

## **DIFFERENCES IN THE EXTENT OF INFLAMMATION CAUSED BY INTRATRACHEAL EXPOSURE TO THREE ULTRAFINE METALS: ROLE OF FREE RADICALS**

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*Nickel and cobalt, which belong to the same elemental group, are known to cause interstitial lung disease and bronchial asthma. The ability of these metals to injure lung cells and cause inflammation is likely to be important in their pathogenicity but comparative studies are rare. Additionally, ultrafine (uf) forms of these metals are used increasingly and there is little available information on their toxicity. Thus the inflammatory response following intratracheal instillation of ultrafine particles of Co, Ni, and TiO<sub>2</sub> was compared. Physiological saline (PS) was used as a vehicle control and DQ<sub>12</sub> quartz as a positive control. Male Wistar rats were intratracheally instilled with the 3 particle types at a dose of 1 mg suspended in physiological saline. At 1, 3, 7, 15, and 30 d after the injection, lung weight and the cellular and biochemical changes in bronchoalveolar lavage fluid (BALF) were determined. By all of the indices, Uf-Ni appeared to be the most injurious to the lung, causing severe and sustained inflammation, cytotoxicity and increased epithelial permeability. The next most toxic material was DQ<sub>12</sub> quartz, with Uf-Co being closely similar in ability to cause inflammation. Uf-TiO<sub>2</sub> was more active than the saline control in all of the indices, but was the least toxic of the particles studied. The present study reveals that three ultrafine particles of the same diameter are dramatically different in their ability to cause inflammation. The three ultrafines were compared as to their ability to cause free-radical damage to supercoiled plasmid DNA, and the result of free-radical activity was found to be Uf-TiO<sub>2</sub> << Uf-Co = Uf-Ni. Difference in free-radical-generation activity therefore could underlie the difference in inflammation of these three ultrafine particle types.*

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Previous studies have shown that ultrafine titanium dioxide (Uf-TiO<sub>2</sub>) and ultrafine Al<sub>2</sub>O<sub>3</sub> (Uf-Al<sub>2</sub>O<sub>3</sub>) could cause more pulmonary toxicity than fine (respirable) titanium dioxide (F-TiO<sub>2</sub>) or Al<sub>2</sub>O<sub>3</sub>(F-Al<sub>2</sub>O<sub>3</sub>), respectively (Ferin et al., 1990, 1991; Oberdörster et al., 1990, 1992; Takenaka et al., 1986). Increased translocation of ultrafine particles from the alveoli into the interstitial space has been considered to be an important factor in toxicity of ultrafines due to their small diameter and subsequent high particle number per unit mass. The high particle number overwhelms macrophage clearance and leads to increased interaction with epithelial cells, which leads to interstitialization (Ferin et al., 1990, 1991; Oberdörster et al., 1990, 1992; Warheit et al., 1990). Moreover, a role for active oxygen species in mediating Uf-TiO<sub>2</sub>-induced pulmonary damage has been suggested (Donaldson et al., 1996; Janssen et al., 1994). However, the mechanisms of toxicity of ultrafine particles and the factors that affect toxicity of ultrafine particles are still not clear. Ultrafine metallic nickel particles (Uf-Ni) and cobalt particles (Uf-Co) with a mean diameter of 20 nm are both new products made by the process of vacuum vapor deposition. Their characteristics, including a high level of surface energy, high magnetism, and low melting point, are anticipated to allow their use in magnetic tape, conduction paste, chemical catalysts, and as sintering promoters (Kyono et al., 1992). Ni and Co are both sensitizers and carcinogens (Elinder & Friberg, 1986; Norseth, 1986) and belong to the same element series.

The goal of the present study was to compare the ability of Uf-TiO<sub>2</sub>, Uf-Co, and Uf-Ni to cause inflammation and determine whether factors other than size could be important in the toxicity of ultrafine particles. Physiological saline (PS) and crystalline quartz (DQ<sub>12</sub>), which is well known to cause inflammation at similar mass, were utilized as a vehicle and a positive control, respectively. The toxicity was compared by observing the activity of lactate dehydrogenase (LDH), lipid peroxides (LPO), total protein, and cells profile in bronchoalveolar lavage fluid (BALF) following instillation.

## METHODS

### Rats

Specific-pathogen-free male Wistar rats, weighing 180–200 g, age 7–8 wk, were supplied by Clear Japan, Inc. (JCL). The animals were housed in an air-conditioned room (temperature 22°C, relative humidity 50 ± 10%) with a 12-h light/dark cycle. Rats were fed on the conventional laboratory diet and had free access to tap water.

### Particles and Characterization of Particles

The following particles were used: (1) metallic Uf-Ni powder with a mean diameter of 20 nm and 43.8 m<sup>2</sup>/g of surface area (ultrafine powder,

INABTA and Co, Ltd., Japan); (2) metallic cobalt powder with a mean diameter of 20 nm and 47.9 m<sup>2</sup>/g of surface area (ultrafine powder, INABTA and Co, Ltd., Japan)—Uf-Co is composed of Co and CO<sub>3</sub>O<sub>4</sub> (Kusaka et al., 1997); (3) Uf-TiO<sub>2</sub> with a mean diameter of 28 nm on average and 45 m<sup>2</sup>/g of surface area (ultrafine powder, lot no. TiO<sub>2</sub>-55-1 INABATA and Co., Ltd., Japan); and (4) European standard quartz (DQ<sub>12</sub>), density 2.65 g/cm<sup>3</sup>, median volume diameter 1.85 μm.

Size distributions of Uf-Ni, Uf-Co, and Uf-TiO<sub>2</sub> were determined with a transmission electron microscope (TEM) (H-8000, Hitachi Co., Japan). The particles were dispersed in distilled water and sonicated for 30 s. The particle suspensions of 1 μl each were placed on an electron microscope (EM) grid with carbon-reinforced collodion film. TEM observations were performed to measure the particles sizes at various magnifications.

### **Treatment**

Rats were divided randomly into five groups. Each experimental group of rats was injected intratracheally with 1 mg particle in 1 ml physiological saline. All of the particle suspensions were ultrasonicated for about 30 min and then sterilized at 0.5 kgf/cm<sup>2</sup> pressure and 120°C for 15 min, prior to injection. Control rats were injected with 1 ml physiological saline alone. Groups of 4–6 rats were killed at 1, 3, 7, 15, and 30 d after exposure to particles.

### **Bronchoalveolar Lavage**

Rats were killed by injection with an overdose of phenobarbital solution (50 mg/ml) into the abdominal cavity. The lung and trachea were then removed as a whole from the body. The wet lung weight was measured after removing the heart and the mediastinal lymphoid and adipose tissue. The lung:body weight ratio (lung index) was calculated for each rat. Bronchoalveolar lavage (BAL) was carried out using 10 ml saline at 37°C. The BAL procedure was repeated four times and lungs were gently massaged. The BALF was then centrifuged (1500 × g, 10 min). The first lavage was kept separate, and after centrifugation the first lavage supernatant was used to measure the activity of the LDH, concentration of total protein, and LPO. Total lung cells, macrophages, neutrophils, and lymphocytes were evaluated for a pool of all four lavages.

### **Biochemical and Cytological Evaluation of BALF**

It is well known that changes in levels of enzymatic activities in the BALF may indicate degree of lung injury (Henderson, 1984). LDH, a cytoplasmic enzyme, occurs extracellularly in BALF only if cell lysis or cell membrane damage occurs. The LDH activity in BALF was determined using LDH C-II test kits (Wako Pure Chemical Industries, Ltd.) by the lactate matrix method (Babson & Phillips, 1965). The concentration of total protein in BALF is also a sensitive index of lung inflammation and dam-

age to epithelium with escape of interstitial fluid. The total protein in BALF was determined by the Lowry et al. (1951) method. The concentration of LPO in BALF is a index of lipid peroxidation in the lung (Petruska et al., 1991; Zhang et al., 1996). LPO (hydroperoxide) was determined using a kit (Kyowa Medical Company). Total number of cells in BALF was determined by conventional hemocytometer counting. Cells were differentiated in Wright-Giemsa-stained cytospin samples.

### Free-Radical Damage to Plasmid DNA

Eight microliters of buffer (pH 7.2) containing 290 ng  $\phi$ X 174 RF1 DNA were added to 10  $\mu$ l of water containing 1, 10, or 20  $\mu$ g of Uf-TiO<sub>2</sub>, Uf-Ni, or Uf-Co particles as previously described and incubated for 8 h at 37°C (Gilmour et al., 1996a). Supercoiled, relaxed coil, and linear fragments of the DNA were then separated by agarose gel electrophoresis (Gilmour et al., 1996a). The amount of supercoiled DNA present after treatment was quantified by image analysis software. The amount of depletion of supercoiled DNA compared to control indicated the level of free-radical damage to the plasmid caused by any of the ultrafine metals. Previous studies with a range of particles demonstrated that the oxidative injury to supercoiled plasmid that is caused by particles is mediated by free radicals (Donaldson et al., 1996). Addition of 200 mM mannitol was used to scavenge hydroxyl radical in some experiments.

### Statistical Analysis

Values were expressed as the means and standard errors. The differences among groups according to exposure to particles were tested by one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison. If a *p* value was less than .05, a difference was considered significant. All statistics were performed using an SAS statistics program (SAS Institute, Inc., 1993).

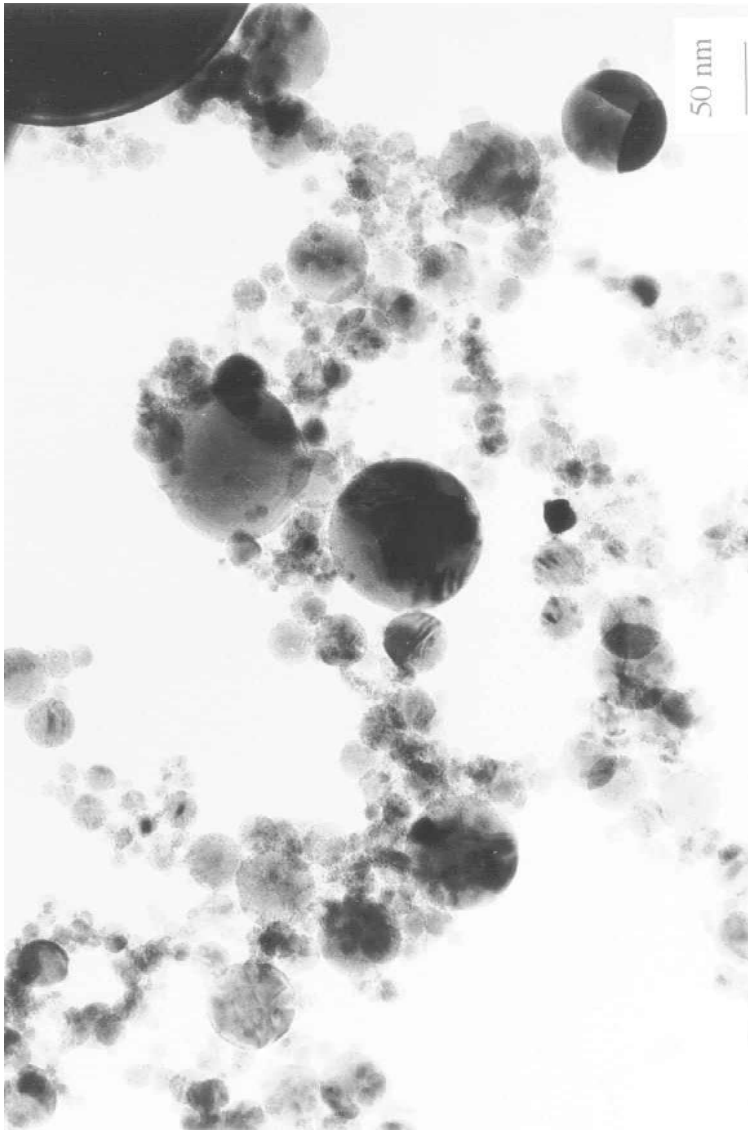
## RESULTS

### Characterization of Ultrafine Particles

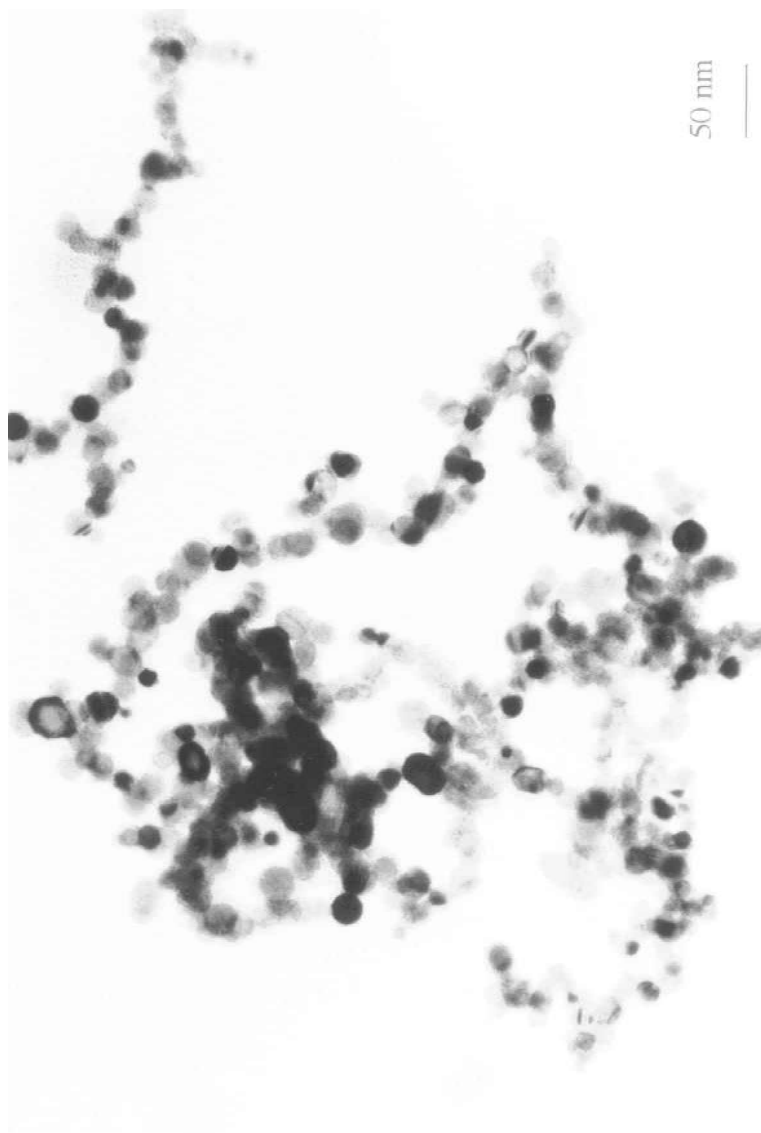
The TEM pictures of Uf-Ni, Uf-Co, and Uf-TiO<sub>2</sub> are shown in Figures 1–3, respectively. The size distributions of Uf-Ni, Uf-Co, and Uf-TiO<sub>2</sub> were as follows: Uf-Ni with a size of <10 nm, 7.62%; >10 nm to <20 nm, 53.36%; >20 nm to <30 nm, 32.32%; >30 nm to <40 nm, 6.7%; Uf-Co particles with a size of <10 nm, 7.84%; >10 nm to <20 nm, 54.87%; >20 nm to <30 nm, 32.29%; >30 nm to <40 nm, 5.0%; and Uf-TiO<sub>2</sub> particles with a size of <10 nm, 5.43%; >10 nm to <20 nm, 36.8 %; >20 nm to <30 nm, 48.9%; >30 nm to <40 nm, 8.87%.

### Recovery of BALF

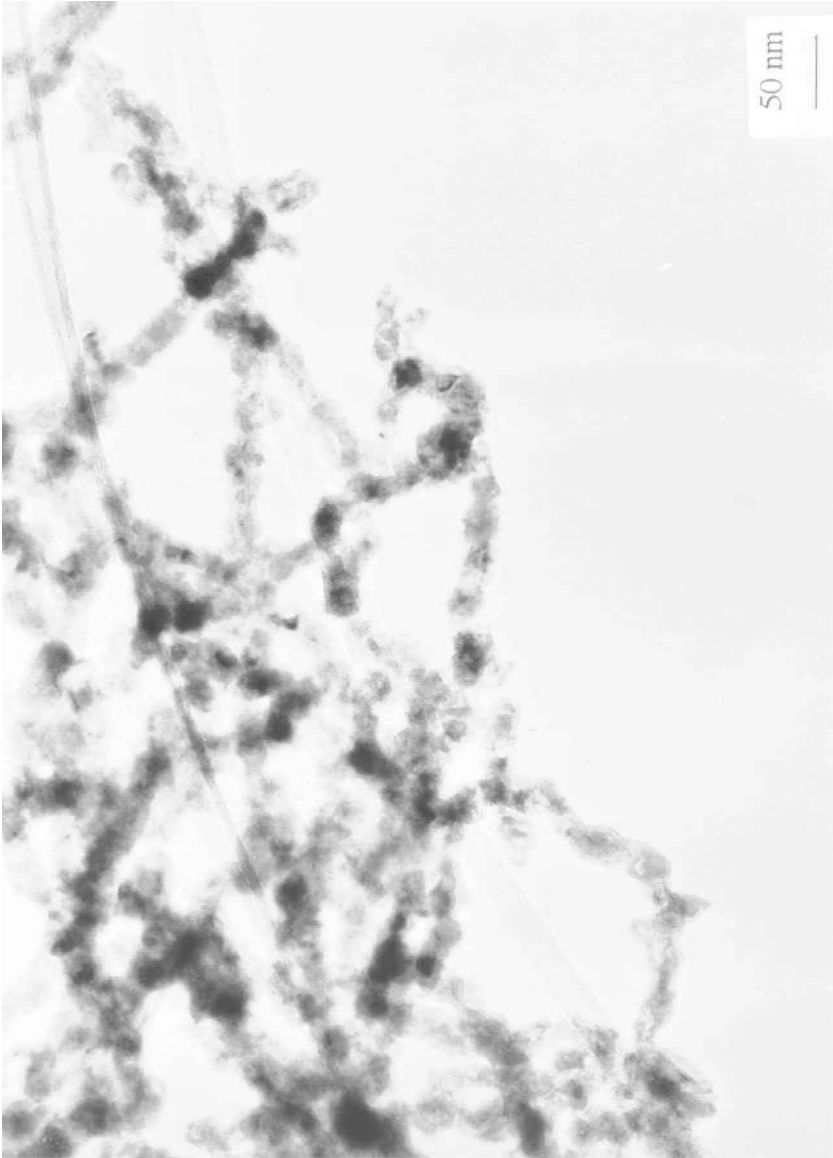
The recovery of BALF did not differ significantly between the experimental group and control groups (data not shown).



**FIGURE 1.** Scanning electron micrograph of Uf-TiO<sub>2</sub>. Bar indicates 50 nm.



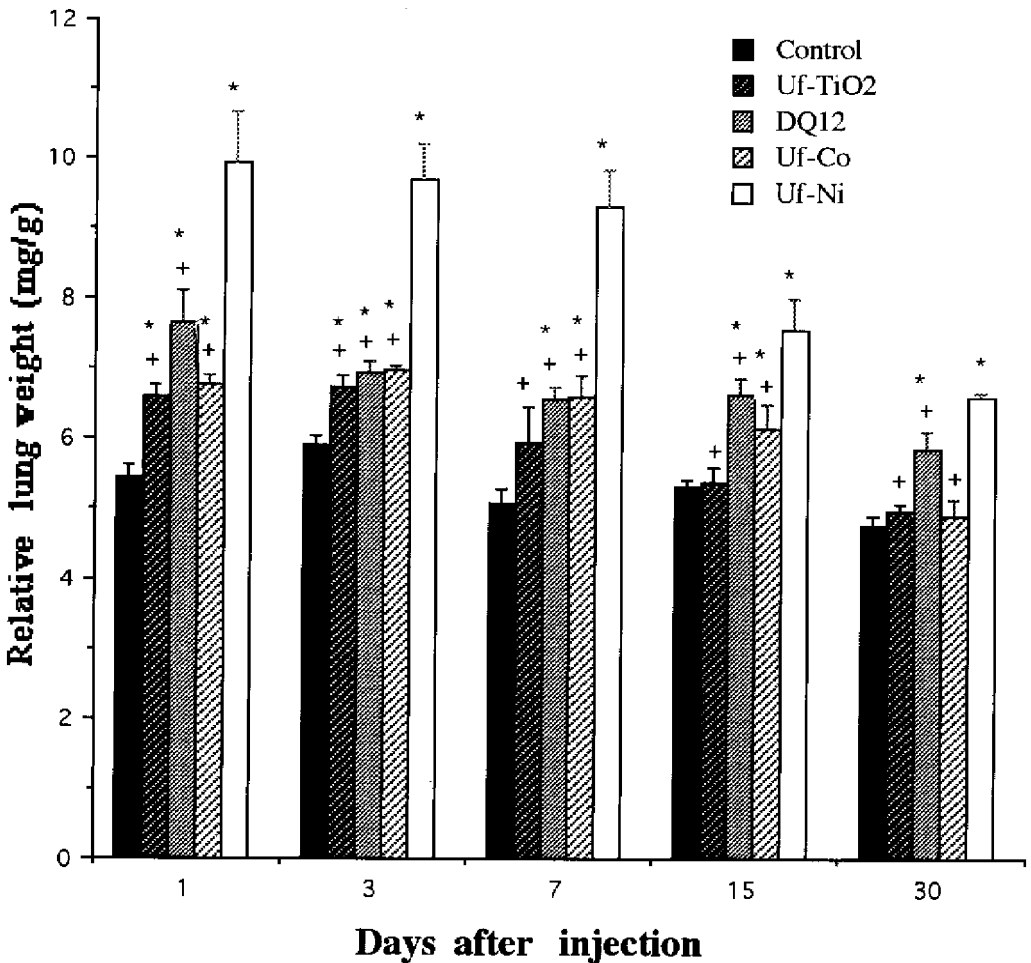
**FIGURE 2.** Scanning electron micrograph of Uf-Ni. Bar indicates 50 nm.



**FIGURE 3.** Scanning electron micrograph of Uf-Co. Bar indicates 50 nm.

### Wet Lung Weight

Absolute lung weight increased with time in all groups, including controls, over the 30 d of the experiment (data not shown), but this was not due to growth since the lung index (lung weight divided by body weight) showed a more flattened line in the PS control. Uf-Ni had by far the greatest effect in increasing the lung index (Figure 4). Of the other 3 particles, all produced a similar rise in lung index, but the Uf-TiO<sub>2</sub>-exposed rats had returned to normal by d 15 whereas this did not occur until 30 d with cobalt; in the case of DQ<sub>12</sub>, the lung index was still significantly elevated at 30 d.



**FIGURE 4.** Lung:body weight ratio of rats up to 30 d after injection of different particles. Values are mean  $\pm$  SE of four to six rats. Asterisk indicates significantly different from the control group,  $p < .05$ . Plus sign indicates significantly different from the Uf-Ni group,  $p < .05$ . Shown by ANOVA with Bonferroni comparison test.

**TABLE 1.** Cellular parameters in BALF up to 30 d after instillation of different particles

Days	Groups	Total cells ( $\times 10^6$ )	Macrophages (%)	Neutrophils (%)	Lymphocytes (%)
1	Control	6.3 $\pm$ 0.4	97.5 $\pm$ 0.3	1.5 $\pm$ 0.4	1.0 $\pm$ 0.2
1	Uf-TiO <sub>2</sub>	12.2 $\pm$ 0.9*+	71.7 $\pm$ 3.0*+	27.2 $\pm$ 2.8*+	1.1 $\pm$ 0.3
1	DQ <sub>12</sub>	29.8 $\pm$ 3.5*+	43.7 $\pm$ 4.0*+	54.7 $\pm$ 3.8*+	1.6 $\pm$ 0.4
1	Uf-Co	30.0 $\pm$ 2.5*+	63.0 $\pm$ 5.1*+	36.3 $\pm$ 4.9*+	0.7 $\pm$ 0.3
1	Uf-Ni	49.0 $\pm$ 3.5*+	38.6 $\pm$ 0.9*	60.0 $\pm$ 1.0*	1.4 $\pm$ 0.2
3	Control	6.1 $\pm$ 0.3	97.6 $\pm$ 0.2	1.2 $\pm$ 0.2	1.2 $\pm$ 0.2
3	Uf-TiO <sub>2</sub>	11.1 $\pm$ 0.5*+	79.8 $\pm$ 2.7*+	18.4 $\pm$ 2.1*+	1.8 $\pm$ 0.4
3	DQ <sub>12</sub>	23.5 $\pm$ 2.8*+	53.2 $\pm$ 1.8*	44.6 $\pm$ 2.4*	2.2 $\pm$ 0.8*
3	Uf-Co	15.8 $\pm$ 2.2*+	61.2 $\pm$ 3.6*+	30.8 $\pm$ 3.3*+	1.0 $\pm$ 0.6+
3	Uf-Ni	40.0 $\pm$ 4.1*	56.7 $\pm$ 3.0*	41.2 $\pm$ 2.5*	2.1 $\pm$ 0.4*
7	Control	6.5 $\pm$ 0.2	97.7 $\pm$ 0.4	1.2 $\pm$ 0.4	1.1 $\pm$ 0.2
7	Uf-TiO <sub>2</sub>	9.6 $\pm$ 0.3*+	84.2 $\pm$ 3.3*+	14.2 $\pm$ 3.9*+	1.6 $\pm$ 0.7+
7	DQ <sub>12</sub>	24.8 $\pm$ 3.3*+	65.6 $\pm$ 2.6*	32.8 $\pm$ 2.7*+	1.6 $\pm$ 0.2+
7	Uf-Co	11.6 $\pm$ 1.8*+	69.5 $\pm$ 1.7*	28.5 $\pm$ 1.1*	2.0 $\pm$ 1.0+
7	Uf-Ni	31.0 $\pm$ 2.0+	67.0 $\pm$ 0.6*	29.8 $\pm$ 0.5*	3.2 $\pm$ 0.4*
15	Control	6.1 $\pm$ 0.2	97.7 $\pm$ 0.4	1.4 $\pm$ 0.4	0.9 $\pm$ 0.1
15	Uf-TiO <sub>2</sub>	7.8 $\pm$ 0.7*+	96.8 $\pm$ 1.4+	2.4 $\pm$ 1.0*+	0.8 $\pm$ 0.4+
15	DQ <sub>12</sub>	16.9 $\pm$ 1.5*+	84.2 $\pm$ 1.3*+	14.6 $\pm$ 2.2*+	1.2 $\pm$ 0.3+
15	Uf-Co	11.8 $\pm$ 2.8*+	85.0 $\pm$ 2.0*+	13.2 $\pm$ 1.6*+	1.8 $\pm$ 0.6*+
15	Uf-Ni	23.0 $\pm$ 2.3*	71.0 $\pm$ 2.8*	25.5 $\pm$ 1.8*	3.5 $\pm$ 0.4*
30	Control	6.0 $\pm$ 0.3	97.6 $\pm$ 0.2	1.2 $\pm$ 0.2	1.2 $\pm$ 0.2
30	Uf-TiO <sub>2</sub>	6.2 $\pm$ 0.1+	95.5 $\pm$ 0.4*+	2.8 $\pm$ 0.4*+	1.7 $\pm$ 0.3+
30	DQ <sub>12</sub>	9.4 $\pm$ 0.2*+	85.2 $\pm$ 2.0*+	11.8 $\pm$ 2.0*+	2.0 $\pm$ 1.0*+
30	Uf-Co	9.8 $\pm$ 1.1*+	86.8 $\pm$ 1.9*+	9.3 $\pm$ 1.1*+	2.5 $\pm$ 0.9*+
30	Uf-Ni	17.0 $\pm$ 1.5*	76.0 $\pm$ 0.6*	14.2 $\pm$ 1.3*	9.8 $\pm$ 0.8*

Note. Values are mean + SE of four to six rats. Asterisk indicates significantly different from the control group,  $p < .05$ . Plus sign indicates significantly different from the Uf-Ni group,  $p < .05$ .

### BALF Profile

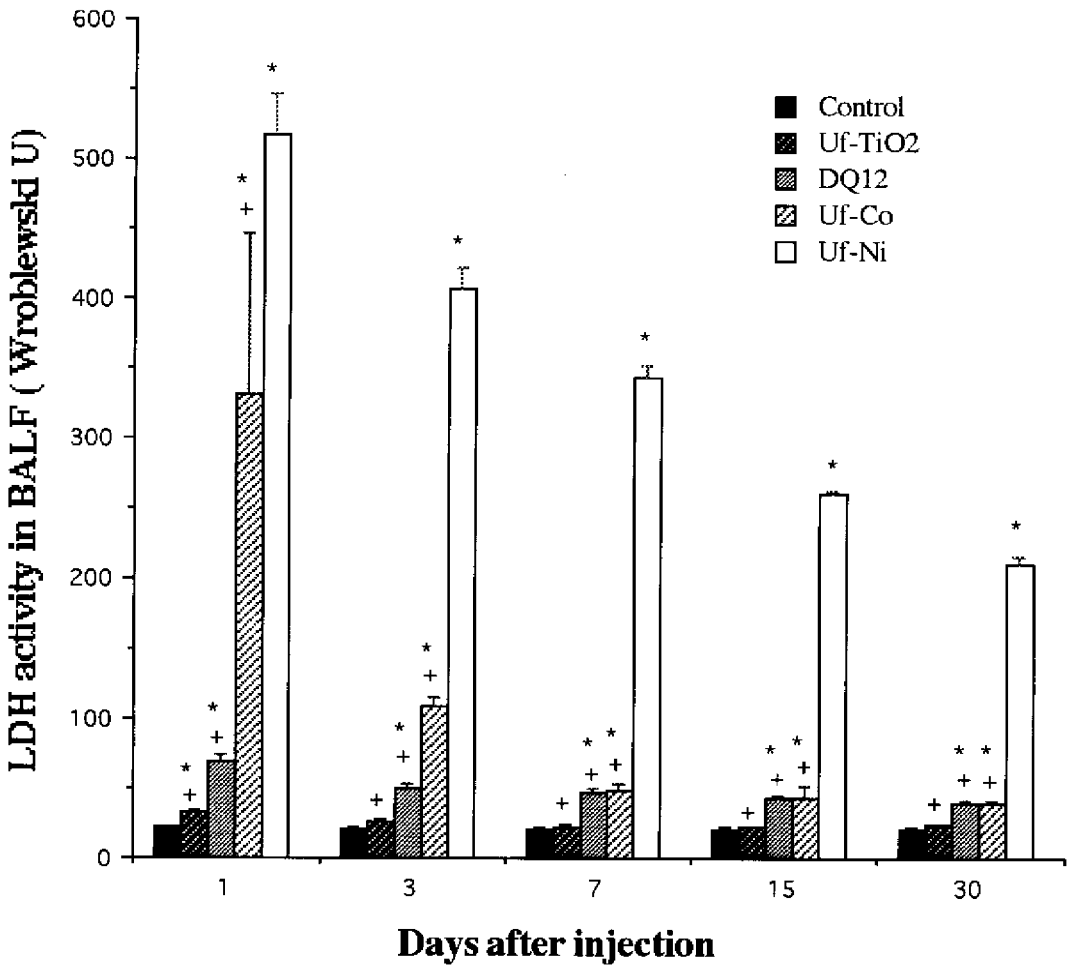
The numbers of total lung cells, macrophages, and neutrophils in the BALF showed a consistent pattern with the severity of the inflammation in the following order of decreasing severity: Uf-Ni > DQ<sub>12</sub> > Uf-Co > Uf-TiO<sub>2</sub> (Table 1). All of the particles induced an increase in total cells and neutrophils that peaked at 1 d and declined over the succeeding 30 d, with Uf-TiO<sub>2</sub> reaching control levels by 30 d while the other 3 particle types still produced significant increases over PS control at this time point. The increases in total cells, neutrophils, and lymphocytes caused by Uf-Ni was marked, and at all of the time points cell recruitment was significantly greater for Uf-Ni than for all the other particles.

Lymphocytes (Table 1) showed a pattern different from that seen with the other cells. DQ<sub>12</sub>, Uf-Co, and Uf-Ni caused a significant sustained increase, but the effect of Uf-Ni was very marked, producing a gradually increasing level over the 30 d of the experiment.

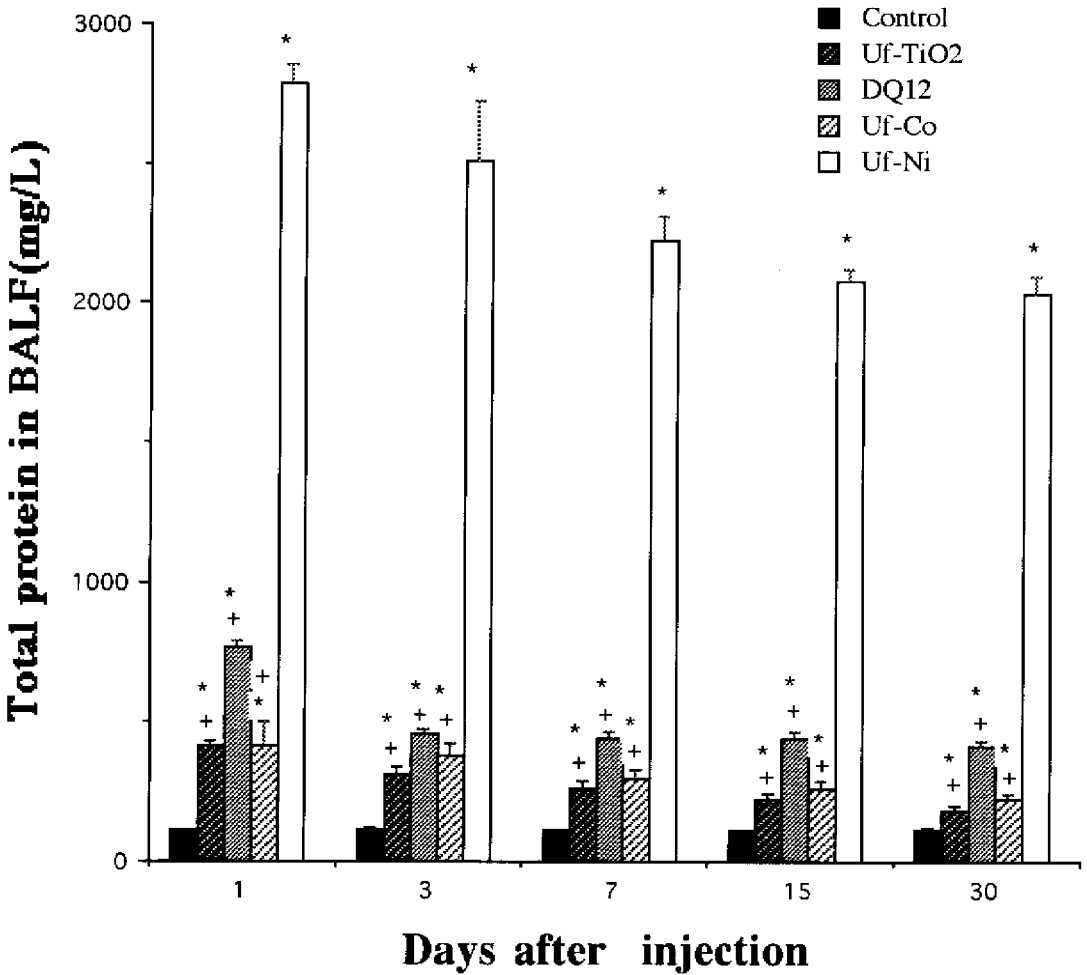
All of the particles caused LDH activity increase at an early stage, indicating cell injury, but this was only sustained in the case of DQ<sub>12</sub>, Uf-Co, and Uf-Ni (Figure 5). However, DQ<sub>12</sub> caused the least toxicity, Uf-Co was intermediate, and Uf-Ni caused the most severe and sustained increase in LDH.

All of the particles caused continued increases in total BALF protein (Figure 6). However, once again there were marked differences in the extent of the protein exudation, with Uf-TiO<sub>2</sub>, Uf-Co, and DQ<sub>12</sub> causing three- to fivefold increases compared to control levels whereas Uf-Ni caused up to 30-fold elevation in total protein in BALF.

Particle types fell into one of two groups when it came to ability to generate LPO in BALF (Figure 7). The first group included control and Uf-



**FIGURE 5.** LDH activity in BALF from rats up to 30 d after injection of different particles. Values are mean  $\pm$  SE of four to six rats. Significant differences indicated as in Figure 4.

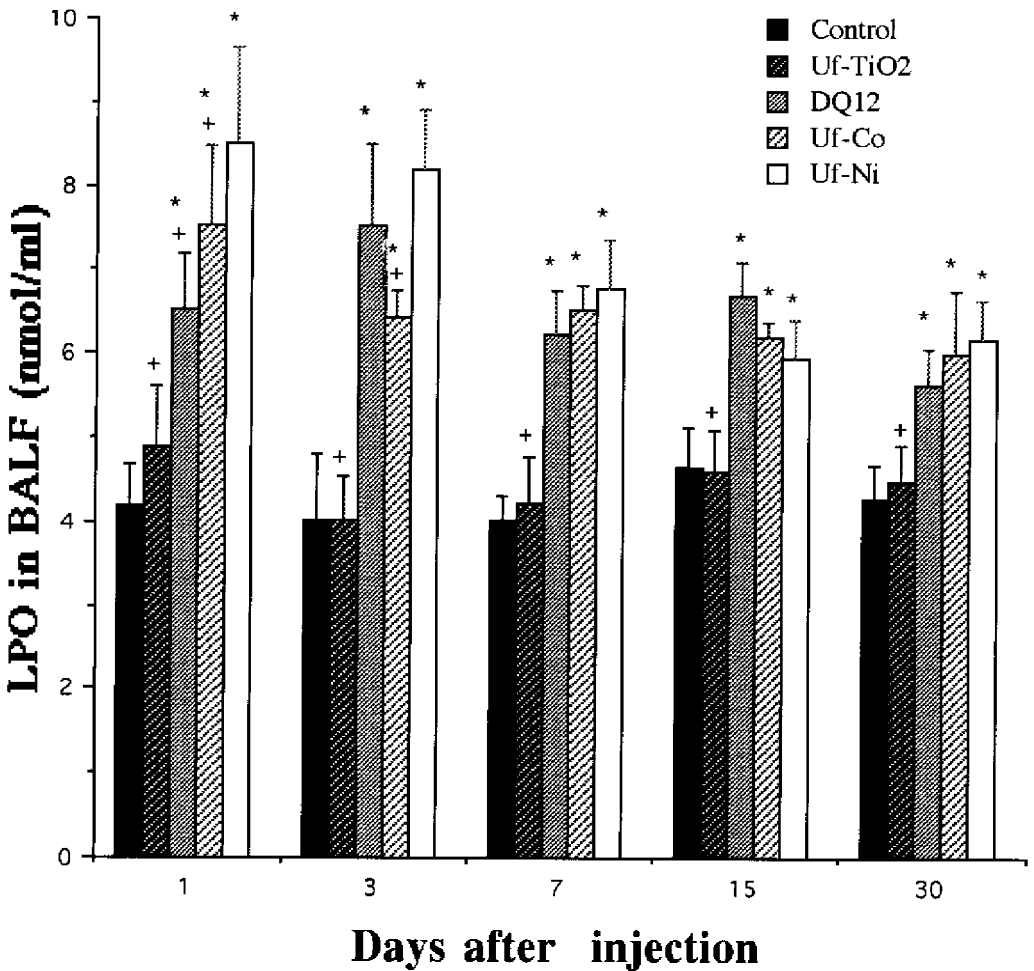


**FIGURE 6.** Concentration of total protein in BALF from rats up to 30 d after injection of different particles. Values are mean  $\pm$  SE of four to six rats. Significant differences indicated as in Figure 4.

TiO<sub>2</sub>, and these produced low levels of LPO. The second group, containing Uf-Ni, Uf-Co, and DQ<sub>12</sub> induced a significant rise in LPO over the 30 d of the experiment.

### Intrinsic Free-Radical Activity of Particles

Figure 8 shows the intrinsic free radical activity of the ultrafine particles as assessed in the supercoiled plasmid DNA assay. It is clear from the images of the gels at the top of the figure, and from the graph, that Uf-TiO<sub>2</sub> has little free-radical activity and that ultrafine Ni and Co displayed marked activity. To demonstrate the role of hydroxyl radical in plasmid DNA depletion, Uf-Co particles were incubated with plasmid DNA in the



**FIGURE 7.** Concentration of LPO in BALF from rats up to 30 d after injection of different particles. Values are mean  $\pm$  SE of four to six rats. Asterisk indicates significantly different from the control group,  $p < .05$ . Plus sign indicates significantly different from the Uf-Ni group,  $p < .05$ . Shown by ANOVA with Bonferroni comparison test.

presence of hydroxyl radical scavenger mannitol (200 mM). This resulted in protection from supercoiled plasmid DNA depletion from 87.7(2.4)% depletion in the absence of mannitol to 44.8(3.4)% depletion in the presence of mannitol (data as mean and SEM of 3 separate experiments).

## DISCUSSION

The ability of ultrafine particles to have enhanced lung-injuring activity as compared to fine (respirable) particles of the same material has now been shown for a number of diverse materials: TiO<sub>2</sub> (Ferin et al., 1990, 1991), carbon black (Li et al., 1996), cobalt (Kusaka et al., 1998), and

Al<sub>2</sub>O<sub>3</sub> (Ferin et al., 1990, 1991; Oberdörster et al., 1990). The mechanism underlying the effect is unknown, but data suggest that free-radical activity may be an important factor due to specific surface free-radical-generating activity of the ultrafines (Donaldson et al., 1996). The present study demonstrated that 3 ultrafine materials, composed of ultrafine particles about 20–30 nm in diameter, exhibit different abilities to cause inflammation and injury following instillation at equal mass. Studies in vitro revealed that the three ultrafines differed in their ability to generate free radicals, with Uf-Co and Uf-Ni having more free radical activity than Uf-TiO<sub>2</sub>. Similar to findings for other pathogenic dusts (Gilmour et al., 1996a), hydroxyl radical was found to be the principal free radical mediating plasmid DNA breakage and depletion (Donaldson et al., 1996; Gilmour et al., 1996b, 1997).

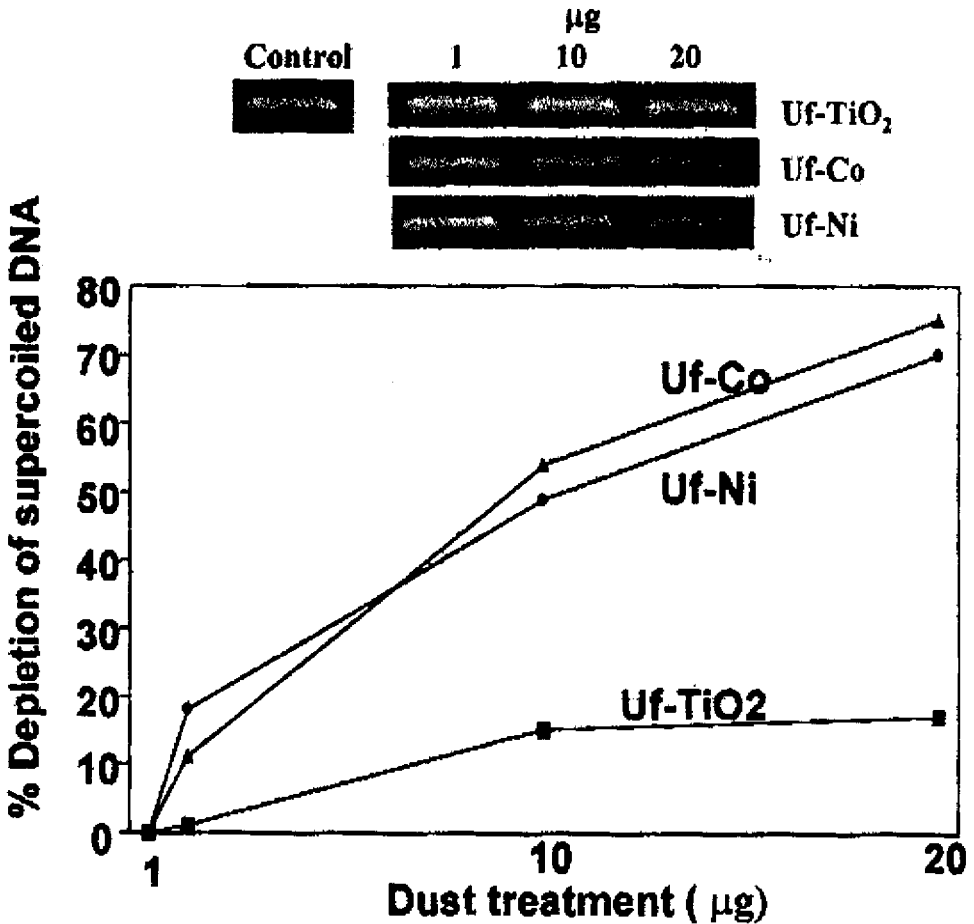


FIGURE 8. Free-radical activity of ultrafine metals as assessed by depletion of supercoiled plasmid DNA. Upper panel: images of supercoiled DNA bands after treatment. Lower panel: quantification of bands. Data from a single representative experiment.

The increase in lymphocytes seen with Ni instillation may be related to the well-documented sensitizing activity of this metal (Norseth, 1986). From our results Uf-Ni may be a potent sensitizing agent by increasing lymphocytes in BALF. Uf-Co was also inflammogenic, but this effect was substantially less than that seen with Uf-Ni. Uf-Co did not show the same ability to cause lymphocyte recruitment as Uf-Ni, although Co is known to induce interstitial pneumonitis and hypersensitivity through cellular and humoral immune responses (Kusaka et al., 1989). However, Co and Ni do show differences in their immunomodulatory activity. Co may cause changes in the homeostasis of the immune response by causing immunotoxicity rather than by being antigenic. In contrast, Ni may have antigenicity, rather than acting as a modulator of the immune system (Nagai et al., 1989). This may account for the different effects between the two in terms of ability to cause lymphocyte recruitment.

The DQ<sub>12</sub> quartz sample was generally intermediate in toxicity between Uf-Ni and Uf-Co, although LDH was a notable exception to this rule, with Uf-Co being more injurious to cells. The toxicity of quartz to cells and fluids is a major factor in leading to its action as an inflammagen (Doll et al., 1983; Donaldson et al., 1992), and free radical activity has been implicated in this toxicity (Vallyathan et al., 1988). Uf-Co and Uf-Ni were more inflammatory than Uf-TiO<sub>2</sub>, and this was in agreement with the intrinsic free-radical data, which showed hydroxyl radicals were the principle free radical. However, Uf-Ni was markedly more inflammatory than Uf-Co, and this was not reflected in differences in the ability of the two ultrafine metals to generate free radicals *in vitro*. We speculate that differences in the ability of Uf-Co and Uf-Ni to redox cycle under the influence of the lung milieu, with Uf-Ni being more active in this respect, could account for the difference between the data for Uf-Ni and Uf-Co on intrinsic free radical activity and ability to cause inflammation.

Free radicals on the surface of particles may also stimulate inflammation via increased transcription of oxidative stress-responsive proinflammatory genes (Donaldson et al., 1996) such as the chemoattractant cytokine IL-8 (Deforge et al., 1993). The finding that Uf-Ni is more inflammagenic than quartz suggests that Uf-Ni has a very active surface in terms of ability to generate free radicals. However, the surface area of Uf-Ni compared to that of quartz, on an equal mass basis, is very much greater, as a result of the smaller particle size of Uf-Ni. Johnston et al. (1996) recently described acute injury to the lung caused by ultrafine polytetrafluoroethylene (PTFE) that was suggestion of an oxidant injury.

Uf-TiO<sub>2</sub> showed the least activity in causing lung inflammation in the present study, with no intrinsic free-radical activity, and caused no LPO production compared to control BALF. This supports the hypothesis that free-radical-generating activity of particle is an important factor in inflammation. However, it is not possible to tell whether the BALF lipid peroxidation seen with Uf-Ni, Uf-Co, and quartz arose from the particles

directly or was a consequence of inflammation. The intrinsic free-radical data, showing similarity of Uf-Co and Uf-Ni, supports the former. It was reported that Uf-TiO<sub>2</sub> from another source does have free-radical-generating activity (Gilmour et al., 1997), and differences between the samples used here, for instance, in terms of transition metal contamination, could explain this difference. Iron was found to be a mediator of free-radical generation for environmental particles (PM10) (Gilmour et al., 1996b), which are considered to have an ultrafine component that mediates this activity (Seaton et al., 1995). Transition metals appear to mediate the pathogenicity of other particles such as quartz (Castranova et al., 1996) and asbestos (Gilmour et al., 1996a). From the point of view of sensitization, Uf-Ni and Uf-Co are likely to be much more allergenic than Ni or Co as fine particles, and these findings have important implications for hygiene regulations.

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