

Experimental studies on the effects of ultrafine particles

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Ultrafine particles are usually defined as particles smaller than 20 or 40 nm and sometimes less than 100 nm. Such particles have, for several reasons, been regarded as a tentative cause of the health effects associated with particles found in a large number of epidemiologic studies (see the section "Epidemiologic Studies on the Health Effects of Ambient Particulate Air Pollution"). Ultrafine particles are produced in combustion processes. They occur in large numbers in the ambient air (see the section "Particles in the Ambient Atmosphere"). The mass fraction of ultrafine particles is, however, small because ultrafine particles rapidly form aggregates. One crucial question concerning the role of ultrafine particles is whether the toxicity of inhaled particles is determined by the number, the surface area, or the mass of particles. If the first two alternatives are correct, it is obvious that ultrafine particles are of great importance. For example, one 2- μm particle has the same mass as 1×10^6 20 nm particles, and the same surface area as 1×10^4 20 nm particles. Furthermore, the deposition in the lungs, especially in the alveolar region, is high for ultrafine particles (see the section "Deposition and Clearance of Particles in the Human Lung"). There are also toxicologic data which can be considered evidence for the importance of ultrafine particles in causing toxic effects. One set of evidence comes from studies of the effect of heated perfluoropolymers such as polytetrafluoroethylene and tetrafluoroethylene-hexafluoropropylene copolymer, which have long been known to be extremely toxic to the lungs. Recent studies indicate that ultrafine particles play an important role in these effects. Another set of evidence comes from studies of exposure of the lung to aggregates of ultrafine particles. These aggregates have been found to be more toxic than solid particles of the same size as the aggregates and of the same material.

Heated perfluoropolymers

Already in 1955, Zapp et al (1) found that the inhalation of heated perfluoropolymers was highly toxic in animal experiments. Inhalation exposure of rodents to heated perfluoropolymer fumes induced massive acute lung injury with focal hemorrhage, pulmonary edema, and necrosis of tracheobronchial epithelium (2, 3). These severe effects on the lung could be avoided when rats were exposed via a filter or by letting the combustion products age (4, 5). The median particle diameter was estimated to be 30 nm by Warheit et al (5). The reduction of the toxic effects by aging was probably

caused by aggregation of the ultrafine particles. These results suggest that severe toxic effects are caused by ultrafine particles, either because of the effect of the particles themselves or because of toxic compounds on the surface of the particles. However, Seidel et al (6) found that 10-minute exposure with a particle concentration of 200 $\mu\text{g}/\text{m}^3$ of fumes from heated perfluoropolymers was mortal to rats, even when the heating temperature was so low (370°C) that no free radicals were present in the fumes.

Lee & Seidel (7) exposed rats to fresh fumes, aged fumes, filtered fumes, and reheated fumes from perfluoropolymers generated at a low temperature (370°C). The particle sizes in the fresh fumes were in the range of 20–70 nm. Exposure to 200 $\mu\text{g}/\text{m}^3$ for 30 minutes killed eight of eight rats. In the aged fumes, there was a small number of individual particles within the size range of 20–70 nm, but many particles formed aggregates ranging from 0.5 to 4 μm . Exposure to a 400 $\mu\text{g}/\text{m}^3$ concentration of the aged fumes for 30 minutes killed 4 of 24 rats. The 18 rats that were exposed for 30 minutes to the filtered fumes showed no abnormal clinical signs, and the four rats that were taken for pathological evaluation had normal lungs. The reheated fumes (240°C) consisted of numerous particles measuring 20–200 nm and aggregates up to 800 nm. Two of three rats died after exposure to 760 $\mu\text{g}/\text{m}^3$ of the reheated fumes for 30 minutes.

Oberdörster et al (8) exposed rats for up to 30 minutes to fumes of heated polytetrafluoroethylene, generated at 405–425°C. The particle concentration varied in the range of 0.74– 1.0×10^6 particles/ cm^3 . The number median diameter was 26.2 nm with a geometric standard deviation of 1.44. On the assumption of unit density (1 g/cm^3), 1×10^6 particles/ cm^3 was calculated to have a mass of 64 $\mu\text{g}/\text{m}^3$. Several rats were killed by exposure to these fumes. The lavage fluids of these rats were largely contaminated with red blood cells, which indicated severe injury to the capillary-epithelial barrier, and the pathological evaluation of the lungs showed acute hemorrhagic inflammatory edema. In the surviving rats, the polymorphonuclear leukocytes in the lavage fluid were markedly increased (by a factor of 100) 4 hours after the exposure.

In a later study by the same group (9), rats were exposed to fumes from heated polytetrafluoroethylene (450–460°C) for 15 minutes. The number median diameter was 18 μm , and the particle concentration was 0.5×10^6 particles/ cm^3 , which is equivalent to about 50 $\mu\text{g}/\text{m}^3$. Four hours after the exposure, the lungs were lavaged. Again, there was a massive increase in

Table 1. Total number of cells and neutrophils in lavage fluid after the tracheal instillation of aggregates of fine or ultrafine particles.

	Control	Fine particles	Ultrafine particles
Total number of cells in the lavage	1.15×10^7	1.48×10^7	4.68×10^7
Neutrophils in the lavage	1.6%	8.9%	50.5%

neutrophils. The exposure also initiated an antioxidant and cytokine response for compounds produced from inflammatory, epithelial, and endothelial cells.

Oberdörster et al (10) reported that fumes from polytetrafluoroethylene generated in heated argon gas consisted of ultrafine particles of the same size as in polytetrafluoroethylene fumes generated in air, but that the gas phase did not contain fluoride, which is a main gas component in air-generated polytetrafluoroethylene fumes. Rats exposed to polytetrafluoroethylene generated in argon gas did not show any inflammatory response. This finding suggests that the earlier reported effects from exposures to perfluoropolymer fumes were not a specific effect of ultrafine particles. One possibility for these effects may be that hydrogen fluoride produced during the pyrolysis was transported down to the alveolar region by the particles and that it damaged this part of the lung. Pure hydrogen fluoride, even at a concentration of more than 1000 ppm, only produced damage in the nasal region (11).

Aggregates of ultrafine particles

In an earlier review article (12), it was evident that aggregates of ultrafine particles of titanium dioxide and carbon black induced more lung tumors in rats than larger particles of the same material. Driscoll (13) compiled a large number of long-term inhalation experiments on rats with particles of different materials and sizes. For rats he found a better correlation between the surface area of the lung burden and lung tumors than between the mass of the lung burden and lung tumors.

Ferin et al (14) instilled aggregates (500 µg) of two types of ultrafine titanium dioxide particles, rutile and anatase, with primary particle diameters of 12 nm and 21 nm, respectively. They also instilled fine titanium dioxide particles of rutile and anatase with primary diameters of 230 nm and 250 nm. The fraction which could not be removed by lavage increased markedly with decreasing particle size, from 19% for 250 nm particles to 61% for 12 nm particles. The same group (15) studied the inflammatory response of aggregates of ultrafine (primary diameter 20 nm) and fine (primary di-

ameter 250 nm) titanium dioxide particles. Twenty-four hours after the instillation, the animals were killed and the lungs lavaged. The estimated dose to the interstitial part of the lung was 2.8 µg for the fine particles and 51.8 µg for the ultrafine particles. The total number of cells obtained in the lavage was markedly enhanced in the rats exposed to the ultrafine particles when compared with the lavages from control rats and rats exposed to fine particles (table 1). Especially the neutrophils were increased in the group exposed to the ultrafine particles. In an additional experiment, the authors instilled around 100 µg of aggregated ultrafine titanium dioxide particles in the form of either free particles, particles coated with rat serum, or phagocytized particles in combination with alveolar macrophages and neutrophils. The free and serum-coated particles both induced a marked and similar increase in neutrophils (neutrophils constituted about 15% of the total cell numbers), whereas the phagocytized particles did not induce any increase in the neutrophils.

Ferin et al (14) and Oberdörster et al (16) also exposed rats via inhalation to aggregates of ultrafine (21 nm) and fine (250 nm) titanium dioxide particles. The mass median diameter was 0.78 µm for the ultrafine aerosol and 0.71 µm for the fine aerosol. The concentration was 23.5 (SD 2.9) mg/m³ for the ultrafine particles and 22.3 (SD 4.2) mg/m³ for the fine particles. The exposure lasted for 12 weeks (6 hours a day, 5 days a week). The animals were followed up to 64 weeks. The lung burdens after 12 weeks were 6.62 (SD 1.22) mg for the fine particles and 5.22 (SD 0.75) mg for the ultrafine particles. The half-time of the retention in the lungs for the ultrafine particles was around 500 days, and for the fine particles it was about 170 days. After 8 weeks, the interstitial burden was markedly higher for the ultrafine than for the fine particles (eg, around 500 µg of the fine particles, and more than 2000 µg of the ultrafine particles was located in this region after 41 weeks). The content in the hilar lymph nodes was also much higher for the ultrafine than for the fine particles (eg, 807 µg of the ultrafine particles compared with 69 µg of the fine particles was located in this region after 41 weeks). With respect to the inflammatory response, the ultrafine particles induced a marked increase in the total number of cells in the lung lavage, especially in neutrophils, but also in alveolar macrophages. After 12 weeks, there was, for example, an increase of neutrophils with a factor of 100 and of macrophages with a factor of 3. The fine titanium dioxide particles induced no clear effect on the total number of cells and macrophages, but an increase in neutrophils occurred with almost a factor of 10 after 12 weeks.

When radiolabeled polystyrene particles were inhaled 12 weeks after the start of the exposure to titanium dioxide particles, the retention half-times were 66

days for the controls, 117 days for the group exposed to fine particles, and 541 days for the group exposed to ultrafine particles. When the dose was expressed as the surface area of retained particles per 1×10^6 alveolar macrophages, the relationship between the dose and the retention half-times for the two particle types was described by one curve (figure 1). When radiolabeled test particles were inhaled 7 months after the start of the exposure, clearance in the group exposed to the fine titanium dioxide particles was similar to that of the controls, whereas the group exposed to ultrafine particles still had a significantly slower clearance than the controls. A histological evaluation after 41 weeks showed early fibrotic reaction in the ultrafine particle group and, to a less degree, also in the fine particle group. This reaction was less prominent 1 year after the exposure.

Single ultrafine soluble particles

Chen et al (17) exposed guinea pigs to a $300 \mu\text{g}/\text{m}^3$ of fine (mass median diameter 300 nm) and ultrafine (mass median diameter 41 nm) sulfuric acid aerosols. Some animals were exposed for a single 3-hour period, and other animals were exposed for 3 hours a day for 3 days. The animals were killed immediately after the exposure or 24 hours later. The lungs were lavaged and cell viability, β -glucuronidase activity, lactate dehydrogenase activity, and total protein concentration was measured. Although there were statistically significant differences in these parameters, they were rather small, and there was no clear pattern of difference between the two aerosols. Changes in the tumor necrosis factor alpha (TNF- α) were also seen, but they did not differ between exposure to the fine and the ultrafine aerosol. Alveolar macrophage phagocytic function was tested and found to be depressed in the animals killed 24 hours after the exposure to the ultrafine aerosol and increased in animals killed 24 hours after the exposure to the fine aerosol.

Summary and concluding remarks

Ultrafine particles are usually defined as particles less than 20 or 40 nm in size and sometimes less than 100 nm. These particles occur in large numbers in the ambient air and are mainly deposited in the alveolar region.

Heated perfluoropolymers are lethal to animals in low concentrations, less than $100 \mu\text{g}/\text{m}^3$, and the fumes consist of ultrafine particles. Therefore, attention toward the evaluation of toxicity caused by ultrafine particles

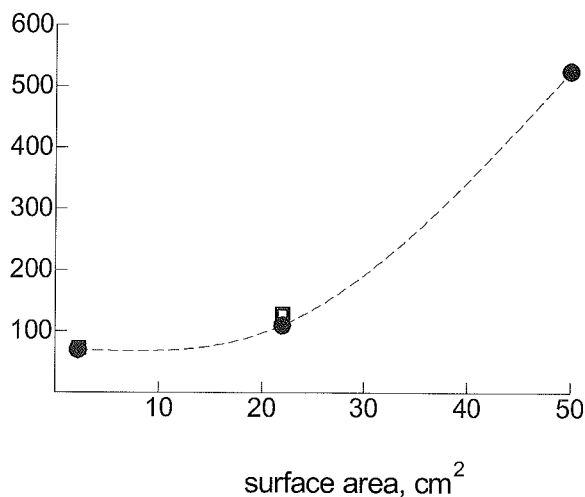


Figure 1. Half-time of inhaled polystyrene particles as the function of the surface area of titanium dioxide particles (fine and ultrafine). Rats had been exposed to the titanium dioxide particles before the study of the retention of the polystyrene particles. [From Oberdörster et al, 1994 (16)]

has increased. The fact that filtered and aged fumes of perfluoropolymers are considerably less toxic than fresh fumes has been taken as an indication that ultrafine particles in general are toxic. However, a recent study showed that, when perfluoropolymers are heated in argon gas, the concentration of ultrafine particles is similar, but the toxicity is lower. This finding indicates that the toxic effects of heated perfluoropolymers may not be a specific effect of ultrafine particles.

Animal experiments show that aggregates of ultrafine particles are more toxic than aggregates of fine particles. When aggregates of ultrafine and fine particles have been instilled via the trachea or inhaled, the ultrafine particles have induced much stronger inflammatory reactions, for example, more neutrophils in the lavage fluid. Furthermore, the ultrafine particles are, to a greater extent, located in the interstitium and in the regional lymph nodes. Interestingly, the relationship between dose and the alveolar retention half-time of test particles could be well described by one curve for both fine and ultrafine particles when the dose was expressed by the surface area of the particles. This finding suggests that the surface area is a better measure of dose than mass is. The difference between solid fine and ultrafine particles in effect contrasts with the difference in effect between fine and ultrafine liquid particles (sulfuric acid), which is small.

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