 (0.51, 0.31) |

[a]*n* = 2 or 3 rats.

**Table 3.** Estimated Ag content in the lung and liver of rats receiving 7 µg $AgNO_3$ (4.4 µg Ag) by intratracheal instillation.[a]

| | Time after instillation | | |
|---|---|---|---|
| | Day 1, µg (SD) | Day 4, µg (SD) | Day 7, µg (SD) |
| Lung | 1,038.4 (445.4) | 333.1 (89.9) | 257.4 (57.9) |
| Liver | 357.4 (13.0) | 186.6 (79.7) | 66.7 (35.5) |

[a]*n* = 4 for each examination.

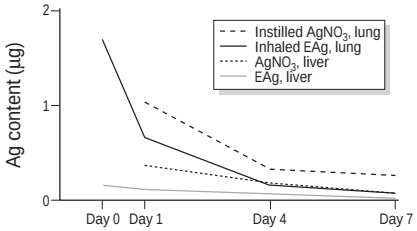


**Figure 5.** Ag content in the lung and liver of rats receiving either inhaled EAg or instilled $AgNO_3$ (see also Tables 1 and 3). Statistically significant difference in pulmonary clearance rate from days 1–7 between 2 groups ($p < 0.05$).

Ag from the lung was observed. A possible mechanism for the fast clearance is as follows: Whereas agglomerated EAg particles remain undissolved in alveolar macrophages, ultrafine EAg particles are dissolved rapidly in the lung and Ag enters the blood capillaries by diffusion (*31–33*). We found that instilled water-soluble $AgNO_3$ was cleared rapidly from the lung, which supports the theory of rapid solubilization of ultrafine EAg in the lung. Nevertheless, the clearance rate for the $AgNO_3$ instillation group from days 1–7 was lower than that for the EAg inhalation group. The retardation of $AgNO_3$ clearance may partly be due to the binding affinity of Ag ions onto proteins. After ingestion of $AgNO_3$ in drinking water or intravenous injection, Ag was detected in the cellular component/basement membrane in the tissues (*34,35*). In our study a portion of instilled Ag may have combined with cellular components in the lung with subsequent retention. As we have no evidence on the binding of Ag in the lung in this experiment, further study is required.

However, another mechanism for rapid clearance of inhaled EAg may also be possible. Ultrafine particles entering the alveolar wall might gain access to the blood capillaries. In this case, no prior dissolution process is necessary. Morrow (*31*), Stradling et al. (*36*), Raabe (*37*), and Ferin et al. (*14*) proposed that the blood capillaries are a likely clearance route for ultrafine particles. Previous studies showed that instilled colloidal carbon particles (30-nm diameter for primary particles) have been found in alveolar macrophages, alveolar type I cells, and the septal interstitium but not in blood capillaries (*12,29*). However, Stearns et al. (*38*), using analytical electron microscopy, demonstrated an unusual presence of ultrafine copper oxide particles in the septal blood capillaries. Kanapilly et al. (*39*) found a difference in pulmonary deposition and retention between 20-nm gallium oxide aerosols and 100-nm aggregates of the same material. In their study 20-nm particles were cleared more rapidly from the lung than 100-nm particles. The authors commented that because the same primary size of the same material was used in the study, the different clearance could not be explained by a difference in solubility and therefore was due to unknown mechanisms. A possible explanation for their results is that because of their very small size, the ultrafine particles were not efficiently phagocytized by macrophages and instead were cleared rapidly through the circulatory system.

In the future, ultrafine particles with other properties, e.g., solubility, size or binding affinity, must be used in studies to further understand the fate of inhaled ultrafine particles.

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