PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Toxicology of ultrafine particles: *in vivo* studies

Güunter Oberdürster

Phil. Trans. R. Soc. Lond. A 2000 **358**, 2719-2740
doi: 10.1098/rsta.2000.0680

10.1098/rsta.2000.0680

Toxicology of ultrafine particles: *in vivo* studies

By Günter Oberdörster

Department of Environmental Medicine, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642, USA

Ultrafine particles (less than 100 nm in diameter) are encountered in ambient air and at the workplace. Normal background levels in the urban atmosphere for ultrafine particles are in the range 1–4 $\times 10^4$ cm^{-3}; however, their mass concentration is normally not greater than 2 μg m^{-3}. At the workplace, ultrafine particles occur regularly in metal fumes and polymer fumes, both of which can induce acute inflammatory responses in the lung upon inhalation. Although ultrafine particles occurring at the workplace are not representative, and, therefore, are not relevant for urban atmospheric particles, their use in toxicological studies can give valuable information on principles of the toxicity of ultrafine particles. Studies in rats using ultrafine polymer fumes of polytetrafluoroethylene (PTFE) (count median diameter *ca.* 18 nm) showed that (i) they induced very high pulmonary toxicity and lethality in rats after 15 min of inhalation at 50 μg m^{-3}; (ii) ageing of PTFE fumes resulted in agglomeration to larger particles and loss of toxicity; (iii) repeated pre-exposure for very short periods protected against the toxic and lethal effects of a subsequent 15 min exposure; (iv) rapid translocation of PTFE particles occurred to epithelial, interstitial and endothelial sites. Since one characteristic of urban ultrafine particles is their carbonaceous nature, exposure of rats to laboratory-generated ultrafine carbonaceous (elemental, and organic, carbon) particles was carried out at a concentration of *ca.* 100 μg m^{-3} for 6 h. Modulating factors of responses were prior low-dose inhalation of endotoxin in order to mimic early respiratory tract infections, old age (22-month old rats versus 10-week old rats) and ozone co-exposure. Analysis of results showed that (i) ultrafine carbon particles can induce slight inflammatory responses; (ii) LPS priming and ozone co-exposure increase the responses to ultrafine carbon; (iii) the aged lung is at increased risk for ultrafine particle-induced oxidative stress. Other studies with ultrafine and fine TiO_2 showed that the same mass dose of ultrafine particles has a significantly greater inflammatory potential than fine particles. The increased surface area of ultrafine particles is apparently a most important determinant for their greater biological activity. In addition, the propensity of ultrafine particles to translocate may result in systemic distribution to extrapulmonary tissues.

Keywords: ultrafine particles; carbon; pulmonary inflammation; dosimetry; adaptation; LPS; age

1. Introduction

Epidemiological studies have shown an association between increased particulate air pollution and adverse health effects in susceptible parts of the population, in particular the elderly with respiratory and cardiovascular diseases (EPA 1996). Urban

airborne particles consist of three modes: ultrafine particles, accumulation-mode particles (which together with the ultrafines form the fine particle mode), and coarse-mode particles. Ultrafine particles (particles less than 0.1 μm in diameter) contribute very little to the overall mass, but they contribute most to the number concentration of airborne urban particles. Any of the three modes could causally be associated with adverse health effects, although epidemiological studies suggest that fine-mode particles are better correlated with such effects than coarse particles (EPA 1996). We are testing the hypothesis that urban ultrafine particles consisting of a carbonaceous core with attached inorganic and organic materials can cause adverse health effects in compromised subjects during episodic high increases in concentration.

Ultrafine particles are also encountered in the workplace as fumes generated from smelting processes of metals and heating of polymers. Resulting acute effects in exposed workers have been well documented as metal-fume fever and polymer-fume fever, consisting of acute pulmonary and inflammatory responses, which can also be accompanied by systemic effects with symptoms of fever, nausea and headaches (Drinker *et al*. 1927; Gordon *et al*. 1992; Goldstein *et al*. 1987; Rosenstock & Cullen 1986). Concentrations of such ultrafine particles at the workplace are very high compared with those in the urban atmosphere, and often the high concentrations encountered at the workplace result in particles which have agglomerated, by classical coagulation phenomena, to larger sized particles.

Although workplace exposures to ultrafine fumes are known to result in potentially severe acute responses, a direct extrapolation to effects of urban ultrafine particles is not possible. The toxicity of carbonaceous urban ultrafine particles is apparently much lower compared with that of freshly generated metal or polymer fumes. However, an important difference between exposures to ultrafine particles at the workplace and in the general environment lies in the exposed population. These are healthy adults at the workplace, and all of the population, including the most sensitive members, in the urban setting. Despite this, it is likely that certain principles and concepts of ultrafine particle toxicology are common between workplace ultrafine particles and urban ultrafine particles. Therefore, it could be very useful to assess the toxicity and behaviour of ultrafine particles encountered at the workplace, which might be helpful for designing studies with ambient ultrafine particles. This paper will first summarize some of our studies with polymer fumes, discuss certain principles of ultrafine particle dosimetry and behaviour, and finally describe results of toxicological studies with carbonaceous and other ultrafine particles as surrogates for ambient particles.

2. Studies with ultrafine particles from polymer thermodegradation

(*a*) *Characterization and toxicity of inhaled polytetrafluoroethylene (PTFE)*

Heating of PTFE (Teflon) to *ca.* 480 °C results in the generation of fumes that consist of ultrafine particles and some gas-phase products, mainly HF. Heating to temperatures above 500 °C will generate additional gas-phase products like perfluoroisobutylene and others, which are highly toxic (Lee & Seidel 1991; Waritz & Kwon 1968; Makulova 1965). Studies in our laboratory were performed at heating temperatures below 500 °C to avoid confounding of the responses due to the toxic gas-phase components. The ultrafine particle size of the PTFE fumes ranges between

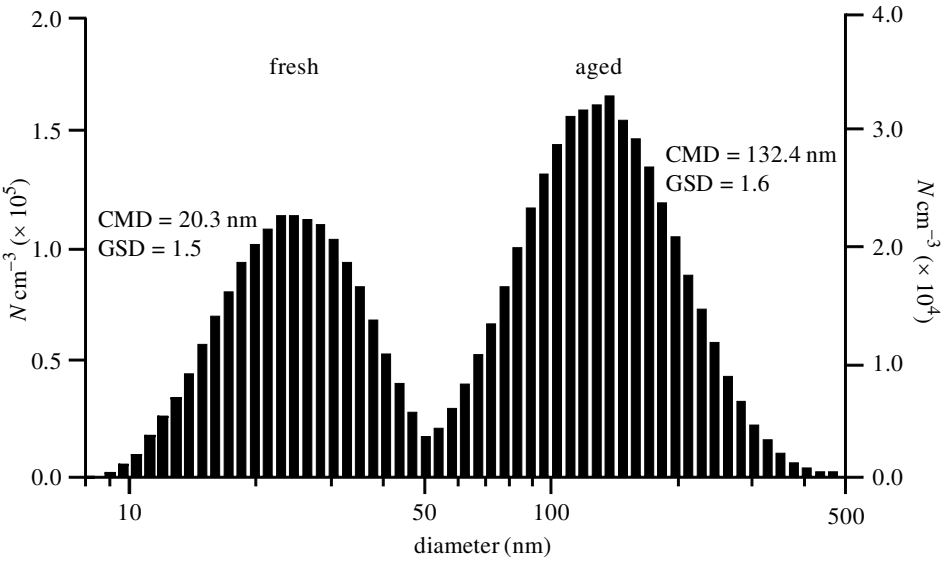

Figure 1. Particle size distribution of freshly generated and aged PTFE fumes showing the shift from ultrafine particle distribution to a size distribution with a median greater than 100 nm due to coagulation after 3.5 min of ageing.

10 and 50 nm. Exposure of rats to a concentration of 5×10^5 particles cm^{-3} (equivalent to *ca.* 50 $\mu g\, m^{-3}$) for 10–20 min results in the development of severe pulmonary inflammation and hemorrhage within 4 h post exposure (Oberdörster *et al.* 1995). High mortality due to the severe pulmonary oedematous response also occurs. Lung lavage fluid shows significantly increased neutrophils, up to 80% of total cells, highly increased protein levels, and increases in lysosomal and cytoplasmic enzymes. Histologically, a high degree of alveolar and interstitial oedema is present and both epithelial and endothelial cell layers are severely disrupted. These ultrafine particles are thought to be the cause of the toxicity of PTFE fumes. Using electron energy-loss spectroscopy (EELS), ultrafine fluorine-containing particles can be found shortly after the exposure in epithelial, interstitial and endothelial sites of both the conducting airways and alveolar regions of the lung (Oberdörster *et al.* 2000). Analysis of lung mRNA shows significant upregulation of proinflammatory cytokines such as *IL*-6, *IL*-1b, *IL*-1a, TNFα, and of antioxidants such as MnSOD, and metallothionein and the chemokine MIP-2. These responses are consistent with injuries due to severe oxidative lung damage.

(*b*) *Effect of ageing on PTFE toxicity*

Particles present in the air at high number concentrations tend to coagulate and thereby form larger particles over time. This coagulation process can occur within fractions of a second if number concentrations exceed 10^8 particles cm^{-3}, and will be progressively slower at lower concentrations (Hinds 1982). Ageing of the freshly generated PTFE fumes for 3–5 min results in a shift of the particle size distribution from a count median diameter (CMD) of 15–20 nm to one above 100 nm (figure 1). Gas-phase products do not seem to change in this ageing process. If the toxicity of PTFE fumes is indeed caused by the presence of ultrafine particles and if larger

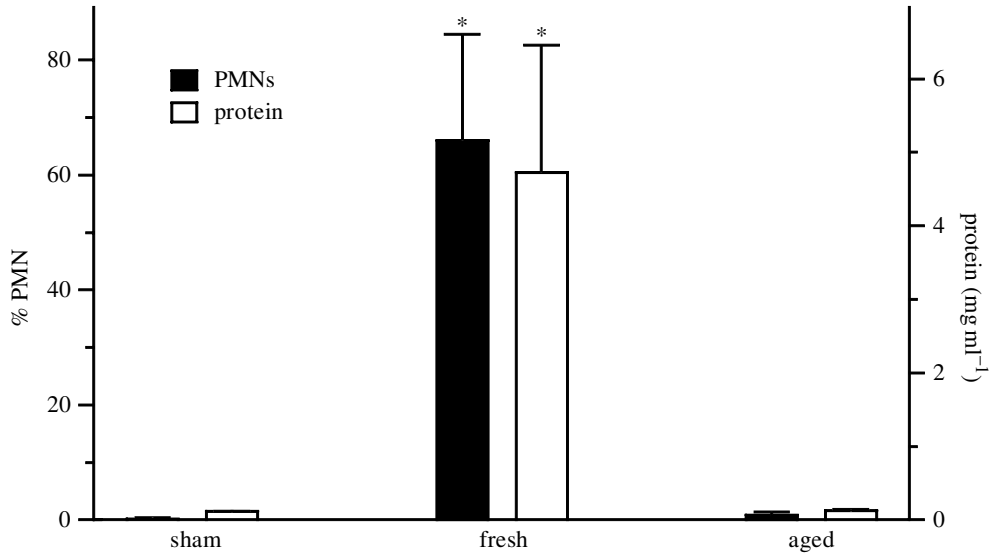

Figure 2. Lung lavage parameters of rats 4 h after a 50 min exposure to fresh and aged PTFE fumes. The percentage of neutrophils of the total lavage cells and the lavage protein content are shown. ($N = 4$ per group; mean ±SD; ∗, significantly different from sham and aged (ANOVA, $p < 0.05$).)

particles are less toxic, one would predict that exposure to the aged PTFE fumes with the larger particles will result in significantly less toxicity. Figure 2 shows the results of a study in which rats were exposed to either the freshly generated PTFE fumes at a concentration of 5×10^5 particles cm^{-3} or to aged PTFE fumes at a concentration of 1.5×10^5 particles cm^{-3}. The mass concentrations of the particles were 50 and 70 μg m^{-3}, respectively. Control rats were separately exposed to filtered air, and inflammatory lung lavage parameters were determined 4 h post exposure. As can be seen from figure 2, the rats exposed to the freshly generated fumes showed the expected high increase in lavage neutrophils and protein, indicating the presence of severe inflammatory pulmonary oedema. In contrast, sham-exposed control rats and rats exposed to the aged fumes for 15 min did not show significant changes in the lavage parameters.

Although these data are consistent with our hypothesis that inhaled ultrafine particles have a significantly greater toxic and inflammatory potential than larger sized particles, it cannot be excluded that the ageing and coagulation of the fresh PTFE fumes for several minutes had altered potential short-lived toxic radicals on the surface of the particles (Pryor *et al*. 1990). Whether the existence of such radicals plays a role in the toxicity of freshly generated polymer fumes is uncertain; earlier studies have shown that fumes generated from different plastic materials all show high toxicity regardless of the presence or absence of short-lived radicals (Seidel *et al*. 1991).

(*c*) *Adaptation to PTFE fume toxicity*

Workplace exposures to fumes of metals such as zinc or cadmium are known to induce a state of tolerance upon repeated exposures (Drinker *et al*. 1927). Such

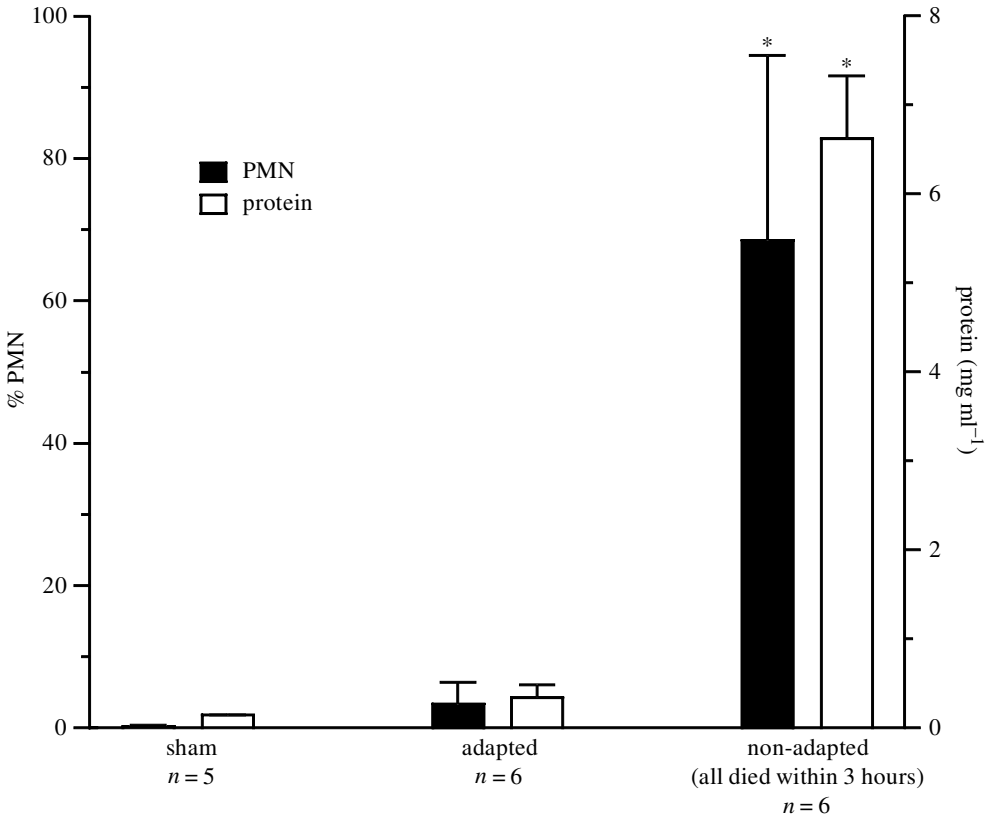

Figure 3. Lavage parameters 4 h after a 15 min exposure to PTFE fumes in adapted and non-adapted F-344 rats. The percentage neutrophils and total lavage protein content are shown. ($N = 6$ per group, mean ±SD; ∗, significantly different from sham and adapted group (ANOVA, $p < 0.05$).)

adaptation is associated with increases of antioxidant proteins including metallothionein (Hart *et al*. 1989), which protect the lung from the toxic effects of subsequent exposure to high concentrations of these fumes. Such adaptive responses have not been demonstrated for polymer fumes. However, since the mechanism for the high acute toxicity of these metal fumes is most likely to be due to oxidative stress, one might expect that similar adaptive changes occur after exposures to polymer fumes consisting of ultrafine particles.

In order to investigate the induction of PTFE fume tolerance, we exposed rats to PTFE fumes containing 5×10^5 particles cm^{-3} (equivalent to *ca.* 50 μg m^{-3}) for 5 min each on three consecutive days, followed by a 15 min exposure to the same concentration on day four. A second group of rats was sham exposed for 5 min on three consecutive days and on day four received the 15 min PTFE fume exposure together with animals from group one. A third group was sham exposed to filtered air on all four days. Four hours after the 15 min exposure, surviving animals were euthanized and their lungs lavaged for measurement of inflammatory parameters. Figure 3 shows the result of this study with respect to lavage neutrophils and lavage protein content. During the three day adaptation, animals of the adapted group

did not show any respiratory symptoms of being affected by these short, 5 min, exposures. Neither did the rats of this group show any clinical signs of respiratory effects after the 15 min exposure of PTFE fumes on day four. In contrast, rats of the non-adapted group were severely affected by the 15 min PTFE fume exposure on day four: all of them had to be euthanized within 3 h post exposure because of severe dyspnea. Sham-exposed control animals, like the adapted animals, did not show any effects (figure 3).

The high inflammatory effects in the lung observed after PTFE fume exposure are likely to also lead to systemic responses. This in turn would affect the rats' behaviour, and work performance is expected to drop significantly. Indeed, exposure of rats to PTFE fumes for 10 min was found to reduce performance on a running wheel by up to 40%, the effect starting immediately after exposure and lasting several days. However, pre-exposure of rats on three consecutive days, as was done in the adaptation study described above, adapted the animals so that the 10 min PTFE exposure on day four did not lead to any reduction in work performance.

The development of a state of tolerance against the deleterious effects of ultrafine PTFE fumes in these experiments demonstrates the importance of pre-exposure history for the induction of pulmonary and probably systemic responses. This may also be important for ambient particles. Adaptation is known to occur after exposure to oxidant gases such as ozone (Van Bree *et al*. 1993; Dodge *et al*. 1994). Such protective responses may be age dependent, and adaptation may not be as complete or may take longer if the host organism is compromised and in a more susceptible state.

(*d*) *Particle versus gas phase toxicity of PTFE fumes*

Since heating of PTFE results in the generation of both ultrafine particles and gas-phase products, the question arises as to whether the ultrafine particles per se can induce the observed highly toxic inflammatory responses. In order to evaluate this, an experiment was performed with exposure of four groups of rats as follows:

group 1: sham-exposed control animals;

group 2: animals exposed to the complete phase (particle plus gas) of freshly generated PTFE fumes;

group 3: animals exposed to the gas phase only after filtering the ultrafine particles; and

group 4: animals exposed to ultrafine PTFE particles only.

For the latter group, the generation of PTFE fumes was changed from heating Teflon in air to heating in argon, which resulted in the generation of ultrafine particles with no detectable gas-phase compounds. The exposure was for 25 min in compartmentalized whole-body exposure chambers at an ultrafine particle number concentration of 5×10^5 particles cm^{-3}. Four hours after exposure, the animals were euthanized and inflammatory lung lavage parameters determined. As shown in figure 4, neither the group exposed to the gas phase alone nor the group exposed to the ultrafine particles alone showed significant responses with respect to lavage neutrophils or protein compared with control animals. In contrast, the group exposed to the complete fume,

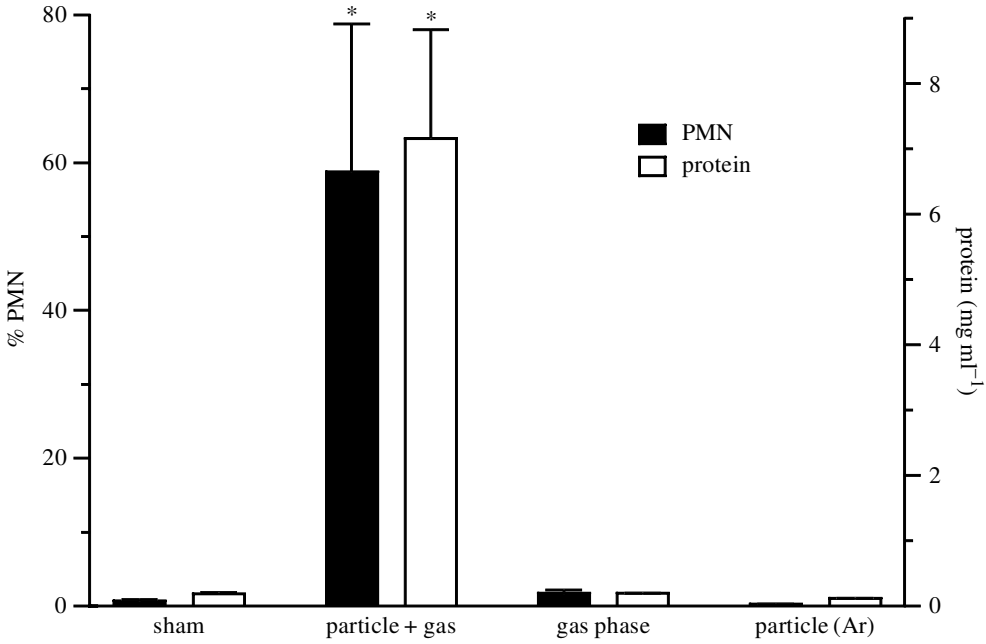

Figure 4. Lung lavage parameters of rats 4 h after a 25 min exposure to the gas or particle phase of PTFE fumes or to the complete particle-plus-gas phase. The percentage neutrophils of total lavage cells and lavage protein content are shown. ($N = 5$ per group, mean ±SE; ∗, significantly different from all other groups (ANOVA, $p < 0.05$).)

particle plus gas phase, confirmed the highly toxic nature of the PTFE fumes by responding with high increases in both lavage neutrophils and protein.

This result indicates that 'clean' ultrafine PTFE particles (generated in argon) do not induce significant toxicity after short-term exposure. However, it is conceivable that the argon-generated PTFE particles may have different surface reactivity compared with the air-generated PTFE particles, which may contribute to their high toxicity. Clearly, the gas-phase constituents alone, when generated at below 500 °C, do not exert pulmonary toxicity, but their combination with the ultrafine particles does. Preliminary attempts to adsorb PTFE gas-phase constituents onto ultrafine carbon particles did not result in acute pulmonary, inflammatory responses. Earlier studies have also found that gas-phase compounds of PTFE fumes do not cause the high toxicity when generated below 500 °C (Waritz & Kwon 1968). However, additional studies may be needed to confirm that gas-phase compounds do not play a role in PTFE fume toxicity.

(*e*) *Conclusions from PTFE fume studies*

The conclusions from these studies with PTFE fumes are that the ultrafine particles are, indeed, key to their high pulmonary toxicity. Furthermore, coagulation to larger particles during the ageing process results in a significant loss of the high ultrafine particle toxicity. The translocation of ultrafine particles deposited in the lung to epithelial, interstitial and endothelial sites also appears to be rapid, confirming earlier observations (Stearns *et al*. 1994). This is probably due to the fact that these

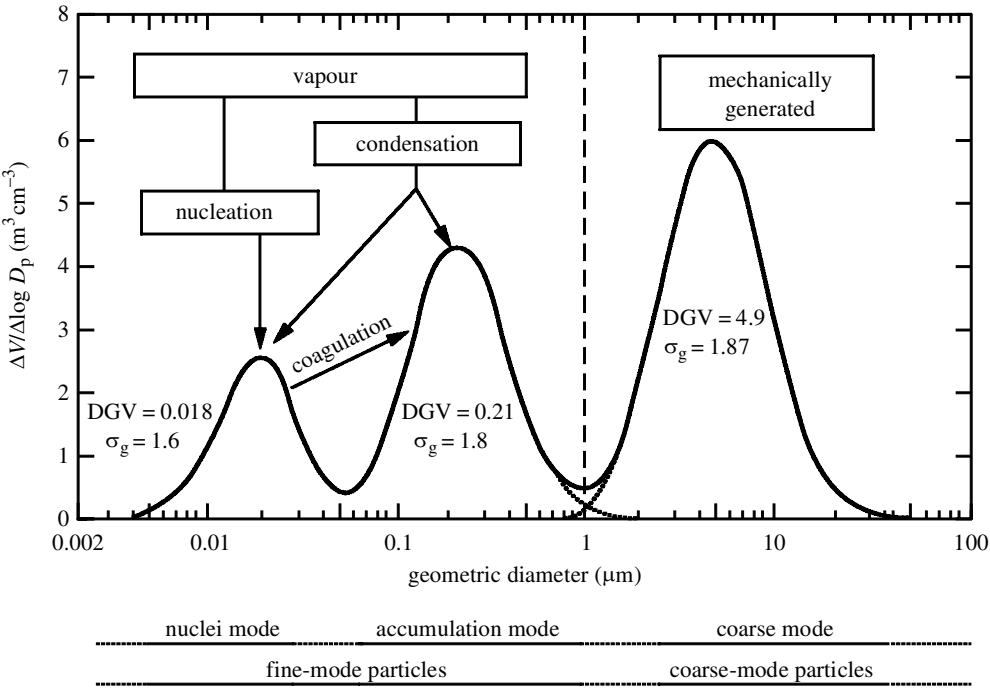

Figure 5. Trimodal urban aerosol, typical size distribution (from EPA 1996).

very tiny particles escape alveolar macrophage surveillance, which is very efficient for larger particles (Hahn *et al*. 1977). Of significance is that the very high, acute toxicity of ultrafine PTFE fumes can be prevented by short-term pre-exposures, leading to protective adaptation phenomena in the lung. Whether these findings can be extrapolated to ultrafine particles of low toxicity, such as those occurring in the urban atmosphere, requires studies with relevant ultrafine particles of low toxicity. The following sections show that some concepts of ultrafine particle toxicity do indeed apply to other ultrafine particles, such as the relative potencies of ultrafine and larger particles, translocation across the epithelium and to interstitial sites, and the importance of combined exposure of ultrafine particles with an oxidant gas.

3. Studies with ultrafine particles of low toxicity

(*a*) *Dosimetric aspects of ultrafine particle toxicology*

As mentioned in § 1, ultrafine particles are one of three particle modes in the urban atmosphere (figure 5). Normal background levels of the ultrafine mode are below 2 μg m^{-3}, with number concentrations in the range 1–4 × 10^4 particles cm^{-3} (Hughes *et al*. 1998). Table 1 summarizes results of measurements which show that episodic events can give rise to much higher concentrations, approaching 1 × 10^6 particles cm^{-3} and mass concentrations of up to 50 μg m^{-3}. In addition to traffic-related increases, other sources seem to be responsible for these high concentrations as well, and further research is needed to determine the composition of these particles. Episodic increases in ultrafine particle concentrations occur frequently and can last for more than a day and it is probably during or after these events that health effects may

Table 1. *Urban atmosphere particle concentrations: ultrafines* (*below* 100 nm)
(Nature of particles: carbonaceous; and others?)

condition	particles (cm^{-3})	($\mu g\ m^{-3}$)	reference
background average (Erfurt, Los Angeles)	$1–5 \times 10^4$	0.8–1.6	Tuch *et al.* (1997), Hughes *et al.* (1998) (Cass: 10 t d^{-1} emitted in Southern California)
episodic event peak (Frankfurt)	3×10^5	*ca.* 50	Brand *et al.* (1992)
episodic events and traffic peak (Atlanta)	1×10^6	?	P. McMurry (2000, personal communication) (preliminary data)

occur. Therefore, these concentrations can serve as a guideline for designing toxicological studies using realistic exposure levels. Importantly, when performing studies with ultrafine particle exposures in experimental animals, one should attempt to estimate deposited lung doses of ultrafine particles with the goal of achieving lung burdens that would also be experienced by humans under a given urban exposure scenario. Exposure concentrations to achieve this may be different between humans and experimental animals, as is discussed below.

Deposition of inhaled ultrafine particles in the respiratory tract occurs by diffusional processes. Several studies have shown that the human nose is highly efficient in collecting inhaled ultrafine particles by this process in the nasal compartment (Cheng *et al.* 1991; Swift *et al.* 1992). However, these data also show that diffusional deposition in the nasopharyngeal compartment is highly dependent on the ultrafine particle size, such that ultrafine particles below *ca.* 10 nm deposit with high efficiency in the nose, whereas this deposition becomes less for particles between 10 and 100 nm. There is thus a misconception that all ultrafine particles deposit efficiently in the nose. However, predictive particle deposition models show that the probability of deposition of 20 nm particles is greatest in the alveolar region (figure 6). Likewise, the tracheobronchial region can also be a significant target for even smaller ultrafine particles, as indicated in figure 6. If the deposited dose is expressed per unit surface area of the epithelium, the tracheo-bronchially deposited dose of ultrafine particles can be up to 50-fold higher than that for the alveolar region. These are important dosimetric aspects in ultrafine particle toxicology, which need to be considered when studying these particles. In addition, an important factor is the fate of deposited ultrafine particles, that is their disposition, which seems to be different from larger particles, as was discussed in the PTFE fume experiments.

Another important aspect of particle toxicology in general relates to comparative dosimetry between experimental animals and humans. Rodents, as the most frequently used experimental animals, have different deposition efficiencies for particles in their respiratory tract compared with humans. Table 2 compares the deposition efficiency of inhaled 20 nm particles—which is the peak of the urban ultrafine particle mode—between rats and humans based on deposition models by Yeh & Schum (1980) for rats and the ICRP (1994) model for humans. As this table indicates, the human equivalent concentration for 20 nm ultrafine particles is about twice that for the rat.

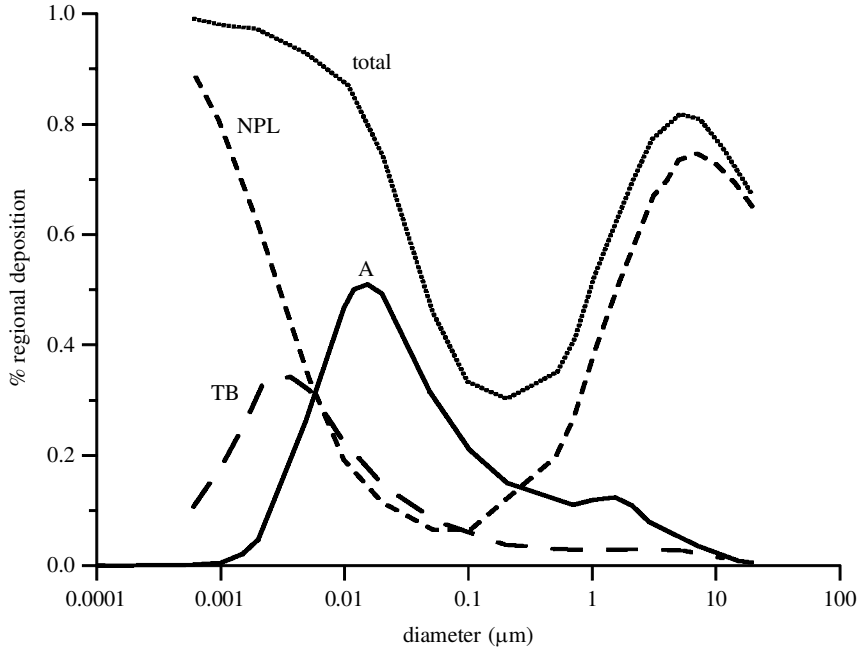

Figure 6. Predicted deposition of inhaled particles of different sizes of unit density in the human respiratory tract during nose breathing, light exercise (ICRP 1994). 'NPL' denotes nasopharyngolaryngeal deposition; 'TB' denotes tracheobronchial deposition; 'A' denotes alveolar deposition; 'total' denotes the sum of particle depositions in the respiratory tract for all three compartments.

Table 2. *Particle dosimetry*

(Human equivalent concentration for inhaled 20 nm particles (ICRP human model and Yeh & Schum rat model).)

	alveolar deposition fraction	deposited dose over 6 h per m^2 alveolar surface at 10 μg m^{-3}	relative dose at same inhaled concentration
rat, nasal breathing at rest	0.27	365 ng	1
human, nasal breathing, light	0.5	692 ng	1.9

The aforementioned high episodic ultrafine particle concentration of 50 μg m^{-3} in urban air would, therefore, be equivalent to *ca.* 100 μg m^{-3} inhaled by rats. Since this comparison is based on predictive deposition models, and since these models have not been validated experimentally for ultrafine particles, there could be considerable uncertainties. For example, significant differences exist between the NCRP and the ICRP deposition model. Thus, there is an urgent need to validate these deposition models by experimental data in rodents as well as humans.

(*b*) *Inflammatory potential of ultrafine versus fine particles*

We postulate that deposited ultrafine particles induce a greater inflammatory response per given mass than larger particles of the size of the accumulation mode.

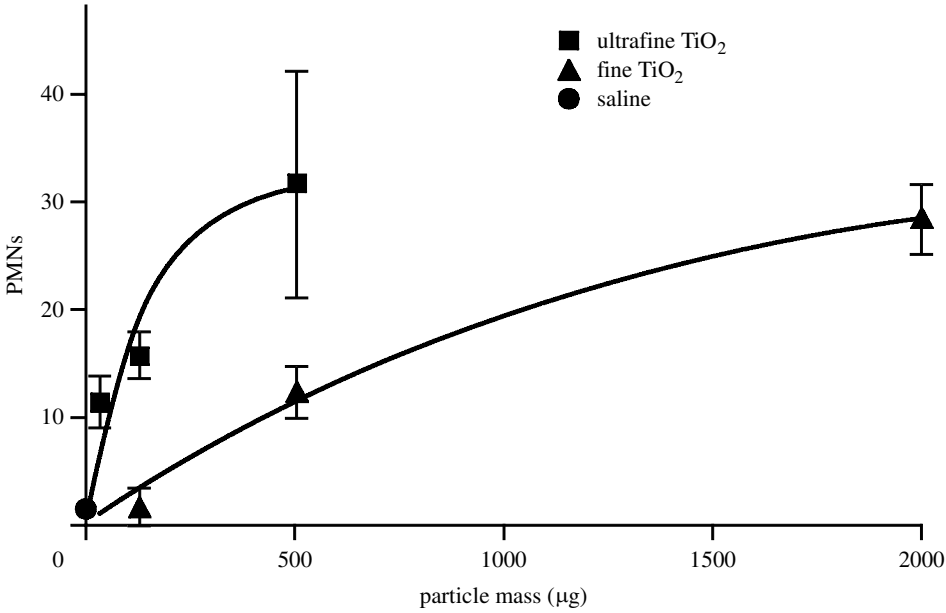

Figure 7. Inflammatory response 24 h after instillation of different doses of ultrafine and fine TiO_2 in rats, expressed as percentage neutrophils in lung lavage as a function of instilled particle mass. Ultrafine TiO_2, *ca.* 20 nm; fine TiO_2, *ca.* 250 nm.

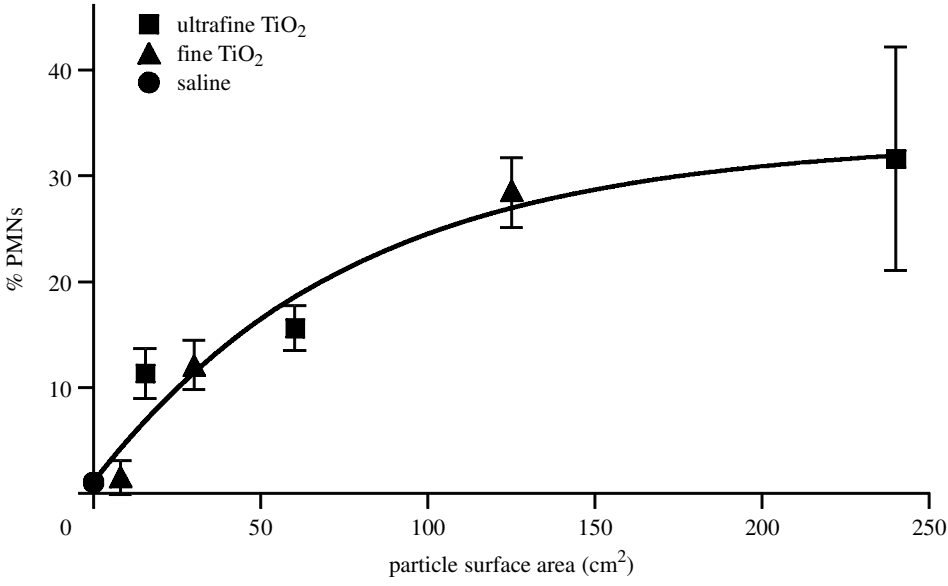

Figure 8. Same data as shown in figure 7 with particle dose expressed as particle surface area in the lung.

Results of our studies with ultrafine freshly generated and larger, aged PTFE fumes described above are consistent with this hypothesis. We tested this hypothesis by dosing rats with two different particle types of a rather benign dust, TiO_2. Ultrafine

Table 3. *Accumulation versus nucleation (ultrafine) mode particles: pulmonary inflammatory potential in humans*

(Assumptions: composition of two particle types is the same, toxicity is proportional to deposited dose, expressed as particle surface area (example: fine and ultrafine TiO_2).)

	accumulation mode particle (*ca.* 250 nm)	ultrafine particle (*ca.* 20 nm)
relative alveolar deposition	1	3.6
relative particle surface area	1	10
relative predicted toxicity	1	36[a]
	(10 μg m^{-3} ultrafine ≡ 360 μg m^{-3} accumulation mode.)	

[a] Additional factors need to be considered: increased interstitial translocation leads to extrapulmonary effects.

TiO_2 with an average particle size of 20 nm and pigment grade (fine) TiO_2 with an average particle size of *ca.* 250 nm were used. Doses ranging from 30 to 2000 μg of TiO_2 were intratracheally instilled into groups of rats. The inflammatory response in their lungs was assessed by analysis of cellular and biochemical lung lavage parameters 24 h later. The result of this dose–response study is shown in figure 7. It is evident from this figure that ultrafine TiO_2 elicited a significantly greater inflammatory cell influx (neutrophils) for the same dose than larger sized TiO_2. However, when the deposited TiO_2 dose was expressed as particle surface area, the result was quite different, as shown in figure 8. Using the particle surface area as dosimetric resulted in virtually identical inflammatory responses of these two different sizes of TiO_2 particles. The importance of particle surface area for eliciting inflammatory responses in the lung has been confirmed by Li *et al.* (1996) with ultrafine and fine carbon-black particles. This concept of particle surface area as the appropriate dosimetric has been recognized as an important principle in particulate matter toxicology (Oberdörster 1996; Donaldson *et al.* 1998).

Considering differences in pulmonary deposition and the importance of dosimetric for characterizing the inflammatory potential of inhaled particles, one can deduce a relative potency ranking for the *in vivo* toxicity of inhaled ultrafine particles versus larger 250 nm particles of the accumulation mode. Assuming that the chemical composition of the two particle sizes is the same and that the toxicity is proportional to the deposited dose expressed as particle surface area, one can derive that the toxicity of ultrafine particles is about 36-fold greater than that of accumulation-mode particles in terms of the inhaled mass concentration. Table 3 shows that this factor is due to the 3.6-fold greater deposition efficiency in the alveolar region and the ten-fold larger particle surface area per given mass for the 20 nm particles compared with 250 nm particles. Additional factors may need to be considered, such as the difference in interstitial translocation between the two particle sizes, and possibly also differences in translocation to extrapulmonary sites.

As mentioned above, TiO_2 particles are of a rather benign nature and have been used in the past in a number of studies as control particles of low toxic potency against which effects of other particle types have been compared. It is likely that the inflammatory response elicited by higher doses of ultrafine TiO_2 is based on a similar oxidative stress mechanism to that underlying the much greater pulmonary toxicity

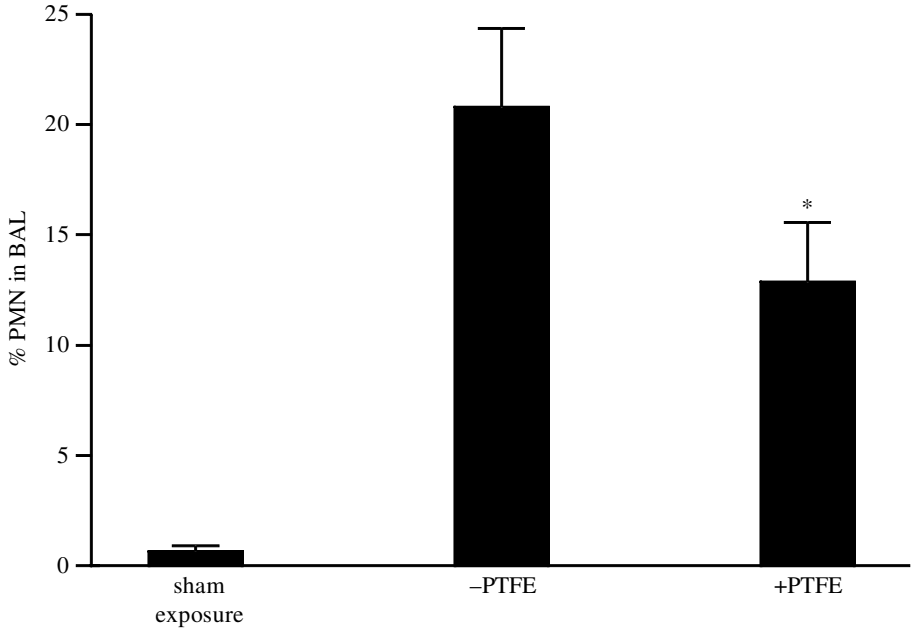

Figure 9. Lavage neutrophil response in rats 24 h after intratracheal instillation of 100 μg of TiO_2 particles in PTFE-fume-adapted and non-adapted rats. ∗, significantly different groups without PTFE and sham exposure (ANOVA, $p < 0.05$)

of PTFE fumes discussed above (Donaldson *et al*. 1998). One might, therefore, expect that adaptive responses observed in our PTFE experiments would also attenuate the inflammatory response of ultrafine TiO_2 based on the existence of cross-tolerance. The result of a study in rats indeed showed a significantly reduced inflammatory response in the lung to intratracheally instilled 100 μg of TiO_2 when the animals had been adapted to PTFE fumes for the previous three days (figure 9).

(*c*) *Deposition studies with ultrafine carbon and other particles*

A major component of ambient particles generated by combustion processes is their carbonaceous core (Hughes *et al*. 1998). These particles consist of different inorganic and organic compounds and we used carbonaceous particles consisting of elemental and *ca*. 30% organic carbon in our initial studies as surrogates to determine whether these ultrafine particles can induce effects in the lung. We used an electric spark discharge system that generates ultrafine carbonaceous particles between two graphite electrodes in an argon atmosphere. Figure 10 shows a typical particle size distribution with a count median diameter of 24 nm and a geometric standard deviation (GSD) of 1.86. One of our goals was to determine the fate of these ultrafine particles in the lung. In collaboration with Dr Godleski (Harvard University), using EELS technology we could show that these ultrafine carbonaceous particles were present in type I and type II alveolar epithelial cells shortly after a 6 h exposure to *ca*. 100 μg m^{-3}.

In order to further evaluate whether ultrafine particles after deposition can also translocate to extrapulmonary tissues, we used ultrafine platinum particles (CMD 13 nm, GSD 1.7) and exposed a rat to these particles for 6 h at a concentration of

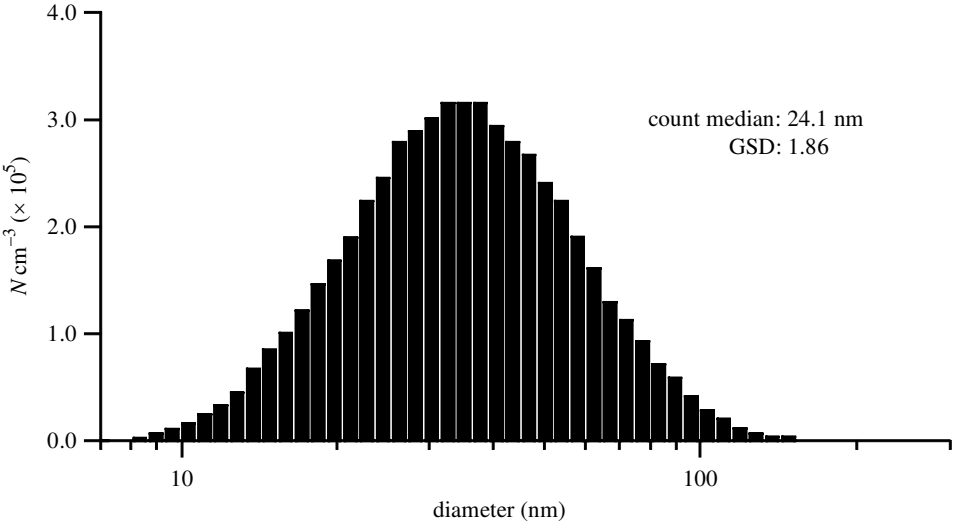

Figure 10. Particle size distribution of ultrafine carbonaceous particles generated by electric spark discharge between graphite electrodes in an argon atmosphere.

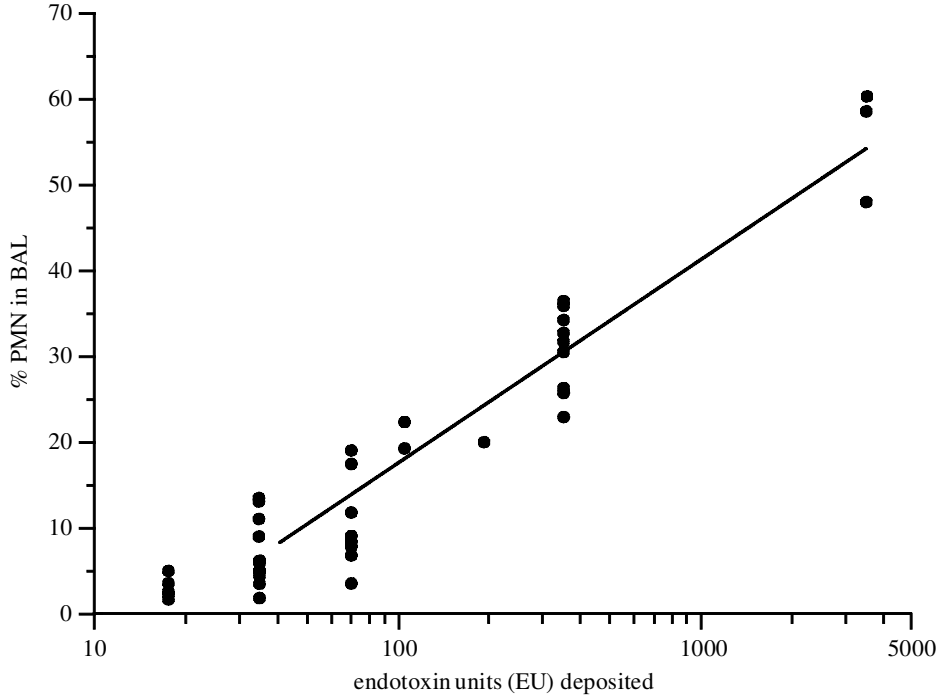

Figure 11. Dose–response relationship of lung lavage neutrophils 24 h after different lung doses of inhaled endotoxin in young rats.

ca. 100 μg m^{-3}. With the use of inductively coupled plasma mass spectroscopy, platinum levels were determined 30 min after the exposure in different lobes of the lung, the trachea and the liver. A total of 2.12 μg of platinum was found to be deposited in

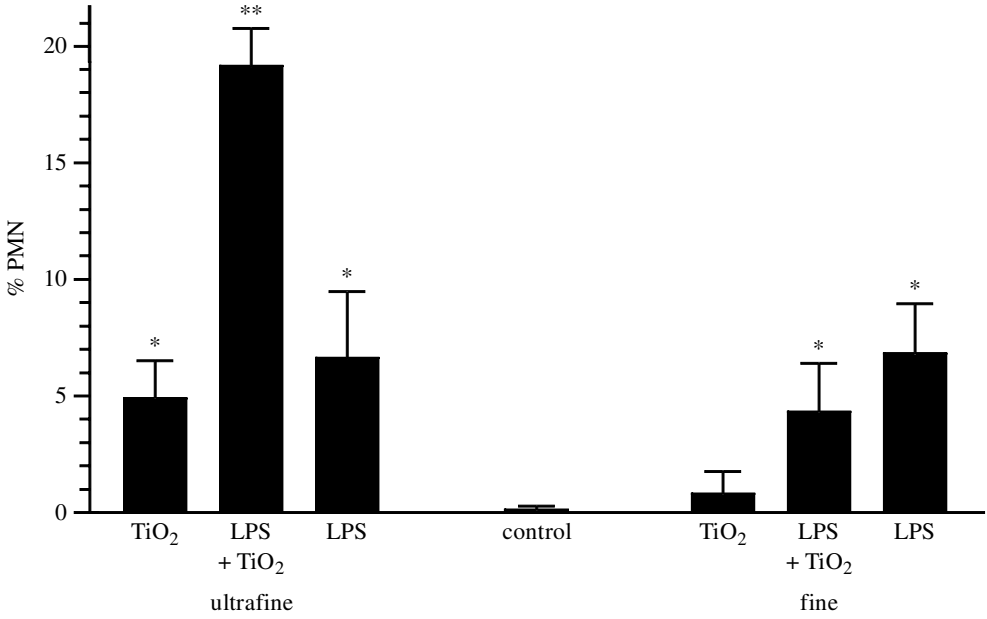

Figure 12. Lung lavage neutrophils 24 h after intratracheal instillation of 50 μg of ultrafine (20 nm) or fine (250 nm) TiO_2 particles into rats with or without prior LPS priming compared with LPS alone and saline-instilled control rats (mean ±SE); *, significant difference to control rats; **, significant difference to LPS-primed group and to ultrafine particle only group ($P < 0.05$; one-way ANOVA).

the lower respiratory tract, which corresponds to an estimated deposition efficiency of 20% of the inhaled ultrafine platinum particles. A significant finding was that platinum was also found in the liver, which amounted to *ca.* 7% of the lung platinum burden. However, it would be premature to conclude that this indicates translocation of the ultrafine particles from the lung, since it cannot be excluded that a small amount of the ultrafine platinum particles has been solubilized in the lung and may have reached the liver as soluble platinum. Thus, although platinum metal is considered to be very poorly soluble, additional studies with insoluble ultrafine particles need to be performed and are planned in our laboratory to determine more precisely the potential for ultrafine particles to reach extrapulmonary tissues.

(*d*) *Animal models of a compromised host to study ultrafine particle toxicity*

Associations between particulate air pollution and adverse health effects have only been observed in susceptible parts of the population and not in healthy people. An important aspect of studying potential causality of such effects includes, therefore, the use of animal models, which mimic a compromised respiratory or cardiovascular condition occurring in humans. Inhalation studies with particles in the past were typically performed in young, healthy animals using high exposure concentrations and doses in order to induce effects that can then be analysed further. With increasing awareness of dosimetry issues (low relevant doses) and of the importance of the impact of diseases on effects, those previous study designs have to be questioned: are mechanisms underlying effects induced by high doses in the healthy mammalian

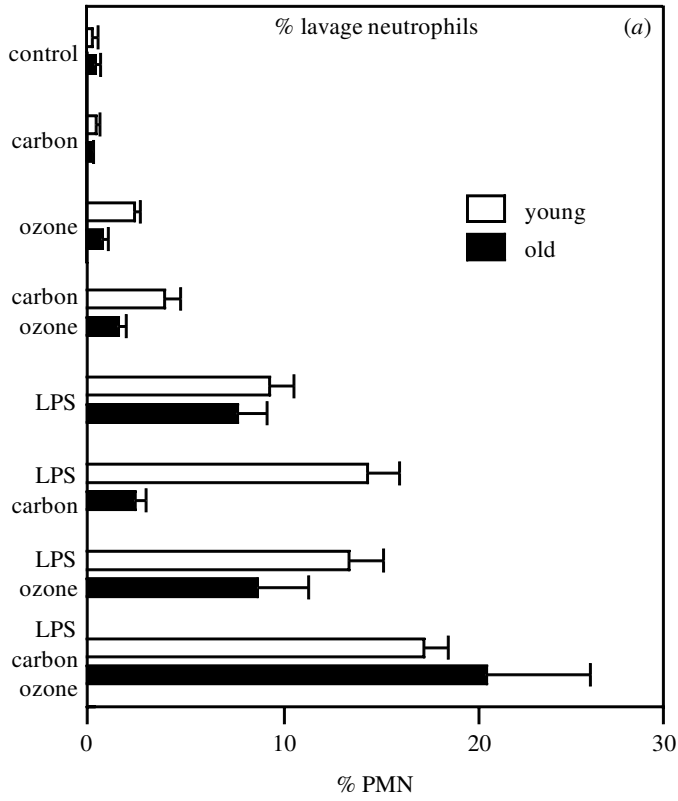

Figure 13. Lung lavage inflammatory cell response of young (10 weeks) and old (22 month) rats following inhalation exposure to ultrafine carbonaceous particles (*ca.* 105 μg m^{-3}) ± O_3 (1 ppm) ± LPS priming. (*a*) Per cent neutrophils of total lavage cells.

organisms really the same as those of low doses in a compromised host? Or should we not rather assume that the dose/dose rate controls the mechanism, as has been concluded from a number of particle overload studies?

A change in particulate matter toxicology is taking place, switching from the use of healthy animals to that in animal models of compromised humans. These models, which need to be characterized and validated, include specific disease models, the use of transgenic animals and of senescent animals. In addition, as already emphasized, relevant, realistic doses both under *in vivo* and *in vitro* experimental study conditions need to be applied. For example, in the aforementioned studies with intratracheally instilled ultrafine and fine TiO_2 particles, high doses were administered that will not be deposited by inhalation of low ambient concentrations in short-term exposures. However, the goal of those TiO_2 instillation studies was to test the concept of the relative toxicities of ultrafine versus fine particles, rather than examining whether ultrafine particles at reported ambient concentrations can cause adverse effects.

The critical issue of appropriate animal models of a human disease is complicated by the fact that many animal models are of an acute nature, whereas respective human conditions have slowly developed into a chronic state. For example, intratracheal instillation of elastase produces a marked pulmonary emphysema in mice or rats, yet this emphysema is most certainly not equivalent to human emphysema

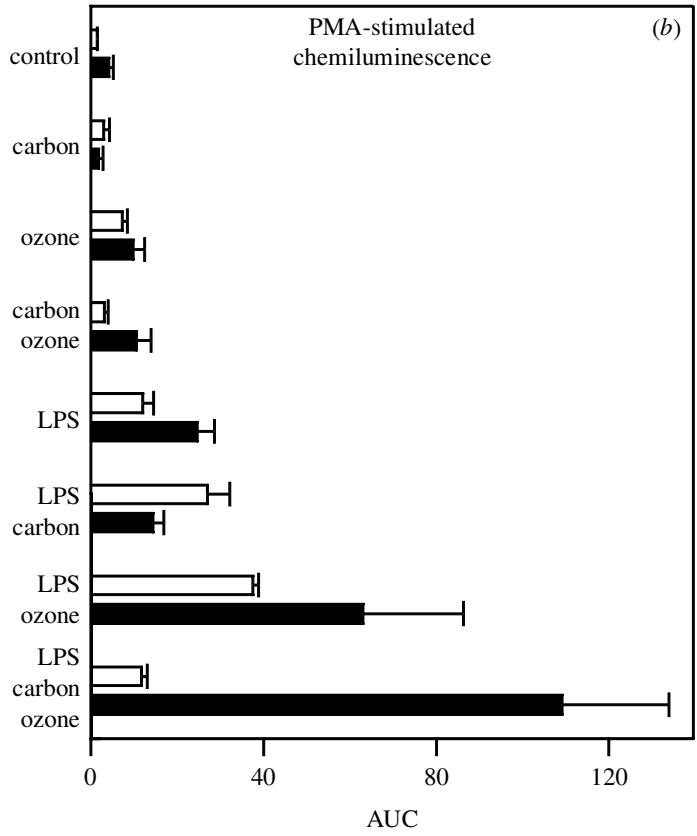

Figure 13. (*Cont.*) (*b*) PMA-stimulated chemiluminescence of lavage cells.

seen in people with chronic obstructive pulmonary disease. In our initial studies with a compromised respiratory tract we used an inhalation model with endotoxin (lipopolysaccharide (LPS)) to mimic the early stages of a respiratory tract infection with gram negative bacteria. People with pneumonia, in particular the elderly, are one susceptible group that has been identified in epidemiological studies (EPA 1996) which we are targeting in our studies.

Figure 11 shows the dose–response relationship of inhaled LPS in rats 24 h after exposure. The neutrophil response in lung lavage fluid after different doses of endotoxin deposited in the alveolar region is shown. The LPS doses were estimated based on the particle size distribution of the inhaled LPS aerosol and the airborne concentration, with the use of predictive deposition models (Yeh & Schum 1980). LPS exposure lasted only for *ca.* 12 min. Depending on the deposited dose, LPS at very high doses can result in a severe ARDS-like pulmonary inflammation, with large amounts of neutrophils and protein in the lavage fluid. However, at lower doses, only a mild inflammatory response in terms of neutrophil influx and no increase in lavage protein occurs. We used this lower dose of *ca.* 70 endotoxin units (EUs) to prime the respiratory tract prior to exposure with ultrafine particles. The mild inflammatory response at this low deposited alveoles dose is characterized by a lavage neutrophil level of *ca.* 10% of the total cells 24 h post exposure.

Before using this LPS model to examine the response to inhaled low concentrations of spark discharge-generated ultrafine carbonaceous particles, we tested it using ultrafine and fine TiO_2 particles via intratracheal instillation. 50 μg of ultrafine and fine TiO_2 were intratracheally instilled in rats that had either received the LPS priming inhalation or a sham inhalation of NaCl aerosol. Instillation of the particles was performed within 30 min after the inhalation. Other rats received inhaled LPS alone or instilled saline (controls). As the result in figure 12 shows, only the ultrafine TiO_2 particles given after LPS induced a significantly greater neutrophil influx compared with TiO_2 alone and LPS alone, whereas the fine TiO_2 particles administered to the LPS-primed lung did not show a greater response than LPS alone. This result shows that priming of the respiratory tract with inhaled LPS can indeed amplify the response to a subsequent particulate stimulus, and it further confirms that for the same lung dose in terms of mass, ultrafine particles are significantly more potent than fine particles.

(*e*) *Age and ozone co-exposure as modulators of ultrafine carbon particle toxicity*

The senescent mammalian organisms may be more sensitive to inhaled toxicants than the younger organism. With respect to the potential effect of ambient particulate matter, it has been suggested that co-exposure to other pollutants, such as oxidant gases, may contribute to the adverse effects observed in the epidemiological studies (Burnett *et al*. 1997*a*, *b*; Samet *et al*. 1997). Our studies with ultrafine PTFE fumes, although inconclusive with respect to a contributory effect of gas-phase compounds, can be interpreted as showing some influence of gas-phase compounds. Our latest study with inhaled ultrafine carbonaceous particles was designed to evaluate their inflammatory effects in the lung of young and old rats with and without ozone co-exposure and with and without LPS inhalation priming. Eight groups of 10-week old and 22-month old rats were exposed to ultrafine carbonaceous particles (concentration *ca.* 105 μg m^{-3}), ozone (1 ppm) or inhaled LPS (*ca.* 70 EU estimated alveolar dose) and to combinations of these compounds. Sham-exposed control rats served as controls. Lung lavage parameters were determined 24 h later and lavaged inflammatory cells were subjected to a chemiluminescence assay *in vitro* to determine their unstimulated and phorbol ester (PMA)-stimulated oxidant release.

Figure 13 shows the result with respect to lavage neutrophils and the PMA-stimulated chemiluminescence. Three-way analyses of variance (ANOVAs) for the groups of young and old rats, as well as a four-way ANOVA for the two age groups combined, were performed; the three factors were ultrafine carbon, ozone and LPS and the fourth factor was age. These analyses showed that each of the three components (ultrafine carbon, ozone and LPS) induced significant effects independently. In addition, the ultrafine carbonaceous particle response in the aged rats was synergistic with the effects of ozone. In both old and young groups, the greatest inflammatory cell response was observed in the LPS-primed group with combined exposure to ultrafine carbonaceous particles and ozone (Elder *et al*. 2000*b*).

There was also a significant age effect, showing that the aged animals responded with greater oxidant release of the lavaged inflammatory cells compared with the young animals in the combined exposure groups. This greater release of reactive oxygen species implies a greater risk of oxidative lung injury in the aged organism under exposure conditions of ultrafine carbon particles in combination with ozone in

the LPS-sensitized respiratory tract. These studies show that ultrafine carbonaceous particles can cause significant pulmonary inflammation. This occurs at inhaled concentrations leading to lung doses which are deposited in human lungs during episodic increases of urban ultrafine particles. Future studies will evaluate whether addition of transition metals to the carbon particles amplifies the inflammatory response.

4. Summary and conclusions

Conclusions from these studies are as follows.

(1) Poorly soluble ultrafine particles cause a significantly greater pulmonary inflammation per given mass than larger particles. The appropriate dosimetric is their high specific surface area rather than the mass of these particles.

(2) Ultrafine carbonaceous particles at relevant inhaled concentrations can cause an inflammatory response in rodents.

(3) Ultrafine particles translocate readily to epithelial and interstitial sites. It is also conceivable that they may be transported to extrapulmonary organs; this needs to be confirmed in future studies.

(4) Specific modulating factors that increase ultrafine particle effects include age and a compromised/sensitized respiratory tract.

(5) Combined exposures with an oxidant gas can enhance ultrafine particle effects.

Studies with ultrafine particles mentioned in this manuscript have been supported by the Health Effects Institute (contract no. 95-11) and by the National Institute of Health Sciences grants (ES 04872 and ES 247). Detailed results of these studies were reported by Elder *et al.* (2000*a*–*c*), Johnston *et al.* (1996, 1998) and Oberdörster *et al.* (1995, 2000).

References

Brand, P., Ruob, K. & Gebhart, J. 1992 Performance of a mobile aerosol spectrometer for an *in situ* characterization of environmental aerosols in Frankfurt city. *Atmos. Environ.* A **26**, 2451–2457.

Burnett, R. T, Brook J. R., Yung, W. T., Dales, R. E. & Krewski, D. 1997*a* Association between ozone and hospitalization for respiratory diseases in 16 Canadian cities. *Environ. Res.* **72**, 24–31.

Burnett, R. T., Cakmak, S., Brook, J. R. & Krewski, D. 1997*b* The role of particulate size and chemistry in the association between summertime ambient air pollution and hospitalization for cardiorespiratory diseases. *Environ. Health Perspect.* **105**, 614–620.

Cheng, Y.-S., Yeh, H.-C. & Swift, D. L. 1991 Aerosol deposition in human nasal airway for particles 1 nm to 20 nm: a model study. *Radiation Protection Dosimetry* **38**, 41–47.

Dodge, D. E., Rucker, R. B. Pinkerton, K. E., Haselton, C. J. & Plopper, C. G. 1994 Dose-dependent tolerance to ozone. III. Elevation of intracellular clara cell 10-k protein in central acini of rats exposed for 20 months. *Toxicol. Appl. Pharmacol.* **127**, 109–123.

Donaldson, K., Li, X. Y. & MacNee, W. 1998 Ultrafine (nanometer) particle mediated lung injury. *J. Aerosol Sci.* **29**, 553–560.

Drinker, P., Thomson, R. M. & Finn, J. L. 1927 Metal fume fever. II. Resistance acquired by inhalation of zinc oxide on two successive days. *J. Ind. Hygiene Toxicol.* **9**, 98–105.

Elder, A. C. P., Johnston, C., Finkelstein, J. & Oberdörster, G. 2000*a* Induction of adaptation to inhaled lipopolysaccharide in young and old rats and mice. *Inhal. Toxicol.* **12**, 225–243.

Elder, A. C. P., Gelein, R., Finkelstein, J., Cox, C. & Oberdörster, G. 2000*b* Endotoxin priming affects the lung response to ultrafine particles and ozone in young and old rats. *Inhal. Toxicol.* (Suppl. I) **12**, 85–98.

Elder, A. C. P., Gelein, R., Finkelstein, J., Cox, C., Oberdörster, G. 2000*c* The pulmonary inflammatory response to inhaled ultrafine particles is modified by age, respiratory tract sensitization, and disease. *Inhal. Toxicol.* (In the press.)

EPA 1996 Air quality criteria for particulate matter, vol. III. EPA/600/P-95/001cF.

Goldstein, M., Weiss, H., Wade, K. *et al.* 1987 An outbreak of fume fever in an electronics instrument testing laboratory. *J. Occup. Med.* **29**, 746–749.

Gordon, T., Chen, L. C., Fine, J. M., Schlesinger, R. B., Su, W. Y., Kimmel, T. A. & Amdur, M. O. 1992 Pulmonary effects of inhaled zincoxide in human subjects, guinea pigs, rats and rabbits. *Am. Ind. Hygiene Ass. J.* **53**, 503–509.

Hahn, F. F., Newton, G. J. & Bryant, P. L. 1977 *In vitro* phagocytosis of respirable-sized monodisperse particles by alveolar macrophages. In *Pulmonary macrophages and epithelia cells* (ed. C. L. Sanders, R. P. Schneider, G. E. Dagle & H. A. Ragen), pp. 424–435. Energy Research and Development Administration Symposium Series.

Hart, B. A., Voss, G. W. & Willean, C. L. 1989 Pulmonary tolerance to cadmium following cadmium aerosol pretreatement. *Toxicol. Appl. Pharmacol.* **101**, 447–460.

Hinds, W. C. 1982 *Aerosol technology*, pp. 235–239. Wiley.

Hughes, L. S., Cass, G. R., Jones, J., Ames, M. & Olmec, L. 1998 Physical and chemical characterization of atmospheric ultrafine particles in the Los Angeles area. *Environ. Sci. Technol.* **32**, 1153–1161.

ICRP (International Commission on Radiological Protection) 1994 *Annals of the ICRP, human respiratory tract model for radiological protection.* ICRP publication 66. Oxford: Pergamon.

Johnston, C. J., Finkelstein, J. N., Gelein, R., Baggs, R. & Oberdörster, G. 1996 Characterization of the early pulmonary inflammatory response associated with PTFE fume exposure. *Toxicol. Appl. Pharmacol.* **140**, 154–163.

Johnston, C. J., Finkelstein, J. N., Gelein, R. M. & Oberdörster, G. 1998 Pulmonary inflammatory responses and cytokine and antioxidant mRNA levels in the lungs of young and old C57BL/6 mice after exposure to Teflon fumes. *Inhal. Toxicol.* **10**, 931–953.

Lee, K. P. & Seidel, W. C. 1991 Pulmonary response to perfluoropolymer fume and particles generated under various exposure conditions. *Fund. Appl. Toxicol.* **17**, 254–269.

Li, X. Y., Gilmour, P. S., Donaldson, K. & MacNee, W. 1996 Free radical activity and pro-inflammatory effects of particulate air pollution (PM_{10}) *in vivo* and *in vitro*. *Thorax* **51**, 1216–1222.

Makulova, I. D. 1965 The clinical picture in acute perfluoroisobutylen poisoning. *Gigiena Truda I Professionalnye Zabolevaniya* **9**, 20–23.

Oberdörster, G. 1996 Significance of particle parameters in the evaluation of exposure–dose–response relationships of inhaled particles. *Inhal. Toxicol.* (Suppl.) **8**, 73–89.

Oberdörster, G., Gelein, R. M., Ferin, J. & Weiss, B. 1995 Association of particulate air pollution and acute morality: involvment of ultrafine particles? *Inhal. Toxicol.* **7**, 111–124.

Oberdörster, G., Finkelstein, J. N., Johnston, C., Gelein, R., Cox, C., Baggs, R. & Elder, A. 2000 Investigator's report: acute pulmonary effects of ultrafine particles in rats and mice. Health Effects Institute final report. (In the press.)

Pryor, W. A., Nuggehalli, S. K., Scherer Jr, K. V. & Church, D. F. 1990 An electron spin resonance study of the particles produced in the pyrolysis of perfluoro-polymers. *Chem. Res. Toxicol.* **3**, 2–7.

Rosenstock, L. & Cullen, M. R. 1986 *Clinical occupational medicine*, pp. 28, 232. Philadelphia, PA: Saunders.

Samet, J. M., Zeger, S. L., Kelsall, J. E., Xu, J. & Kalkstein, L. S. 1997 Particulate air pollution and daily mortality: analysis of the effects of weather and multiple air pollutants. The phase I.B. report of the particle epidemiology evaluation project. Cambridge, MA: Health Effects Institute report.

Seidel, W. C., Scherer Jr, K. V., Cline Jr, D., Olson, A. H., Bonesteel, J. K., Church, D. F., Nuggehalli, S. & Pryor, W. A. 1991 Chemical, physical, and toxicological characterization of fumes produced by heating tetrafluoroethene homopolymer and its copolymers with hexafluoropropene and perfluoro(propyl vinyl ether). *Chem. Res. Toxicol.* **4**, 229–236.

Stearns, R. C., Murthy, G. G. K., Skornik, W., Hatch, V., Katler, M. & Godleski, J. J. 1994 Detection of ultrafine copper oxide particles in the lungs of hamsters by electron spectroscopic imaging. In *Proc. Int. Conf. on Electron Microscopy, ICEM 13, Paris*, pp. 763–765.

Swift, D. L., Montassier, N., Hopke, P. K., Karpen-Hayes, K., Cheng, Y.-S., Su, Y. F., Yeh, H. C. & Strong, J. C. 1992 Inspiratory deposition of ultrafine particles in human nasal replicate cast. *J. Aerosol Sci.* **23**, 65–72.

Tuch, T. H., Brand, P., Wichmann, H. E. & Heyder, J. 1997 Variation of particle number and mass concentration in various size ranges of ambient aerosols in eastern Germany. *Atmos. Environ.* **31**, 4193–4197.

Van Bree, L., Koren, H. S., Devlin, R. B. & Rombout, P. J. A. 1993 Recovery from attenuated inflammation in lower airways of rats following repeated exposure to ozone. *Am. Rev. Respir. Dis.* **147**, A633.

Waritz, R. S. & Kwon, B. K. 1968 The inhalation toxicity of pyrolysis products of polytetrafluoroethylene heated below 500 degrees centigrade. *Am. Indust. Hygiene Ass. J.* **29**, 10–26.

Yeh, H. C. & Schum, M. 1980 Theoretical evaluation of aerosol deposition in anatomical models of mammalian lung airways. *Bull. Math. Biol.* **42**, 1–15.

Discussion

M. S. Bingley (*Cobham, Surrey, UK*). The European Community is about to remove lead (Pb) from solder. Replacement solders will have a higher melting point. Electronic circuitry makes extensive use of PTFE. In view of what you have said about the toxicity of ultrafine particles generated by heated PTFE, are electronic engineers to be put in danger by future EC legislation?

G. Oberdörster. The particles in fumes from solder seem to be bigger than ultrafines, and you can actually see those fumes in contrast to PTFE fumes. If PTFE is present on electronic circuitry and is heated at the same time by the solder, the emitted ultrafine PTFE particles will most likely coagulate onto the larger particles of the dense solder fume. Experiments adding PTFE fumes to diesel smoke or wood smoke have shown that these combined particles were 80 times less potent in their toxicity than PTFE fumes.

D. Costa (*US EPA, NC, USA*). Your last figure, showing the combined effects of ultrafines, ozone and LPS showed that 100 $\mu g\,m^{-3}$ of ultrafines alone had little impact on PMNs, and likewise, the ozone exposure also had little effect, where many published results show a significant effect. Yet, the interaction (combined) effect seems larger. Do you have an explanation for this?

G. Oberdörster. Indeed, the response of neutrophils in BAL was rather low after ozone alone and after ultrafine carbon alone. However, the combined exposure in the

LPS-primed animals showed a large response, which emphasizes the importance of establishing animal models of increased susceptibility for evaluating otherwise subtle effects of inhaled ultrafine carbon. I think that the development and use of specific animal models—with a compromised pulmonary or cardiovascular system—is crucial for future progress in PM research.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES