

## SHORT REPORT

## Stimulation of IL-8 release from epithelial cells by gas cooker PM<sub>10</sub>: a pilot study

C A J Dick, M Dennekamp, S Howarth, J W Cherrie, A Seaton, K Donaldson, V Stone

### Abstract

**Objective**—To measure the effect of matter collected by a method that has a 50% efficiency for particles with an aerodynamic diameter of 10 µm (PM<sub>10</sub>), generated by gas and electric cooking, on A549 epithelial cells with and without nitrogen dioxide (NO<sub>2</sub>).

**Method**—Multiple indoor PM<sub>10</sub> samples were collected on Teflon filters during the use of gas or electric cookers. Interleukin-8 (IL-8) concentrations were measured with a sandwich enzyme linked immunosorbent assay (ELISA) system.

**Results**—Treatment of A549 cells with PM<sub>10</sub> generated from gas cooking resulted in increased concentrations of IL-8 compared with untreated cells; particles from the electric cooker had no effect. NO<sub>2</sub> did not alter the concentration of IL-8.

**Conclusion**—PM<sub>10</sub> generated by gas cooking has the potential to cause proinflammatory effects in lung cells. This may have implications for susceptible people.

(Occup Environ Med 2001;58:208–210)

Keywords: indoor air pollution; PM<sub>10</sub>; interleukin-8

Studies of air pollution have until recently concentrated on outdoor air. However, time budget studies have shown that 90% of our time is spent indoors<sup>1</sup> and increased levels of pollution are common within public and private buildings. In this study, we have examined the impact of particles (collected in a kitchen as matter collected by a system that has a 50% efficiency for particles with an aerodynamic diameter of 10 µm (PM<sub>10</sub>)) and nitrogen dioxide (NO<sub>2</sub>) on cultured lung epithelial cells.

Epidemiological studies have shown a range of adverse health effects to be associated with increased concentrations of outdoor particulate matter (PM) and of NO<sub>2</sub> in the air, including respiratory and cardiovascular mortality, hospital admissions, and asthma attacks.<sup>2</sup> Until recently few mechanistic studies have focused on the effects of indoor particles, even though this exposure probably contributes a large proportion of personal exposure. With human monocytes, Monn and Becker<sup>3</sup> did not identify any significant changes in either cytotoxicity or

cytokine gene expression on exposure to indoor PM collected in a laboratory.

Nitrogen dioxide is produced by gas cooking at concentrations that may reach 1000 ppb or more in kitchens<sup>4</sup> and has been shown to cause proinflammatory cytokine release by epithelial cells in vitro.<sup>5</sup> In this study we have examined the effects on human cells in vitro of PM<sub>10</sub> collected during cooking with gas and electricity, both with and without concomitant NO<sub>2</sub> exposure.

### Methods

The human epithelial type II cell line, A549 (American type culture collection) was cultured in Dulbecco's modified eagle medium with 10% foetal calf serum at 37°C and 5% CO<sub>2</sub>. Cells were grown to confluence in tissue culture flasks (Helena Biosciences, UK), detached with trypsin, and centrifuged at 600g for 2 minutes. The cells were resuspended in 3 ml of medium before being counted and assessed for viability with 0.5% trypan blue (Sigma, Poole UK). They were then diluted to yield 498 cells/mm<sup>2</sup>. To each 3.5 cm plate (Bibby Sterilin, UK) 2 ml cell suspension in Dulbecco's modified eagle medium with 10% foetal calf serum was added and cultured for 24 hours at 37°C and 5% CO<sub>2</sub>. After this period the medium was replaced with Dulbecco's modified eagle medium without foetal calf serum for a further 24 hours before treatment.

A particle sampling head (IOM, Edinburgh) was used to collect indoor PM<sub>10</sub>. The sampling heads were loaded with 37 mm Gelman TF1000 filters (Gelman Sciences, Northampton, UK), extracts of which had been tested previously for their low inflammogenicity in the rat lung in vivo and cytotoxicity in vitro.<sup>6</sup> Sampling was carried out in a laboratory (70 m<sup>3</sup>), equipped with a gas and electric cooker. There was no mechanical ventilation and the windows were closed during collection; there were no sources of particles in the laboratory apart from the cooking. The sampler was attached to an SKC sidekick pump set to draw air through the filter at 2 l/minute. The samples were collected on multiple filters over a period of 2–3 weeks during the use of either the gas or electric cooker. Cooking experiments, detailed elsewhere,<sup>4</sup> were duplicated on the two cookers. The filters were then placed into a bijoux tube with 1 ml Dulbecco's modified eagle

Napier University,  
School of Life  
Sciences, 10 Colinton  
Road, Edinburgh,  
EH10 5DT, Scotland,  
UK

C A J Dick  
K Donaldson  
V Stone

University of  
Aberdeen, Department  
of Environmental and  
Occupational  
Medicine, Aberdeen,  
AB25 2ZD, Scotland,  
UK

M Dennekamp  
S Howarth  
J W Cherrie  
A Seaton

Correspondence to:  
Dr C A J Dick  
c.dick@napier.ac.uk

Accepted 19 October 2000

medium containing 25 mM HEPES and vortexed for 2–4 minutes to allow aqueous suspension of the particles to form. Sufficient filters to give a mass of 600–700 µg for each experiment were used. Estimations of the concentration of indoor particles in solution were calculated with optical density reading at 600 nm compared with a carbon black standard. The concentration was adjusted to give 0.05 µg/mm<sup>2</sup>.

To treat the cells with NO<sub>2</sub>, each 3.5 cm dish was placed onto an angled rotating platform inside the exposure chamber. The NO<sub>2</sub> was then passed into the exposure chamber (90 seconds at 5 psi), the outflow and inflow vents were sealed and the platforms rotated at 3 rpm for 4 hours to allow contact between cells and gas. The control chamber contained zero grade air. The treatment chamber was filled with 476 ppb NO<sub>2</sub> in zero grade air (BOC Special gases, UK) for 90 seconds (5 psi). The chamber was then securely sealed. The NO<sub>2</sub> concentration was maintained at a range of 300–450 ppb over the 4 hour treatment period. Concentrations of NO<sub>2</sub> were measured with the ML 9841A NO<sub>x</sub> chemiluminescence analyser (Monitor Labs, Inglewood, CO, USA).

Interleukin-8 protein was measured with a “DuoSet” sandwich ELISA system (R and D Systems, UK). Total glutathione was measured with the 5,5'-dithio-bis-(2-nitrobenzoic acid) method described by Baker *et al.*<sup>7</sup>

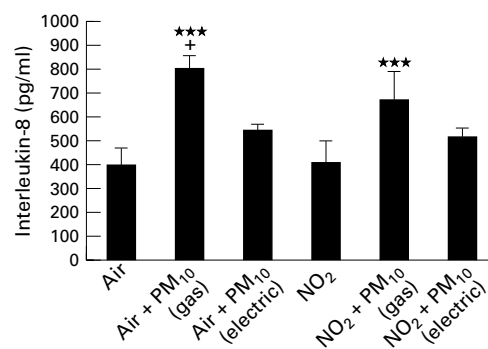
Of 12 filters available from each cooker type, six were intended for cultures that were to receive NO<sub>2</sub> and six for cultures that were to receive zero grade air. In fact loading of filters was sufficient in four cases to allow duplicate cultures to be treated. Therefore the data are described for six to eight values.

Values are expressed as mean (SEM). The data were analysed by one way analysis of variance (ANOVA) with a Tukey's multiple comparison test.

## Results

Only treatment of A549 cells for 4 hours with indoor PM<sub>10</sub> (0.05 µg/mm<sup>2</sup>) collected during periods of gas cooking resulted in release of increased concentrations of IL-8 compared with untreated cells; particles from electric cooking had no significant effect (figure). A549 cells dosed with indoor PM<sub>10</sub> from gas cooking released significantly more IL-8 protein than control cells (804.1 (55.8) pg/ml compared with 397.7 (73.2) pg/ml) and this was not influenced by coexposure to NO<sub>2</sub>. Indoor PM<sub>10</sub> generated from electric cooking did not significantly increase IL-8 concentrations: untreated in air 397.7 (73.2) pg/ml; electric generated PM<sub>10</sub> 542.8 (27.7) pg/ml in the presence of air; and 516.3 (38.8) pg/ml in the presence of NO<sub>2</sub>.

The cells were also assessed for total glutathione concentrations, as a change in this intracellular antioxidant would indicate oxidative stress. After treatment of the A549 cells for 4 hours with PM<sub>10</sub> generated by gas or electric cooking, with or without NO<sub>2</sub>, there was no change in intracellular glutathione (data not shown), indicating that the cells had not undergone oxidative stress at this time.



The effect of indoor PM<sub>10</sub> (0.05 µg/mm<sup>2</sup>) from gas or electric cooking with and without NO<sub>2</sub> (300–450 ppb) for 4 hours on secretion of interleukin-8 protein by A549 cells. All values are means (SEM), n=6–8 (see methods and material for explanation) (\*\*\*)p<0.001 v air alone, +p<0.001 v air with PM<sub>10</sub> (electric).

Limited toxicity testing was carried out with the 3–4,5-dimethylthiazol-2,5-diphenyl-tetrazolin bromide (MTT) assay, as a measure of metabolic competence, and no toxicity was found.

## Discussion

Interleukin-8 is a chemokine which has been shown to be produced by lung epithelial and other cells, release of which leads to neutrophil recruitment.<sup>8</sup> Its release by the epithelial cell line is used here as an indicator of potential to induce inflammation *in vivo*. We have shown that, by contrast with PM<sub>10</sub> generated by electric cooking, PM<sub>10</sub> generated by gas cooking resulted in increased IL-8 production by A549 cells. We used NO<sub>2</sub> coexposure because gas cooking also generates this gas, the concentrations being comparable with those detected in kitchens with gas cookers.<sup>4</sup> However, NO<sub>2</sub> alone or its addition to PM<sub>10</sub> had no effect on IL-8 release, even though the rocking exposure system used allowed cell/gas contact. These results are not consistent with the results of Devalia *et al.* (1993), which indicated an increase in IL-8 after treatment of NO<sub>2</sub> (400 ppb) for 6 hours. However, their study was conducted on a bronchial epithelial cell line and our study used an alveolar cell line. This, along with the difference in exposure time may account for the difference in results. Although dose-response studies would have been desirable, the small concentration of airborne particulate prevented this (84 weeks of sampling were required to produce enough data for the figure).

It has been shown that residual oil fly ash and PM<sub>10</sub> can induce IL-8 expression in epithelial cells with concomitant oxidant activation of NF-κB, a transcription factor that controls expression of proinflammatory genes.<sup>9</sup> Activation of NF-κB was suppressed in the presence of antioxidants, such as dimethylthiourea (DMTU), suggesting the involvement of reactive oxygen species.<sup>10</sup> In view of this role of NF-κB in the control of IL-8 expression, we expected a decrease in glutathione concentrations, an indicator of oxidative stress. That this was not found in our model, perhaps due to

many factors, such as the time chosen or the abundance of glutathione present in A549 cells.<sup>11</sup>

In summary our results indicate the potential of PM<sub>10</sub> generated by gas cooking to cause proinflammatory effects in lung cells. Although NO<sub>2</sub> had no apparent enhancing effect, further studies with a more abundant supply of PM<sub>10</sub> would be desirable to allow dose-response and time-response studies to investigate any possible interactions. The small amounts of PM<sub>10</sub> that were generated from the cooking posed a problem for obtaining sufficient material for the study, and this implies that any risk might be small from such small concentrations of airborne mass. Further research is warranted but high volume collection systems will be necessary to obtain sufficient particulate matter.

We thank Andrea Pringle for her technical assistance. This study was funded by the United Kingdom Department of Health, through the Institute for Environment and Health. MD is supported by the British Lung Foundation. KD is the British Lung Foundation/Transco fellow in air pollution and respiratory health.

- 1 Robinson J, Nelson WC. *National human activity pattern survey database*. Research Triangle Park, NC: United States Environmental Protection Agency, 1995.
- 2 Department of Health Committee on the Medical Effects of Air Pollutants. *Handbook on air pollution and health*. London: The Stationary Office, 1997.
- 3 Monn C, Becker S. Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine (PM<sub>2.5</sub>) and coarse particles (PM<sub>10-2.5</sub>) in outdoor and indoor air. *Toxicol Appl Pharmacol* 1999;155:245–52.
- 4 Alberts WM. Indoor air pollution: NO, NO<sub>2</sub>, CO, and CO<sub>2</sub>. *J Allergy Clin Immunol* 1994;94:289–95.
- 5 Devalia JL, Campbell AM, Sapsford RJ, et al. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells in vitro. *Am J Respir Cell Mol Biol* 1993;9:271–8.
- 6 Dick CAJ, Stone V, Brown DM, et al. Toxic and inflammatory effects of filters frequently used for the collection of airborne particulate matter. *Atmosphere and Environment* 2000;34:2587–92.
- 7 Baker M A, Cerniglia G J, Zaman A. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. *Anal Biochem* 1990;190:360–5.
- 8 Standisford TJ, Kunkel SL, Basha MA, et al. Interleukin-8 gene expression by a pulmonary epithelial cell line. *J Clin Invest*. 1990;86:1945–53.
- 9 Jimenez LA, Thompson J, Brown DA, et al. Activation of NF-κB by PM<sub>10</sub> occurs via an iron-mediated mechanism in the absence of IκB degradation. *Toxicol Appl Pharmacol* 2000;166:101–10.
- 10 Simeonova PP, Luster MI. Asbestos induction of nuclear transcription factors and interleukin 8 gene regulation. *Am J Respir Cell Mol Biol* 1996;15:787–95.
- 11 Rahman I, Li XY, Donaldson K, et al. Glutathione homeostasis in alveolar epithelial cells in vitro and lung in vivo under oxidative stress. *Am J Physiol* 1995;269:285–92.