Temporal variation of hydroxyl radical generation and 
8-hydroxy-2′-deoxyguanosine formation by coarse and 
fine particulate matter

T Shi, A M Knaapen, J Begerow, W Birmili, P J A Borm, R P F Schins

Aims: To determine the induction of 8-hydroxy-2′-deoxyguanosine (8-OHdG) by fine (<2.5 µm) and coarse (10–2.5 µm) particulate matter (PM) sampled over time at one sampling location, and to relate the observed effects to the hydroxyl radical (⋅OH) generating activities and transition metal content of these samples, and to meteorological parameters.

Methods: Weekly samples of coarse and fine PM were analysed for H₂O₂ dependent ⋅OH formation using electron spin resonance (ESR) and formation of 8-OHdG in calf thymus DNA using an immunodotblot assay. Immunochemistry was used to determine 8-OHdG formation in A549 human epithelial lung cells. To determine temporal effects, samples from six weeks in summer and six weeks in autumn/winter were compared using ESR and the dotblot assay. Concentrations of leachable V, Cr, Fe, Ni, and Cu were determined by inductively coupled plasma mass spectrometry.

Results: Both PM fractions elicited ⋅OH generation as well as 8-OHdG formation in calf thymus DNA and in A549 cells. 8-OHdG formation in the naked DNA was significantly related to ⋅OH generation, but not to metal concentrations except for copper. A significantly higher ⋅OH generation was observed for coarse PM, but not fine PM collected during the autumn/winter season; this was not due to differences in sampled mass or metal content. Specific weather conditions under which increased ⋅OH formation in the coarse mode was observed suggest that other, as yet unknown, anthropogenic components might affect the radical generating capacity of PM.

Conclusions: Both coarse and fine PM are able to generate ⋅OH, and induce formation of 8-OHdG. When considered at equal mass, ⋅OH formation shows considerable variability with regard to the fraction of PM, as well as the sampling season. The toxicological implications of this heterogeneity in ⋅OH formation by PM, as can be easily determined by ESR, need further investigation.

Increased exposure to ambient particulate matter (PM) has been associated with respiratory, cardiovascular, and malignant lung disease. In vitro studies indicate that the effects of PM may be due to its chemical composition or the size fraction of the particulates. In lung epithelial cells, the cytotoxicity of residual oil fly ashes and Utah-Valley dust has been linked to their transition metal content. In macrophages, the toxicity of PM10 was also shown to involve transition metals, but interestingly this effect was merely observed in its coarse (2.5–10 µm) fraction, and not in its fine (<2.5 µm) fraction. Despite this observation, higher metal concentrations are usually found in the fine mode, which is largely composed of particles of anthropogenic origin. Metals have also been implicated in the inflammatory effects of PM, as well as in the ability of PM to induce oxidative DNA damage. Transition metals that are present in PM are considered to exert their effects predominantly via formation of hydroxyl radicals (⋅OH), generated by available iron via the Fenton reaction. In addition to iron, several other “Fenton active” transition metals that usually occur in PM, such as chromium, vanadium, and copper, are also known to induce the ⋅OH specific DNA adduct 8-hydroxy-2′-deoxyguanosine (8-OHdG), albeit with considerably varying efficiency. As such, these metal specific findings are difficult to extrapolate to the ⋅OH generating properties of PM as an entirety, since this will depend on the concentration, bioavailability, and chemical speciation and oxidation state of each individual metal. Furthermore, ⋅OH generation may also be modified by other agents of this complex mixture. Therefore, we have recently developed a method using electron spin resonance (ESR) to measure the generation of ⋅OH by PM in the presence of H₂O₂ and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a specific spintrap, as an integrate of the Fenton reactivity of a given PM sample.

Since the chemical composition of PM (for example, metal concentrations) is well known to vary with time, sampling location, and size fraction, we have anticipated that this would also be reflected in variability of ⋅OH generating capacities, as well as ⋅OH associated effects. Therefore, the aim of our present study was to determine the variation in ⋅OH generation and formation of 8-OHdG by coarse and fine PM, sampled over time at one sampling location, in relation to sampled mass and transition metal content, as well as to meteorological data that could affect its chemical composition.

METHODS
Collection and sample processing of particulate matter
Coarse (PM 10–2.5 µm) and fine (PM <2.5 µm) fractions of PM10 were sampled weekly in Düsseldorf, Germany in the period July to December 1999. Coarse and fine PM were collected on Teflon filters using Graseby-Anderson dichotomous low volume samplers at a flow of 16.7 l/min. Filters were stored in the dark in a dry atmosphere until further analysis. The PM was removed from the filters by agitation (five minutes) in 1 ml of ultrapure water, and the suspensions were sonicated for five minutes. Resulting PM concentrations were estimated using comparative turbidometry against a standard dilution curve using a carbon black suspension. Comparative analysis of gravimetric and turbidometric analysis of a total of 86 samples from another study (unpublished) revealed the...
Table 1  Sample characteristics of ambient PM sampled in Düsseldorf

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Fine mode</th>
<th>Coarse mode</th>
<th>Meteorological parameters</th>
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<tbody>
<tr>
<td>Period</td>
<td>Conc. ‡ (mg/ml)</td>
<td>ESR ‡ (AU)</td>
<td>Conc. ‡ (mg/ml)</td>
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<td>I</td>
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<td>23.1</td>
<td>0.71</td>
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</table>

*Experiment (see method section for details); †Mass of PM extracted from filters; ‡OH generation determined by ESR; §Weekly sum at station Brüggen; ¶Mixing layer height; **No. of days when the local winds pointed predominantly from the core of the Ruhr area.

outcomes are expressed as the total amplitude in arbitrary units (AU).

8-Hydroxydeoxyguanosine induction by PM in calf thymus DNA

Induction of the OH specific DNA lesion 8-hydroxydeoxyguanosine (8-OHdG) by PM in isolated calf thymus DNA was estimated via a dotblot assay that we developed, based on a method described by Musarrat and Wani. Freshly prepared suspensions of PM were incubated with 50 µg of calf thymus DNA dissolved in Tris-HCl (10 mM, pH 8.0) and H2O2 (1 mM). In each experiment, DNA incubated without PM and H2O2, as well as DNA incubated with 0.1 mM FeSO4 and 1 mM H2O2, were included respectively as negative and positive controls. Samples were incubated in the dark for 90 minutes at 37°C in a shaking water bath, and then immediately centrifuged (6000 rpm, 5 minutes). A 400 µl aliquot of the supernatant was transferred to a fresh tube, and DNA was precipitated by the addition of 1/10 vol NaAc (1.5 M, pH 6.0) and 20% vol. 100% ice cold ethanol. The DNA was then washed twice using 70% ethanol (13000 rpm, 5 minutes), dried in the vacuum and dissolved in 50 µl of Tris-HCl buffer, and stored overnight at 4°C. DNA concentrations were determined spectrophotometrically and the samples were diluted to a final concentration of 2.56 µg/ml in 20x SSC. Of each sample, replicate two-fold dilutions were blotted on a nitrocellulose membrane using a dotblot apparatus. To each blot, both negative and positive controls were added. The DNA was cross linked by baking of the membrane for 90 minutes in a prewarmed oven at 80°C. Blocking of the membrane was performed overnight using casein. Immunolocalisation of 8-OHdG was performed using the N45.1 monoclonal antibody,17 and using the Vectorstain-ABC kit with dianaminobenzidine staining according to the recommended protocol (Vector Laboratories). The blots were analysed by computer assisted densitometry scanning (BioRad), and expressed relative to the density of the negative controls.

Measurement of 8-hydroxydeoxyguanosine in A549 epithelial cells

A549 cells (American Type Culture Collection), were grown in Dulbecco’s Modified Eagle’s Medium (DMEM; Life Sciences), supplemented with 10% heat inactivated fetal calf serum (FCS; Life Sciences), 1-glutamine (Life Sciences), and 30 IU/ml penicillin-streptomycin (Life Sciences) at 37°C and 5% CO2. Induction of 8-OHdG in the epithelial cells was measured by immunocytochemistry as follows. A549 cells were seeded in four-chamber Slides (Falcon) at a concentration of 120 000 cells/chamber. After two days, cells were exposed to PM...
suspended in Hanks balanced salt solution (HBSS) for two hours. Immunocytochemistry was performed using the Vectorstain-ABC kit (Vector Laboratories), and the same antibody as used for the dot blot assay. 8-OHdG was quantified using a digital imaging software analysis system (SIS, Münster, Germany). Therefore, at least three chambers were analysed per treatment, counting at least 100 cells per chamber.

Analysis of metals by ICP-MS
ICP-MS was used to determine the concentrations of V, Cr, Fe, Ni, and Cu in the aqueous suspensions of PM. Therefore, the freshly prepared suspensions of PM were filtered through a 0.2 µm Millipore filter (Minisart RC15 syringe filter, Sartorius AG, Göttingen, Germany). The filtrate was diluted with deionised water (1/5), and filtered again. The transition metals were analysed by sector field ICP-MS (ELEMENT by Finnigan MAT, Bremen, Germany) in the medium resolution mode (m/Δm ≅ 4000) using the standard addition procedure for calibration. A 50 µl aliquot of the filtrate was diluted with 500 µl 0.08 N HNO₃ and 2000 µl ultrapure water, and spiked with 50 µl of standard solutions containing 5–20 µg/l Ni, Cu, V, and Cr and 50–200 µg/l Fe, respectively.

Meteorological analysis
To identify eventual influences of meteorological parameters on properties of the sampled PM, the synoptic scale weather situation, atmospheric stability, local winds, and precipitation in the Düsseldorf area were assessed using three types of data: (a) wind direction, wind speed, temperature, and rainfall measured at the meteorological stations Brüggen (51.20 N; 6.13 E), and Gütersloh (51.93 N; 8.32 E); (b) daily radiosonde ascents at Essen (51.40 N; 6.97 E) at 1200 UTC; and (c) four day backtrajectories (850 hPa level) based on model data from the European Centre for Medium-Range Weather Forecasts. The radiosoundings were used to estimate the mixed layer height of the planetary boundary layer.

Statistical analysis
Comparison between fine and coarse PM or for the PM samples of different sampling periods were made by t test, or the non-parametric Mann-Whitney test (for 8-OHdG only). Spearman rank correlation was used to determine the relations between ESR activity, transition metals, and the formation of 8-OHdG. Therefore, the relative staining intensities as determined for 8-OHdG were ranked for each separate experiment (that is, A or B, see table 1). Such rankings were made per experiment, respectively for coarse and fine PM together (that is, ranking from 1 to 12) or for coarse and fine separately (that is, ranking from 1 to 6).

RESULTS
ESR measurements showed that suspensions of both fine and coarse PM caused formation of -OH in the presence of H₂O₂ (see fig 1). Dose-response curves were generated with both coarse and fine PM sampled in three randomly chosen weeks, to determine -OH generating capacities of the PM suspensions at different concentrations (fig 2). For all curves a highly significant fit (r² > 0.97, p < 0.005) was observed when the concentration was expressed on a logarithmic scale. Interestingly, a large variation was observed in the -OH generating capacities of fine as well as of coarse PM, sampled in different weeks. However, coarse PM had greater ability to generate -OH than fine PM when compared at equal mass.

![Figure 1](http://oem.bmj.com/)

Figure 1 DMPO-OH signal as measured by ESR of coarse and fine PM. Following incubation with H₂O₂ and DMPO, ESR analysis showed the -OH specific 1:2:2:1 quartet pattern for fine PM as well as coarse PM. (A) blank Teflon filter; (B) fine PM (2.2 mg/ml); (C), coarse PM (2.2 mg/ml).

![Figure 2](http://oem.bmj.com/)

Figure 2 Hydroxyl radical generation by serial dilutions of suspensions of coarse and fine PM, sampled in three different weeks. The three different graphs represent -OH generation of PM sampled in three different weeks as indicated. Each graph shows -OH generation for serial dilutions of a single weekly sample of respectively coarse or fine PM. Data are expressed as the intensity of the resulting DMPO-OH signal (see also fig 1) in arbitrary units.
To see whether the observed •OH generation relates to the induction of 8-OHdG, coarse and fine PM were incubated with calf thymus DNA and analysed using an immunodotblot assay (see fig 3). Both fine and coarse PM, as well as FeSO₄, used as positive control, caused formation of 8-OHdG in the presence of H₂O₂. In agreement with the observations using ESR, per unit mass the effects of coarse PM were stronger than the effects of fine PM. In order to determine the relevance of these acellular assays in a biological system, A549 human alveolar epithelial cells were treated with coarse and fine PM. Figure 4 shows representative pictures. Both fractions of PM were able to induce 8-OHdG in the A549 cells on two hours exposure. However, unlike the dotblot assay, no clear differences in the induction of 8-OHdG could be observed between coarse and fine PM.

To determine the possible influence of temporal variation, samples from two different periods (2 × 6 weeks) were analysed for •OH generation using ESR as well as formation of 8-OHdG in calf thymus DNA (see table 2). As can be seen in the table, the •OH generating capacities of the coarse particles sampled during the second period were significantly higher than those sampled in the first period. However, no differences in •OH generation were found between both periods for the fine PM. A similar trend was observed for the formation of 8-OHdG although the differences did not reach significance. The observed temporal effects were not due to differences in sample storage time, since repeated measurements of samples collected with three parallel PM samplers during the same week, did not show changes in •OH generation due to sampling storage.

When the samples of both periods were considered together, coarse PM was found to have significantly higher •OH formation than fine PM (n = 12, p < 0.01, t-test), and also caused a significantly higher 8-OHdG formation (n = 12, p < 0.001, Mann-Whitney). Figure 5 shows the correlation between •OH generation and 8-OHdG formation for all samples. A significant correlation between •OH formation and 8-OHdG was observed (n = 24, Spearman’s r = 0.743, p < 0.001), indicating that the •OH generating properties of PM determine its ability to elicit oxidative DNA damage. Interestingly this association was found both with the coarse mode and the fine mode, although the latter did not reach significance (coarse PM: n = 12, r = 0.580, p < 0.05; fine PM: n = 12, r = 0.537, p = 0.060). Neither •OH generation nor 8-OHdG formation were correlated with the extracted mass.

To determine the role of transition metals in the oxidative properties of the PM in relation to the observed differences for both sampling periods, the suspensions of the PM were analysed for leachable Ni, Fe, Cu, V, and Cr by ICP-MS. As shown in table 2, no differences in metal concentrations were observed for the PM sampled for both periods, with the exception of Cu which was significantly lower in the fine PM of the second period. Furthermore, whereas both •OH generation and formation of 8-OHdG were higher for the coarse PM, the concentrations of V, Cr, Fe (all p < 0.01) and Ni (p < 0.05) were found to be significantly lower for the coarse PM in comparison to the fine PM. Thus, the observed differences in hydroxyl radical generating capacities for both sampling periods as observed for the coarse PM were unlikely to be explained by different metal contents. Interestingly however, Cu was significantly correlated with •OH generation (n = 24, r = 0.644, p < 0.001), and to a lesser extent with the formation of 8-OHdG (n = 24, r = 0.510, p < 0.05). This association was also observed for the coarse PM (hydroxyl radicals: n = 12, r = 0.657, p < 0.05; 8-OHdG: n = 12, r = 0.608, p < 0.05), but not for the fine PM. None of the other metals was found to correlate with •OH or 8-OHdG, with the exception of Cr which showed a correlation with •OH formation by the fine PM (n = 12, r = 0.706, p < 0.05). Finally, the particle mass extracted from the filters was not different between both periods (see table 2) and did not correlate with any of the metal concentrations.

Effects of meteorological parameters could be seen in several ways. The highest mass concentrations in the fine particle mode occurred in period I, in the weeks starting on 02.09.99 and 09.09.99 (see table 1). Trajectory analysis showed that the air masses sampled during these weeks originated over South Russia, and South Germany, respectively. Similar continental trajectory influence prevailed during the week starting on 29.07.99 (see table 1), but this time the fine particle mode mass concentration was low. During this week, the mixed layer height of the boundary layer was extremely high on average, eventually allowing for regeneration of the surface based aerosols with clean air from aloft. However, the
inflow of winds with a northern component, often combined with a passage of the air across the Ruhr area, featuring numerous industrial and other anthropogenic sources of PM. While a source contribution of the Ruhr area was evident from a meteorological point of view, there were no signs for increased metal concentrations during the period of concern (data not shown). The influence of recent precipitation was apparent, for example, in the week starting on 09.12.99. The mass concentrations of the fine and the coarse mode were low (0.57 and 0.71 mg/ml, respectively) during this week, which was characterised by heavy rain (51.5 mm). For the entire campaign, the coarse mode mass concentrations seemed to be more susceptible to the recent occurrence of precipitation, whereas the fine mode mass concentrations seemed rather influenced by backtrajectories. However, neither precipitation nor backtrajectories correlated significantly with -OH generation.

DISCUSSION

Although associations between PM exposure and adverse health outcomes have been established in epidemiological studies, there is still a debate on the actual constituents or characteristics of PM that play a role in these effects. Transition metal dependent -OH formation has been considered an important feature of the inflammatory effects of PM. More recently, we and others showed that PM can induce oxidative DNA damage, including formation of the -OH specific lesion 8-OHdG, and that this damage could be prevented with the iron chelator deferoxamine and hydroxyl radical scavenger DMSO. These observations suggest a key role for Fenton reaction driven -OH formation in the induction of DNA damage. This is further supported by our current data, showing a strong association between -OH generation by both coarse and fine PM samples from different sampling periods, and their abilities to induce 8-OHdG in calf thymus DNA. Since 8-OHdG represents a premutagenic DNA adduct which has been implicated in carcinogenesis, the above data should also be viewed in relation to the recently established associations between PM10 and lung cancer.

Obviously, 8-OHdG formation in cell free test systems—that is, using naked DNA, differs considerably from the induction of this DNA lesion in cell culture. However, in the present study we also showed that both coarse PM and fine PM can induce 8-OHdG in A549 human epithelial cells. Notably, unlike the acellular assays (ESR, dotblot), where H2O2 was added to elicit Fenton-like reactions, DNA damage in the A549 cells occurred in the absence of extracellularly added H2O2. This indicates the contribution of endogenous H2O2 to 8-OHdG formation in the epithelial cells. Indeed, it has been shown that DNA damage by PM can be inhibited by catalase and physiological levels of H2O2 have been shown to enhance -OH generation by PM in an H2O2 concentration dependent manner. Organic constituents within the PM have recently been proposed as another source of transition metal derived -OH formation via formation of H2O2 from redox cycling of semiquinone radicals. Our findings are also in agreement with observations by Prahalad and colleagues. Using various (model) PM with highly different metal availability, such as coal and oil fly ashes, they showed a clear association between 8-OHdG induction in calf thymus DNA and in the DNA of BEAS-2B human bronchial epithelial cells. In our current study, the semiquantitative nature of immunohistochemical staining did not allow us to determine clear differences in 8-OHdG formation in the A549 cells for PM samples differing in size fraction or sampling period. Quantitative measurement of 8-OHdG using HPLC/ECD could not be performed, as the isolation of sufficient amounts of cellular DNA would require at least 50 times the amount of PM as used in the present study. Our current method, however, allowed us to test low mass PM samples as typically collected on conventional low

continental nature of the backtrajectories had no visible effect on -OH generation (table 1).

As shown above, the highest rates of -OH generation occurred in the coarse particle mode in period II, especially during the first three weeks of sampling period II. All sampling weeks of period II featured weak vertical exchange and inversions, in contrast to period I, where atmospheric mixing was intense. The particular feature of the three weeks showing the highest -OH generation was the predominant inflow of winds with a northern component, often combined
In the present study we used ICP-MS to determine the role of transition metals in the observed effects. Since previous studies indicate that availability rather than concentrations of the metals are important for the induction of oxidative effects, readily leachable metal contents were determined—that is, on filtration of particle suspensions. Among the metals commonly present in PM, we chose V, Cr, Fe, Ni, and Cu because of their established role in the induction of 8-OHdG. For instance, Lloyd and colleagues showed that Cr(III) and Fe(III), and to some lesser extent V(III), Cu(II), and Cr(IV) caused induction of 8-OHdG in salmon sperm DNA, whereas Ni(II) and some other transition metals had no significant effect. Another study investigating the effectiveness of 8-OHdG formation by metals showed a ranking decreasing from V(IV), Fe(II), V(III), Fe(III), to Ni(II). Recent ESR experiments in our laboratory with soluble metals or with talc samples, this method does not allow determination of the (coarse) PM and also tended to be higher in the coarse PM than in the fine PM, this does not fully explain why coarse PM showed higher 8-OHdG generation for a number of reasons. Firstly, for fine PM no clear association was found between oxidative DNA damage and copper. Secondly, due to the multitude of transition metals usually present in the PM, and due to the intrinsic differences in the Fenton reactivity of each individual metal species, it would be unlikely that a single metal would account for the observed oxidative effects. Most importantly however, although ICP-MS allows detection of low concentrations of metals as typically occurring in low mass environments, this method does not allow determination of the chemical speciation of the metal. For instance, Prahalad and colleagues showed that the residual oil fly ash (ROFA) was 40-fold more potent in causing 8-OHdG than oil fly ash (OFA), despite similar metal content and availability. Finally, the body of meteorological observations in this study also points to specific weather conditions under which increased 8-OHdG generation in the coarse mode was observed, in sampling period II. All sampling weeks of this period featured weak vertical exchange and inversions, in contrast to period I, where atmospheric mixing was intense. It is well established that conditions such as in period II lead to an accumulation of atmospheric pollutants near the surface. The particular features of the three weeks showing the highest 8-OHdG generation were trajectories that are notably influenced by regional

![Figure 5](image-url)  
Figure 5 Correlation between hydroxyl radical generating properties and 8-OHdG formation in calf thymus DNA for fine and coarse PM. Hydroxyl radical generation as determined by ESR is expressed in arbitrary units, and for 8-OHdG data are expressed according to ranking of the samples as determined by densitometry analysis (see methods section for details).

| Table 2 Hydroxyl radical generation, 8-OHdG formation in calf thymus DNA, and transition metal concentrations of fine and coarse PM sampled during six weeks in summer (period I) and six weeks in autumn/winter (period II) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Fine PM Period I | Period II       | Coarse PM Period I | Period II       |
| Mass (µg)       | 1.56 (0.56)     | 1.20 (0.37)     | 1.13 (0.38)     | 1.28 (0.43)     |
| ESR (AU)        | 20.1 (7.3)      | 21.1 (6.3)      | 29.2 (9.2)      | 54.6 (23.3)     |
| 8-OHdG (rank)‡  | 3.3 [2–6.5]     | 3.5 [1.8–5.9]   | 7.5 [5.0–10.3]  | 9.5 [8.8–12.0]  |
| V (µg/g)        | 214 [91]        | 200 [100]       | 81 [60]        | 48 [57]         |
| Cr (µg/g)       | 143 (47)        | 166 (56)        | 70 (71)        | 30 (12)         |
| Fe (µg/g)       | 2724 [1197]     | 1752 [985]      | 1161 [1103]    | 239 [155]       |
| Ni (µg/g)       | 130 (63)        | 192 (63)        | 105 [71]       | 92 [73]         |
| Cu (µg/g)       | 429 (96)        | 287 [112]       | 570 (456)      | 582 [240]       |

*Mass of PM extracted from filters; †OH generation determined by ESR; ‡Relative induction of 8-OHdG.
All data are expressed as mean (SD), with the exception of 8-OHdG which is shown as median [25th–75th centile] rank.

§Significantly different from period I (p<0.05, t test).
Radical generation by particulate matter

anthropogenic sources, such as from the Ruhr area. The observed lack of correlation of this meteorological situation with increased metal concentrations (V, Cr, Fe, Ni, Cu) eventually points to other, yet unknown, anthropogenic components in the particle coarse mode that can affects its overall -OH generating capacity. The high mass accumulations as observed for some weeks for the fine particle mode, which were ascribed to accumulation of primary and secondary aerosols in slow and non-precipitating air masses,20 and the continental nature of the backtrajectories had no visible effect on -OH generation.

Taken together these data support the use of a measurement that integrates the intrinsic redox activity of all different constituents within the PM. The ESR method described here is relatively simple and can be applied to low mass samples of (different size fractions of) PM. The observed high correlation between -OH formation and the induction of 8-OHdG indicates that this overall measurement of -OH generating activity of a PM sample, is a better predictor for the induction of oxidative DNA damage in vitro as shown previously20 18 15 20 21 and in the current study, than determination of the concentrations of individual transition metals. Since reactive oxygen species including -OH are also implicated in transcriptional activation of nuclear factor (NF) κB and associated up-regulation of inflammatory genes,22 the observed variation in terms of sampling season and the size fraction, may also be reflected in the inflammatory effects of PM. For instance, temporal differences have been described for the induction of inflammatory mediators in a murine macrophage cell line,23 and more recently we showed considerable regional variability in inflammatory mediator release from A549 cells.24 Whether these effects are related to the intrinsic -OH generating properties which can be determined using the ESR method as described, is currently under investigation.

ACKNOWLEDGEMENTS

We acknowledge Martina Turfeld for the ICP-MS analysis and Tobias Georgi for his contributions to development of the dot-blot assay. WB wishes to thank the British Atmospheric Data Centre (Didcot, UK) and the Met Office (Bracknell, UK) for providing meteorological data and backtrajectories.

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*Occup Environ Med* 2003 60: 315-321
doi: 10.1136/oem.60.5.315

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