Pulmonary antioxidants exert differential protective effects against urban and industrial particulate matter

L L GREENWELL*, T MORENO and R J RICHARDS

Cardiff School of Biosciences, Cardiff University, PO Box 911, Museum Avenue, Cardiff CF10 3US, UK

**Corresponding author (Fax, 44-29-2087-4116; Email, Greenwellll@cardiff.ac.uk)*

This investigation focuses on the application of an *in vitro* assay in elucidating the role of lung lining fluid antioxidants in the protection against inhaled particles, and to compare the toxicities of different airborne particulate matter (PM), PM_{10}, collections from South Wales, UK.

PM collections from both urban and industrial sites caused 50% oxidative degradation of DNA *in vitro* at concentrations as low as $12{\cdot}9 \pm 2{\cdot}1$ μg ml^{-1} and $4{\cdot}9 \pm 0{\cdot}9$ μg ml^{-1} respectively. The primary source of this bioreactivity was found to be the soluble fraction of both particle collections. The coarser $PM_{10-2{\cdot}5}$ fraction also showed greater oxidative bioreactivity than the $PM_{2{\cdot}5-0{\cdot}1}$ in both cases. When repeated in the presence of a low molecular weight fraction of fresh pulmonary lavage fluid, as well as in artificial lung lining fluid (200 μM urate, glutathione and ascorbate), the DNA damage was significantly reduced in all cases ($P < 0{\cdot}05$). The antioxidants exerted a greater effect on the industrial samples than on the urban samples, and on the $PM_{10-2{\cdot}5}$ fractions than on the $PM_{2{\cdot}5-0{\cdot}1}$ fractions, supporting the previous findings that respirable PM and urban samples contain fewer free radical sources than inhalable PM and industrial samples.

[Greenwell L L, Moreno T and Richards R J 2003 Pulmonary antioxidants exert differential protective effects against urban and industrial particulate matter; *J. Biosci*. **28** 101–107]

1. Introduction

In recent years epidemiological investigations have highlighted a possible link between increases in air pollution and adverse health effects. A widely accepted hypothesis is that ambient airborne particulate matter (PM) may have a causal role in both acute and chronic conditions. Thus, consistent statistical increases in both morbidity and mortality have been linked to pollution episodes during which the mass of particles is elevated (Pope *et al* 1995; Department of Health 1998; Brunekreef *et al* 2000). PM with diameter of 2·5 μm or less ($PM_{2{\cdot}5}$) perhaps has a greater causal role in the observed health effects than PM with diameter of 10 μm ($PM_{10-2{\cdot}5}$) (Schwartz and Meas 2000). More over, besides particle size, particles with different chemical properties have been implicated in causality or exacerbation of health effects. These include sulphates (Schwartz and Meas 2000), particles such as those from diesel exhaust, or from ambient collections containing transition metals (Richards 1997; Dye *et al* 1997; Gavett *et al* 1999; Adamson *et al* 2000). However, it is likely that the combined effects of the many different types of particles in the airborne mixture form the biological basis of the observed epidemiological findings (Brunekreef *et al* 2000). It is therefore becoming increasingly important that particle collections undergo physicochemical analysis in order that progress can be made in determining the factors that may be causative/exacerbate pulmonary/cardiovascular health conditions.

One popular hypothesis is that PM exerts toxic effects by way of an oxidative process. The mechanism by which particles cause oxidative damage remain unresolved

Keywords. Oxidation; PM_{10}; pulmonary antioxidants

Abbreviations used: ELF, Epithelial lining fluid; PM, particulate matter; PUF, polyurethane foam; sELF, surrogate ELF; ROFA, residual oil fly ash.

although the bioavailability of metal complexes associated with PM surfaces may be important. Permeability changes and inflammation have been produced in rat lungs following the instillation of metal-rich, residual oil fly ash (ROFA) – a material which is 94% soluble (Dreher *et al* 1995, 1996). Similar effects have been noted in mice instilled with EHC93, a Canadian urban sample (Adamson *et al* 1999). The active component of EHC93 was identified as zinc, which could be removed in a water-soluble fraction from the particle surface (Adamson *et al* 2000). Earlier studies have also established that soluble (bioavailable) zinc initiates pulmonary damage (Richards *et al* 1989). Other bioavailable metals (particularly iron) are commonly hypothesised to have a causative role in oxidative damage, whether directly or by formation of reactive oxygen species (ROS) through their interaction with other molecules (Costa and Dreher 1997; Donaldson *et al* 1997). Previous studies have suggested that the formation of superoxide and hydroxyl radicals by bioavailable metals could be responsible for oxidative damage induced by PM *in vitro* and *in vivo* (Donaldson *et al* 1997; Gutteridge *et al* 1979; Marx and Von Asbeck 1996). It has also been hypothesised that these reactive species are the toxic mechanism of diesel exhaust particles (DEP), a major constituent of urban PM (Vogl and Elstner 1989; Sagai *et al* 1993).

In order to ascertain oxidative capacity of PM and then semiquantify its bioreactivity, an *in vitro* assay monitoring damage of supercoiled DNA by free radicals was employed in the present investigation. This plasmid assay has also been used to examine the loss of supercoiled DNA by PM at relatively high doses (150 μg/ml) (Donaldson *et al* 1996). The basis of the assay is that any free radicals associated with particle surfaces can damage the supercoiled DNA by 'nicking' the strands. This initially causes the DNA to unwind from the supercoiled form into a relaxed coil. Further, damage results in complete linearisation followed by fragmentation. This change in quaternary structure alters the electrophoretic mobility of the DNA, thus allowing separation and quantitation on an agarose gel.

Cardiff and Port Talbot, both in South Wales, UK were chosen for this study. Separated by approximately 30 miles, they are comparable in that both are coastal and bisected by a busy motorway. However, Port Talbot is heavily industrialised, encompassing an extensive steelworks factory. Port Talbot registers regular exceedences (20 days exceedence in 1999) of the UK particulate air pollution mass standard (50 $\mu g\ m^{-3}$) from the UK network TEOM situated at the Groeswen Hospital, which is northeast of the town centre and the steelworks. This site was therefore considered optimal for PM heterogeneity and mass. The roadside collection site in Cardiff civic centre was selected for its heavy urbanization and distance from any local industrial activity.

The primary aim of this study was to ascertain and compare the oxidative bioreactivities of PM collections from an urban and an industrial site using an *in vitro* assay. A secondary aim was to then compare the protective effects of different pulmonary antioxidants from natural and artificial sources on each of the different collections.

2. Experimental methods

2.1 *Collections*

PM samples were collected with a High Volume Cascade Impact Elutriator (HVE), run continually at an operational rate of 1100 l/min for periods of 4 to 11 days (Kavouras *et al* 2000), after which the polyurethane foam (PUF) substrates were changed (Kavouras and Koutrakis 2001). PUF substrates (n = 5 per study), were subjected to gravimetric analysis to determine the mass of $PM_{10-2\cdot5}$ collected. Substrates were weighed pre- and post-collection on an analytical balance. A set of control substrates (1 control for every 5 sample substrates) was taken to the field, and used to monitor the reproducibility of the analytical balance and storage conditions; they were stored in sterile petri dishes. Control substrate weights did not increase in mass and were consistently reproducible with a variance in mass of ± 0·00001 g, indicating that there was no need for substrate pre- and post-conditioning.

2.2 *Gravimetric analysis*

The 24 h total mass collected was measured using an analytical balance (Fisher, Southampton, UK, 5 decimal places). The gravimetric measurements in conjunction with the flow rates of each respective collector were used to determine the concentration ($\mu g\ m^{-3}$ air), for each collection, based on the mass collected on the substrate and the device flow-rate.

2.3 *Sample preparation*

Substrates were vortexed in equal volumes of sterile HPLC-grade water for 1 h. The substrates were then sonicated for 2 min and the resulting solutions were freeze-dried and re-weighed to determine yield (mean recovery 86%), and then resuspended in sterile water to a stock concentration. Whole $PM_{10-2\cdot5}$ was reconstituted by recombining relative mass proportions of each of the $PM_{10-2\cdot5}$ and $PM_{2\cdot5-0\cdot1}$ collections noted at the time of collection.

In order to separate the water soluble and biopersistant fractions of each collection, PM samples were suspended in sterile, HPLC-grade water and agitated for 4 h. After this time, the particles were centrifuged at 10,000 g for

1 h and the supernatant (soluble fraction) carefully removed. The remaining particle pellet (washed sample) was resuspended in the same volume of fresh water and mixed thoroughly. All samples were stored at – 80°C until required.

2.4 *The* in vitro *oxidation assay*

Adapted from the protocol of Donaldson *et al* (1995), icosahedral bacteriophage φX174-RF DNA (Promega, Southampton, UK) was selected as the optimum plasmid due to its size and sensitivity to oxidative damage. All control and test incubations were carried out according to the protocol of Greenwell *et al* (2002). Gels were imaged using the Syngene Genesnap program (Synoptics Ltd., Cambridge, UK) and densitometric quantification was performed using the Syngene Genetools program. Bioreactivity was calculated as the amount of damaged DNA as a proportion of the total in each lane. Four replicates of each sample concentration were quantified in this way and the means were plotted against particle mass concentration. Densitometry was performed on the relevant areas of each lane, and any instances in which the densitometer did not register any variance from the background throughout the entire lane (cases of complete DNA fractionation) were assumed to be 100% degradation. The greater sensitivity of the densitometer allows it to measure very subtle bands, which may not be readily detectable to the human eye. A regression equation of the dose-dependence data for each sample facilitated the calculation of the toxic mass of PM necessary to cause 50% of the DNA to become damaged (TM_{50}).

2.5 *Preparation of epithelial lining fluid*

Fresh concentrated epithelial lining fluid was obtained by pulmonary lavage from specific pathogen-free male Sprague-Dawley rats (Charles River, Kent, UK), using approximately 8 ml 0·15 M NaCl. The resulting epithelial lining fluid (ELF) was separated using the Microcon filter unit system (Millipore, Watford, UK) (Greenwell *et al* 2002).

Surrogate ELF (sELF) comprising of 200 μM ascorbate (Sigma, Gillingham, UK), 200 μM urate (Sigma, Gillingham, UK) and 200 μM reduced glutathione (Sigma, Gillingham, UK) was made in sterile, chelex-treated, HPLC-grade water (pH 7·4) and stored at – 80°C until required (Greenwell *et al* 2002).

2.6 *Statistical analysis*

Data was tested for normality using an Anderson-Darling test, then statistical significance was tested using the 2-paired *t*-test or the non-parametric Mann-Whitney test from Minitab 13 (CI = 95%) (Microsoft, USA).

3. Results

3.1 *Gravimetric analysis*

PM samples were collected from both Cardiff and Port Talbot in South Wales. Both lie close to the coast and are bisected by a busy motorway, but they differ in character, with Cardiff being an urban site and Port Talbot being predominantly industrial. In Cardiff the HVE was located in a parking area in Cardiff University. The location is less than 500 m north of the city centre and 300 m east of the A470, but its immediate surroundings have relatively low volume of traffic. The collection period lasted for 43 days, starting in January and finishing in March (table 1). The amount of sample collected varied according to the weather conditions, with the highest amounts obtained on cold and dry days and the longest collection period corresponding to the wettest weather (76 mm of rain, Meteorological Office Data). Up to 697·05 mg of total $PM_{10–2·5}$ and $PM_{2·5–0·1}$ were collected over this 43 days period, always with higher amounts of $PM_{2·5–0·1}$ than $PM_{10–2·5}$ ($PM_{2·5–0·1}/PM_{10–2·5}$ ratio varying from 2·8 to 4·3).

In Port Talbot, the selected collection site lay between the busy M4 motorway (75 m NE), and a large steelworks factory (600 m SW). The traffic flow is estimated to be 50,000 to 55,000 vehicles per day (DETR 2002). Airborne particles were collected over 25 days from April to May, for periods of 5 to 7 days. The total amount of sample was 553·38 mg with a minimum average amount of total PM per day of 19·72 mg. Although these collections were greater in mass than the Cardiff collections, the difference was not statistically significant ($P > 0·05$). In comparison with Cardiff, the Port Talbot samples show a smaller $PM_{2·5–0·1}/PM_{10–2·5}$ ratio (1·1 to 1·9), due to a much higher number of particles in the $PM_{10–2·5}$ substrate. This ratio is significantly lower than the comparative ratio pertaining to the Cardiff collections ($P = 0·002$).

3.2 In vitro *toxicology*

The comparative bioreactivity of the 4 urban and 5 industrial collections was examined in the plasmid assay first as whole $PM_{10–2·5}$. The complete industrial (Port Talbot) collections ($TM_{50} = 2·7 \pm 0·4$) were found to be significantly ($P = 0·018$) more bioreactive than the complete urban (Cardiff) collections ($TM_{50} = 10·6 \pm 1·5$ μg/ml) (data not shown).

Subsequently, the $PM_{10–2·5}$ (coarse) and $PM_{2·5–0·1}$ (fine) were tested individually. Both the coarse and the fine

fractions emulated the results previously found with the complete PM_{10} samples, with the industrial PM proving more bioreactive than the urban PM.

Size-dependant comparison of the PM collections produced controversial findings. The coarse fraction demonstrated a significantly greater oxidative potential than the fine fraction in both the industrial ($P = 0{\cdot}012$) and urban ($P = 0{\cdot}048$) PM collections (see table 2), thus not supporting common hypotheses which suggest that the finer, more respirable fraction is the most bioreactive. However, further fractionation of the PM samples into water-soluble fraction and water-insoluble fraction, indicated that the coarse $PM_{10–2{\cdot}5}$ fractions had significantly greater bioreactivity than the fine $PM_{2{\cdot}5–0{\cdot}1}$ fractions (see table 2). In addition, the industrial samples were more bioreactive than the urban samples in all fractions. Finally, in both the urban and industrial PM samples, the whole fractions demonstrated greater oxidative capacity, but they were not significantly different from the water-soluble material ($P > 0{\cdot}05$) from which the durable particles had been removed. The water-insoluble fractions (durable particles) in both cases were significantly less bioreactive than either the whole fraction or the water-soluble fraction. All results are summarised in table 2.

Inclusion of the low molecular weight fraction of fresh pulmonary lavage in the incubations significantly reduced the effect of the PM samples. A typical separation of DNA conformations on a gel is shown in figure 1. Overall results indicated that in both industrial and urban samples, there was a significantly greater reduction in the effect of the $PM_{10–2{\cdot}5}$ compared to the $PM_{2{\cdot}5–0{\cdot}1}$ ($P = 0{\cdot}019$ and $P = 0{\cdot}019$ respectively) (figure 2). There was also a significantly larger amelioration of the effects of the industrial $PM_{10–2{\cdot}5}$ compared to the urban $PM_{10–2{\cdot}5}$ ($P = 0{\cdot}050$), but this was not echoed in the comparison of the $PM_{2{\cdot}5–0{\cdot}1}$ fractions (figure 2). Incubation of the samples

Table 1. Collection details and gravimetric data for PM samples collected in Cardiff and Port Talbot.

Period	PM weights (mg)		Mean mass (mg/day)	$PM_{2{\cdot}5–0{\cdot}1}$: $PM_{10–2{\cdot}5}$
	$PM_{10–2{\cdot}5}$	$PM_{2{\cdot}5–0{\cdot}1}$		
Urban				
22/01/01–01/02/01	26·63	75·73	10·23	2·8
01/02/01–12/02/01	26·00	75·74	9·25	2·9
12/02/01–19/02/01	41·34	134·53	25·12	3·2
19/02/01–26/02/01	19·80	78·11	13·99	3·9
26/02/01–02/03/01	14·49	48·85	15·83	3·4
02/03/01–06/03/01	29·58	126·25	38·96	4·3
Mean ± Std. Error			18·7 ± 4·7	3·4 ± 0·2
Industrial				
26/04/01–02/05/01	57·35	61·84	19·86	1·1
02/05/01–09/05/01	58·42	79·64	19·72	1·4
09/05/01–14/05/01	51·12	99·85	30·19	1·9
18/05/01–25/05/01	52·04	93·12	20·73	1·8
Mean ± Std. Error			22·6 ± 2·5	1·5 ± 0·2
P value			0·334	0·002

The *P* values relate to the comparison between the urban and industrial collections (CI = 95%).

Table 2. Toxicological TM_{50} data calculated for each urban and industrial PM fractions.

	Urban (Cardiff) [TM_{50} (μg/ml) ± Std. Error]			Industrial (Port Talbot) [TM_{50} (μg/ml) ± Std. Error]		
	$PM_{10–2{\cdot}5}$	$PM_{2{\cdot}5–0{\cdot}1}$	*P* value	$PM_{10–2{\cdot}5}$	$PM_{2{\cdot}5–0{\cdot}1}$	*P* value
Whole	12·9 ± 2·1	19·7 ± 3·7	0·048	4·9 ± 0·9	9·4 ± 2·9	0·012
Water soluble	17·1 ± 3·0	32·8 ± 4·9	0·019	6·6 ± 1·2	14·1 ± 3·9	0·050
Water insoluble	41·4 ± 5·9	55·9 ± 14·3	0·028	38·2 ± 9·8	47·1 ± 9·6	0·054

The *P* values relate to the comparison between the $PM_{10–2{\cdot}5}$ and $PM_{2{\cdot}5–0{\cdot}1}$ data in each instance (CI = 95%).

with sELF solution showed an increased protective effect compared to the ELF (figure 3). It also demonstrated that the oxidative capacity of the soluble fraction of both PM_{10} and $PM_{10–2·5}$ was the most significantly diminished compared with whole samples. Investigation with the sELF solution also reaffirmed that the artificial antioxidant mixtures have the least protective effect against the bioreactivity of the $PM_{2·5–0·1}$ fractions.

4. Discussion

This study has shown that PM collected from urban and industrial sites produce differential oxidative damage in a simple *in vitro* assay. Furthermore, such damage may be ameliorated by antioxidants known to be present in pulmonary lining fluid.

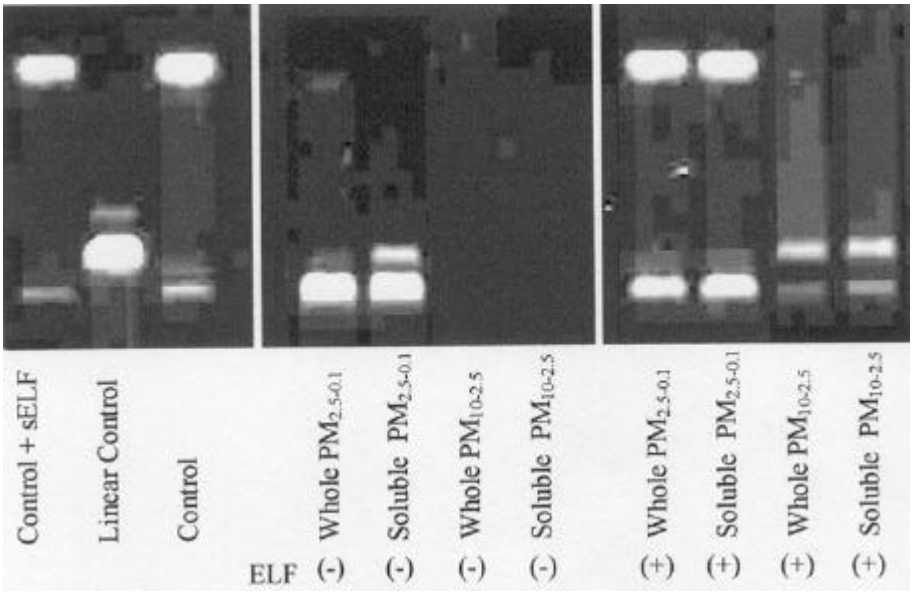

Figure 1. Gel showing < 3 kDa fraction of ELF protection against PM-induced damage. PM concentration was 100 μg ml^{-1}.

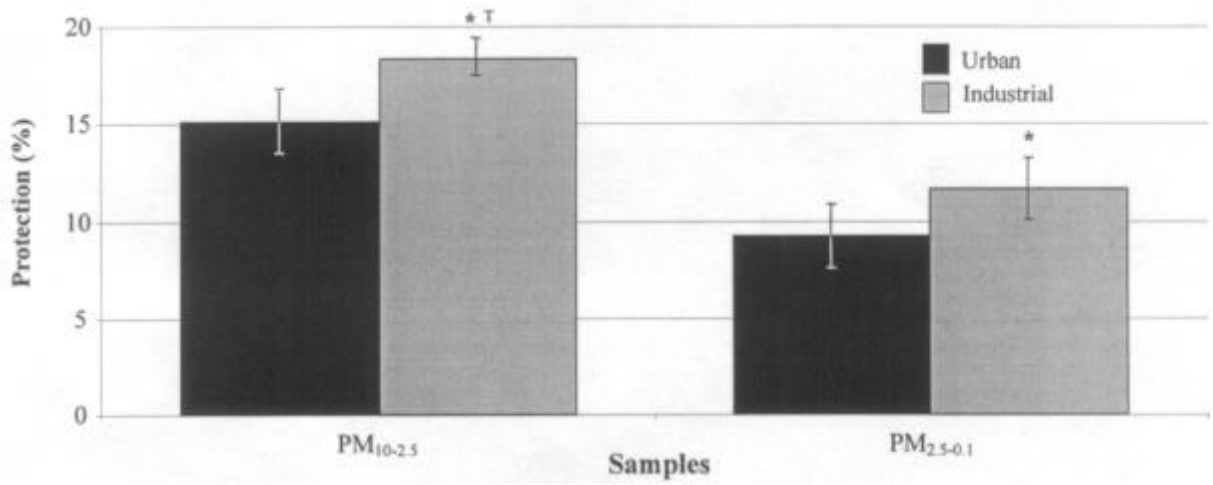

Figure 2. The comparative effect of ELF on urban and industrial PM collections. PM concentration was 100 μg ml^{-1}. Statistical significance between $PM_{10–2·5}$ and $PM_{2·5–0·1}$ is represented by (*), statistical significance between urban and industrial is represented by (^{T}) (CI = 95%).

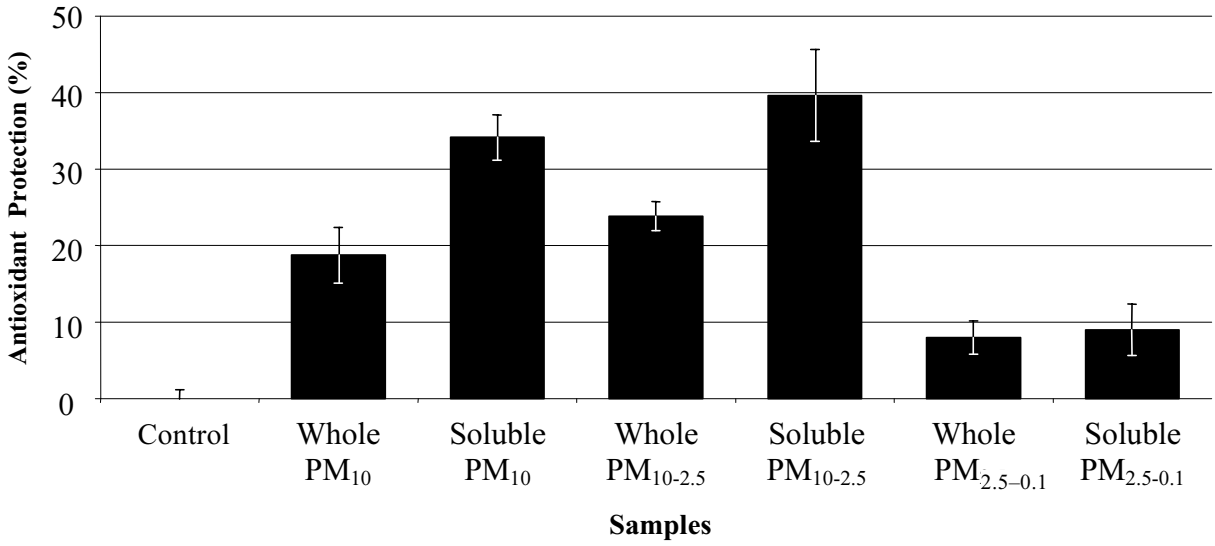

Figure 3. The protective effect of sELF on PM suspensions. PM concentration was 100 μg ml^{-1}.

Gravimetric analysis of PM samples demonstrated that although there was no significant difference between the mean daily pollution masses calculated from the two locations, the samples from the industrial collection site had a significantly lower $PM_{10–2·5}$: $PM_{2·5–0·1}$ ratio. This is postulated to be a result of the industrial collections containing a high proportion of heavy, metal-based industrial particles. This gravimetric difference could also be the cause of the observed greater total collection mass of the industrial samples compared to the urban collections. Further, morphological analysis supported this hypothesis by demonstrating that the urban, Cardiff collections had a greater proportion of soot particles than the industrial collections, and fewer other industrial particles such as iron spherules (which comprise up to one-third of the industrial samples by number) (data not shown), coal fly ash, and glassy spheres. These data also showed that, due to inaccuracies in the size fractionation by the collector, a large number of iron spherules smaller than 2·5 μm in diameter are commonly found in the $PM_{10–2·5}$ substrate. This could explain not only the observed differences in $PM_{10–2·5}$: $PM_{2·5–0·1}$ ratio, but also the differences in oxidative bioreactivity between the coarse and fine fractions if iron is considered a putative prooxidant. The difference in season, and, therefore, prevailing weather conditions could also effect the comparative composition of the PM collections.

The results of the toxicological analysis of these collections therefore supported the popular hypotheses that metal particles could be responsible for the generation of radicals in solution, as there was no significant difference between the mass of PM_{10} collections, but there was a three-fold difference in oxidative capacity. Finding that the coarse fraction of the PM samples from both locations was more bioreactive than the finer fractions was somewhat controversial, disputing common epidemiological hypotheses that suggest $PM_{2·5–0·1}$ has a greater effect in exposed populations than $PM_{10–2·5}$. The observation that the majority of the oxidative capacity of the samples from both locations was retained in the soluble fraction leads to the conclusion that in the pulmonary milieu, the primary toxicological mechanism could be through soluble radicals in the ELF, rather than via direct particulate contact. This would support earlier studies on ROFA (Dreher *et al* 1995, 1996) and EHC93 (Adamson *et al* 1999) whereby the soluble fraction of PM samples has proved bioreactive. Indeed the conclusions from such studies, that toxicological effects may be linked to bioavailable metals, would seem to apply to the urban and industrial PM samples used in this study.

The primary conclusion from the investigations involving both the ELF and sELF solutions is that pulmonary antioxidants are significantly less effective in protecting DNA from oxidative attack by $PM_{2·5–0·1}$ than $PM_{10–2·5}$. Quite why this should be or the significance of this observation is unknown. It was also noted that low molecular weight ELF was less effective at ameliorating the oxidative effects of PM in comparison with the sELF. This may be explained simply by the fact that sELF contains an approximate physiological concentration (therefore approximately 5 μM). It is also highly likely that the rat lavage contains a myriad of other low molecular weight antioxidant and pro-oxidant molecules e.g. superoxide dismutase, cellular debris and neutrophil respiratory burst end products. Equally possible is that rat ELF contains different quantities of the three major low molecular

weight antioxidants compared with the sELF chosen to mimic human epithelial lining material.

In summary, this study has provided further support that components of PM cause oxidative damage. This effect lies primarily with a coarse particle fraction and with the water-soluble component of that fraction. The PM sample from the industrial site, which proved approximately three times as active (on an equivalent mass basis) as PM from the urban collection, is enriched with iron components with potential prooxidant activity (Donaldson *et al* 1997). Further studies are now underway to determine the chemical components, particularly in the soluble fraction, producing oxidative damage alongside more detailed studies on the interaction between particles and low molecular weight pulmonary antioxidants (Zielinski *et al* 1999).

Acknowledgements

We gratefully acknowledge the National Assembly for Wales (ref. 035/99) and the MRC (ref. G9901020) for financial support.

References

Adamson I Y R, Prieditis H and Vincent R 1999 Pulmonary toxicity of an atmospheric particulate sample is due to the soluble fraction; *Toxicol. Appl. Pharmacol.* **157** 43–50

Adamson I Y R, Prieditis H, Hedgecock C and Vincent R 2000 Zinc is the toxic factor in the lung response to an atmospheric particulate sample; *Toxicol. Appl. Pharmacol.* **166** 111–119

Brunekreef B, Hoek G, Fischer P and Spieksma F T M 2000 Relation between airborne pollen concentrations and daily cardiovascular and respiratory-disease mortality; *Lancet* **355** 1517–1518

Costa D L and Dreher K L 1997 Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models; *Environ. Health Perspect.* **1055** 1053–1061

Department of Health 1998 *Quantification of the Effects of Air Pollution on Health in Great Britain* (The Stationery Office UK) pp 18–36

Donaldson K, Gilmour P S and Beswick P H 1995 Supercoiled Plasmid DNA as a Model Target for Assessing the Generation of Free Radicals at the Surface of Fibres; *Exp. Toxic. Pathol.* **47** 235–237

Donaldson K, Beswick P H and Gilmour P S 1996 Free Radical Activity Associated with the Surface of Particles: A Unifying Factor in Determining Biological Activity?; *Toxicol. Lett.* **88** 293–298

Donaldson K, Brown D M, Mitchell C, Dineva M, Beswick P H, Gilmour P and MacNee W 1997 Free Radical Activity of $PM_{10-2.5}$: Iron-Mediated Generation of Hydroxyl Radicals; *Environ. Health Perspect.* **1055** 1285–1289

Dreher K, Jaskot R H, Lehmann J R, Richards J H, McGee J K, Ghio A J and Costa D L 1995 Soluble transition metals mediate residual oil fly ash induced acute lung injury; *J. Toxicol. Environ. Health* **50** 285–305

Dreher K, Jaskot R, Kodavanti U, Lehmann J, Winsett D and Costa D 1996 Soluble transition metals mediate the acute pulmonary injury and airway hyperreactivity induced by residual oil fly ash particles; *Chest* **1093** 541–554

Dye J, Adler K B, Richards J H and Dreher K L 1997 Epithelial injury induced by exposure to residual oil fly-ash particles: role of reactive oxygen species?; *Am J. Resp. Cell Mol. Biol.* **175** 625–633

Gavett S H, Madison S L, Stevens M A and Costa D L 1999 Residual oil fly-ash amplifies allergic cytokines, airway responsiveness and inflammation in mice; *Am. J. Resp. Crit. Care Med.* **1606** 1897–1904

Greenwell L L, Moreno T, Jones T P and Richards R J 2002 Particle-induced oxidative damage is ameliorated by pulmonary antioxidants; *Free Rad. Biol. Med.* **329** 898–905

Gutteridge J M, Richmond R and Halliwell B 1979 Inhibition of the iron-catalysed formation of hydroxyl radicals from superoxide and of lipid peroxidation by desferrioxamine; *Biochem. J.* **184** 469–472

Kavouras I, Ferguson S T, Wolfson J M and Koutrakis P 2000 Development and validation of a high volume low cut-off inertial impactor; *Inhalation Toxicol.* **12** 35–50

Kavouras I G and Koutrakis P 2001 Use of polyurethane foam as the impaction substrate/collection medium in conventional inertial impactors; *Aerosol Sci. Tech.* **341** 46–56

Marx J J and von Asbeck B S 1996 Use of iron chelators in preventing hydroxyl radical damage: Adult respiratory distress syndrome as an experimental model for the oxygen-radical-mediated tissue damage; *Acta Haematol.* **95** 49–62

Pope C A 3rd, Thun M J, Namboodiri M M, Dockery D W, Evans J S, Speizer F E and Heath C W Jr 1995 Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults; *Am. J. Respir. Crit. Care Med.* **151** 669–674

Richards R J, Atkins J, Marrs T C, Brown R F R and Masek L C 1989 The biochemical and pathological changes produced by the intratracheal instillation of certain components of zinc hexachloroethane smoke; *Toxicol. Lett.* **54** 79–88

Richards R J 1997 Small particles, big problems; *Biologist* **44** 249–251

Sagai M, Saito H, Ichinose T, Kodama M and Mori Y 1993 Biological effects of diesel exhaust particles. 1. *in vitro* production of superoxide and *in vivo* toxicity in mouse; *Free Rad. Biol. Med.* **14** 37–47

Schwartz J and Meas L M 2000 Fine particles are more strongly associated than coarse particles with acute respiratory health effects in schoolchildren; *Epidemiology* **11** 6–10

Vogl G and Elstner E F 1989 Diesel soot particles catalyse the production of oxy-radicals; *Toxicol. Lett.* **47** 17–23

Zielinski H, Mudway I S, BeruBe K A, Murphy S, Richards R J and Kelly F J 1999 Modeling the interactions of particulates with epithelial lining fluid antioxidants; *Am. J. Physiol.* **2774 Pt 1** L719–726