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Nanoparticles from photocopiers induce oxidative stress and upper respiratory tract inflammation in healthy volunteers

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Abstract

Photocopiers emit large quantities of nanoparticles (NPs); however, their toxicological properties have not been studied. Here we investigate for the first time early human responses following a day's exposure to NPs from photocopiers. Nine healthy subjects spent 6 h at a busy photocopy centre on 2–3 randomly selected days. Matched nasal lavage and urine samples were collected before and at different time points post-exposure. Nasal lavage samples were analysed for 14 cytokines, inflammatory cells and total protein. Urine samples were analysed for 8-hydroxydeoxyguanosine (*8-OH-dG*). Exposure assessment was conducted using a suite of instruments. The mean total particle number on exposure days was >5 times higher than background, with size distributions in nanoscale range (peak 30–40 nm). Following exposure, *8-OH-dG* and several pro-inflammatory cytokines were elevated 2–10 folds compared with pre-exposure levels and remained elevated for up to 36 h. We conclude that NPs from photocopiers induce upper airway inflammation and oxidative stress.

Keywords: Engineered nanoparticles, photocopiers, printers, inflammation, oxidative stress, biomarkers

Introduction

Printers and photocopiers have become an integral part of modern life. It is now well established that they emit nanoparticles (NPs). (NPs; <100 nm diameter) (He et al. 2007; Lee & Hsu 2007; Wensing et al. 2008). Peak exposures as high as 1×10^8 particles/cm^3 have been reported (Lee & Hsu 2007). The chemical composition of these NPs remains poorly understood. NPs emitted from office printers have been the focus of several investigations in the past few years. They seem to be formed primarily from the condensation of semi volatile organic compounds (SVOCs) evaporated from the toner during the printing process and include different classes of poorly characterised organic compounds, such as alkanes and siloxanes (Morawska et al. 2009; Wensing et al. 2009). More recently, Barthel et al. 2011 reported on emission of other elements, including silicon (Si), sulphur and several metals such as titanium (Ti), iron (Fe), chromium (Cr), nickel (Ni) and zinc (Zn). They estimated that solid inorganic particles accounted for <2% of the total number of emitted particles. In contrast, no reports exist on the chemical composition of NPs emitted from photocopiers. Due to the similarities in the technology of (black and white) laser printing and photocopying, one would expect similar chemical composition of the emitted nano aerosols.

In a companion paper (Bello et al. 2012), we report for the first time on extensive physicochemical and morphological characterisation of airborne size-fractionated aerosol emissions from commercial grade photocopiers from one major manufacturer, focusing primarily on the nano fraction (<100 nm). A size-selective gravimetric and chemical analysis was conducted for the nanoscale fraction (<100 nm, also commonly referred to as ultrafine particulate matter fraction $PM_{0.1}$), fine particulate matter fraction <2.5 m ($PM_{0.1–2.5}$) and the coarse fraction <10μm ($PM_{2.5–10}$). Chemical analysis included organic and elemental carbon, total and water-soluble metals, alkanes and other SVOCs. It was found that NP emissions form photocopiers were chemically complex mixtures of SVOCs, inorganic compounds, carbon black and engineered metal oxides – especially, titanium dioxide, fumed silica and iron oxide. The distribution of these classes of analytes varied with the size fraction. Several elements (iron, titanium, zinc, aluminium, manganese, silicon and sulphur were found in concentrations ranging from 0.01% to 6% of the total mass of the nanoscale fraction. Mass concentrations of each fraction varied in the order of 1–10 μg/m^3, mostly concentrated in the nano and fine fractions. Organic carbon constituted 50–68% of the total mass of the nanoscale and fine PM fractions. Emissions from photocopiers are chemically complex and exposure levels are potentially much higher than that of printers. In this paper, we will

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be using the terms NPs and ultrafine particles synonymously to refer to particles <100 nm as is commonly done in the literature, and engineered NPs to distinguish them from the incidental NPs.

The toxicological significance of exposures to NPs from printing and photocopying is unclear. Several recent studies on cohorts of photocopy employees have found elevated biomarkers of DNA damage compared with controls (Balakrishnan & Das 2010; Gadhia et al. 2005; Goud et al. 2004; Manikantan et al. 2010). Theegarten et al. (2010) presented an interesting case study implicating NPs from printers as possible causative agents. Additionally, several case reports suggest that the use of photocopiers and toner dust may be related with diseases such as sarcoidosis (Rybicki et al. 2004), granulomatous pneumonitis (Armbruster et al. 1996) and antiphospholipid syndrome (Bar-Sela & Shoenfeld 2008). Despite these studies, data that establish a more definitive link between exposure to NPs from photocopiers and any ensuing disease incidence in humans are currently lacking. Moreover, nanotoxicology is a comparatively new field of investigation and methods applicable to human epidemiological studies of NP exposure are underdeveloped (Eisen et al. 2011; Li & Nel 2011). Therefore, there is growing interest in studying the effects of acute exposure to NPs and immediate host responses that may indicate toxic effects.

The respiratory tract is particularly susceptible to cellular assaults caused by inhaled NPs, which is the primary exposure pathway in workplaces and the environment. Damage caused by NPs typically elicits the activation of oxidative stress and inflammatory responses, including the activation of pro-inflammatory cytokine signalling pathways (Bonner 2010; Nel et al. 2006; Oberdorster et al. 2005; Xia et al. 2009). As examples, inhalational studies performed in humans and rodents have shown that acute exposure to incidental or engineered NPs lead to oxidative stress, production of inflammatory mediators and leucocyte infiltration into the pulmonary bed (Frampton 2007; Frampton et al. 2006; Oberdorster 2001; Oberdorster et al. 2005). The pro-inflammatory cytokines and chemokines produced by respiratory epithelium and infiltrating inflammatory cells play important roles in the development of respiratory diseases (Driscoll et al. 1997; Laskin 2007). In animals, polymorphonuclear leucocytes (PMNs) are the predominant inflammatory cells recruited during NP-induced acute inflammation and their infiltration has been used as a marker of acute cellular response in the upper airways (Payne & Cheng 1990). In addition, oxidative stress is a good predictor of inflammation and is known to play a vital role in the development of various diseases and cellular aging (Li et al. 2008; Rushton et al. 2010). DNA is one of the most biologically significant targets of oxidative attack; once it is damaged by reactive oxygen species, numerous oxidative DNA damage by-products are formed. One of them is 8-hydroxydeoxyguanosine (*8-OH-dG*), a predominant and ubiquitous marker of oxidative stress (Loft et al. 1993; Donaldson et al. 2005; Dalle-Donne et al. 2006), which is excreted without further metabolism into urine (Shigenaga & Ames 1991). Increased levels of urinary *8-OH-dG* have been reported in response to increased number concentration of ultrafine particles and other environmental toxicants (Song 2010; Abder-Rahman & Nusair 2007; Wu et al. 2004; Pilger & Rüdiger 2006).

In terms of assessing tissue damage caused by NPs, the nasal cavity provides an easily accessible portion of the upper respiratory tract that is amenable to morphological and pathophysiological evaluation. Moreover, this is a site of significant NP deposition, in particular of smaller NPs, for example <10 nm (Oberdorster et al. 2005; Yang et al. 2008). Since collection of nasal lavage (NL) is a simple, rapid and well-tolerated procedure, this can be used to investigate toxicological impacts at the nasal cavity and to probe the potency of such exposures to other target organs, especially deep airways, and it can be applied to a large sample population. Importantly, studies performed in humans with respiratory disease show a significant correlation in the nature of cytokines measured in NL samples and those obtained from highly invasive bronchoalveolar lavage (Pitrez et al. 2005; Prakash 2005). Additionally, nasal and bronchoalveolar epithelial cell line cultures exposed to various NPs secreted similar inflammatory mediators (McDougall et al. 2008). Based on these considerations, we hypothesised that acute exposure to NPs from photocopiers may induce oxidative stress and airway inflammation in healthy humans and the combined analyses of NLs from humans exposed to NPs together with adequate exposure characterisation, will provide a comprehensive model system for assessing acute responses to NPs.

Methods

Study design

The study design is shown schematically in Figure 1. Young, healthy volunteers (described later) were asked to spend a day (6 h, 9:00 AM to 3:00 PM) in a printer-free office environment (background indoor environment) for three individual days (6 h, 9:00 AM to 3:00 PM) in the photocopy centre. Individuals would spend most of the time reading and/or studying (Figure S1). We did not attempt to control or influence photocopying activity (hence exposures) during study days. Because exposures in the photocopy centre were highly variable, whereas background levels were much less so, each subject was asked to volunteer three individual days in the copy centre. These were non-consecutive days, spaced randomly over several weeks. Therefore, each individual was monitored one day in a background environment and three non-consecutive days in the photocopy centre. Four subjects volunteered only two exposure days. Replicate measures on each subject allows for the investigation of intra- and inter-individual variation in target biomarkers, which provides important information for optimal study design in subsequent future epidemiological studies. Matched NL and urine samples were collected from all volunteers on a background (non-exposure) and exposure days. On background (non-exposure) days samples were collected at morning (9 AM) and afternoon (3 PM). Background morning (termed NL_{AM}, U_{AM}) and afternoon (termed NL_{PM}, U_{PM}) samples were collected to check for diurnal variations in these biomarkers. For exposure day's matched NL and urine samples were collected before (time zero, NL_0, U_0) and immediately after

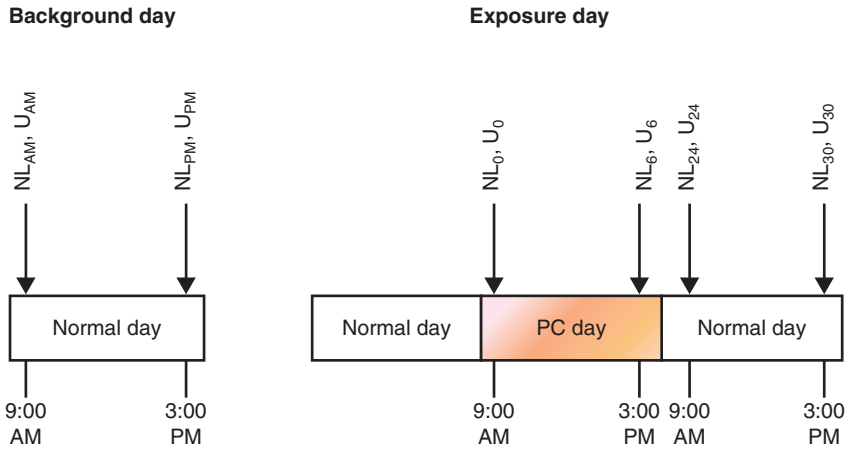

Figure 1. Schematic of the study design for collection of nasal lavage and urine samples on background (office environment) and exposure days (PC environment).

(6 h, $NL_{6,}$ U_6) exposure, and followed at 24 h (next day morning, $NL_{24,}$ U_{24}) and 30 h (next day afternoon, $NL_{30,}$ U_{30}). NL was analysed for a panel of cytokines, inflammatory cells and total protein, whereas urine was analysed for 8-*OH-dG* and creatinine.

Subject recruitment

Nine healthy non-smoker individuals (7 men and 2 women) over the age of 18 were recruited for the study. The mean age of the participants was 26.2 (range 19–40 years). None of subjects suffered from flu, allergies or any other respiratory illnesses. No anti-inflammatory or other drugs, especially nasal sprays or drops, body perfumes and anti-oxidant supplements, were permitted during the study. All participants gave informed written consent before their participation in the study. Study participants were asked to fill in a brief questionnaire about their age, gender, respiratory health status, use of medications and the potential non-occupational exposures to NPs. Study protocols were approved by the UMass Lowell Institutional Review Board.

Exposure characterisation

Real-time instruments monitored size distribution and total number concentration of airborne particulate matter during background days in the office environment and exposure days at the photocopy centres. Since validated personal monitors for NPs are still lacking, the only available technology at the moment is area NP monitoring instrumentation. The inlets of these instruments were positioned in the breathing zone of study volunteers (see Figure S1). More information on instrumentation, exposure assessment approach and the rationale is provided in an accompanying publication (Bello et al. 2012) together with a detailed chemical and morphological analysis of airborne NPs and the photocopy centre environment. Instrumentation used for airborne NP monitoring included: i) a fast mobility particle sizer (FMPS Model 3091, 5.6–560 nm range, 1 sec response time; TSI Inc. Shoreview, MN, USA); ii) an aerodynamic particle sizer (APS Model 3321, 0.5–20 μm range, 1 sec response time; TSI Inc.) and iii) a condensation particle counter (CPC Model 3007, total number concentration in 0.1–1 μm, 1 sec response time; TSI Inc.). In addition to airborne particulate matter, we also monitored in real time for gaseous co-pollutants such as ozone (ozone analyser Model 205, 2B Technologies, Boulder, CO, USA), total volatile organic compounds (tVOCs, ToxiRae Plus PID, RAE Systems, San Jose, CA, USA) and other important indoor environmental quality parameters such as temperature, relative humidity, carbon dioxide and carbon monoxide (Q-Trak Model 8550 and 7565, TSI, Inc.). The data were recorded continuously during the study period. Similarly, exposures were also evaluated in the printer-free office (control environment). A high correlation (Spearman rho 0.94) existed between the total number concentration measured by the CPC and the FMPS. In addition, airborne size distributions in this photocopy environment were similar across different days. The limited access to and portability of FMPS and APS restricted their use to about one-third of all monitoring days. The CPC and other instruments' data were available for all background and exposure days. Chemical and morphological characterisation of the size-selective airborne particulate matter in this photocopy environment was also completed (Bello et al. 2012) and the results are briefly summarised in the Results section. Since NPs have very little mass associated with them, it was not possible to collect sufficient mass for subsequent gravimetric and chemical analysis from one single 6 h exposure period. Therefore, sampling for chemical analysis lasted between 1 and 3 weeks, and both chemical and gravimetric exposure data represent longer-term averages and are complementary to real-time number concentrations and size distributions.

Nasal lavage sample collection

Previous studies using the nasal wash techniques to collect NL have demonstrated high variability in the amount of saline solution recovered after instillation between subjects (Howarth et al. 2005). Therefore, to collect more consistent NLs, we applied the nasal aspiration technique to reduce the variability among subjects and found a saline recovery of about 90% after aspiration. The NL technique was adopted from a previous report (Fujimoto et al. 2009) with minor modifications (as shown in Figure S1). The nasal cavity was misted over with saline using a

specifically designed disposable plastic sprayer, which was compressed with the fingers 2–3 times for each nostril of the subject. NL fluid was collected by aspiration from both nostrils for 1 min on each side through a medical grade 2.7 mm diameter tube and trapped in a mucus trap tube connected with an evacuator. An inserted tube was moved at the depth of 2–4 cm from nostrils backwards and forwards for 1 min on each side. The mean total volume of saline sprayed into the nose for a single lavage was 0.5 ± 0.04 mL and the mean volume of aspirated NL fluid containing both sprayed saline and nasal secretion was 0.46 ± 0.07 mL. These volumes were determined independently using gravimetric technique. The inside of the tube was rinsed with 0.5 mL of saline to maximise lavage fluid collection. The whole process was repeated to collect a second lavage. This protocol was well tolerated by the subjects. Two consecutive NLs were employed routinely per nostril because it was found from pilot studies that 93% of cytokines recovered from multiple consecutive lavages were contained in the first two lavages.

Cytokine recovery from the adapted nasal lavage technique

One issue that has not been sufficiently investigated in the literature is the overall recovery of cytokines from a single NL procedure. This is important to know, in order to interpret the accuracy of cytokine measurements and correct for poor recoveries, but also for understanding and interpreting cross-day (am-pm) differences in cytokine levels. Three consecutive NLs were performed on 7 subjects and the amount of each cytokine in each NL was calculated as percentage of the total recoverable amount in the three lavages. The mean cytokine recovery from each of the three consecutive NLs was 64.8 ± 5.5%, 27.7 ± 4.7% and 7.5 ± 2.5%, respectively, with little variation between different cytokines. For all subsequent sampling, two consecutive lavages, yielding ~93% of the total recoverable cytokine content, were collected from each subject.

Sample processing

Following collection, the specimens were immediately cooled on ice, vortexed for 15 sec and centrifuged at 500 × *g* for 10 min at 4°C. A protease inhibitor cocktail 0.01% v/v (Complete mini EDTA-free, Roche diagnostics, Indianapolis, IN, USA) was added to the supernatant and the samples were stored at −80°C until use. Supernatants were used for cytokine and total protein estimation. The pellet of cells was resuspended in 250 µl of 1 × PBS, 0.1% BSA and 0.1% mucus lysine solution; the mixture was used to obtain total and differential cell counts. Total cell counts were obtained using a haemocytometer. For performing differential cell counts, smears were obtained from 75 to 100 µl of cell suspensions using a cytocentrifuge. Cells on slides were stained with Wright and Giemsa, air dried and permanently mounted with Permount (Fisher Scientific, Pittsburgh, PA, USA) and a cover slip (Prat et al. 1993). Cell morphologies were studied using bright field microscopy under 400× magnification; counts were performed randomly from each slide.

Target inflammatory biomarkers

Twenty initial NL samples originating from a parallel case–control study on chronically exposed photocopy centre workers and healthy controls (12 NL samples were from 5 chronically exposed individuals and 8 samples from 8 controls) were assayed using a 42-plex high sensitivity human cytokine LINCOplex Kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. The NL samples were analysed for the panel of 42-cytokines using a Luminex 200IS System (Luminex Corporation, Austin, TX, USA). Luminex uses a bead based technology which can detect multiple proteins in each sample with very high sensitivity. Cytokine concentrations were calculated using Upstate Beadview (Temecula, CA) software. Based on these preliminary results, 14 cytokines that were frequently overexpressed in chronically exposed workers, compared with the control group, were selected for routine analysis for the current study of 9 health volunteers using 14-plex kits from the same manufacturer. The 14 cytokines included eotaxin, Epidermal Growth Factor (EGF), Granulocyte-Colony Stimulating Factor (G-CSF), Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), Interlukin-1α (IL-1α), Interlukin-1β (IL-1β), Interlukin-6 (IL-6), Interlukin-8 (IL-8), Interlukin-10 (IL-10), Interferon-(IFN γ), Monocyte Chemoattractant Protein-1(MCP-1), Tumour Necrosis Factor (TNF-alpha), fractalkine and Vascular Endothelial Growth Factor (VEGF). Below limit of detection (LOD) cytokine values in sample were substituted with LOD/$\sqrt{2}$ for that particular cytokine. Eotaxin was dropped from the final analysis because the majority of samples in the human volunteers were below the method's LOD.

Total protein concentrations

Total protein concentrations were measured from the supernatant in all the NL samples using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions, with measurements performed using a 96-well microplate reader (SPECTRAmax+384, Molecular Devices).

Urine sample collection

Urine samples were collected in sterile, medical grade 90 mL containers specifically designed for this purpose. After collection, the samples were centrifuged at 5000 × *g* for 10 min at 4°C. The samples were stored at −80°C until use.

Urinary 8-OH-dG and creatinine analysis

Urine analysis for *8-OH-dG* was performed using a commercially available ELISA kit (Japan Institute for the Control of Aging, Fukuroi, Japan) according to the manufacturer's instructions, with quantitative absorbances obtained from a microplate reader. The *8-OH-dG* values from the samples were calculated based on calibration sigmoid plots of the absorbance (450 nm) of an *8-OH-dG* standard at various concentrations. Urine samples were also analysed for creatinine, and *8-OH-dG* levels were reported as creatinine-adjusted concentrations (e.g., micrograms/grams creatinine) to account for any variation due to urine dilution in the body.

Creatinine analysis was performed using a calorimetric assay kit (Oxford biomedical, Rochester Hills, MI, USA) following the manufacturer's instructions, with measurements taken at 490 nm using an absorbance plate reader. Urinary creatinine-adjusted *8-OH-dG* concentrations (micrograms/gram creatinine) were used in all analyses.

Statistical analysis

Exposure and biomarker data were examined for the underlying distributions using the Shapiro–Wilks statistics and by graphing probability plots and histograms using the SAS System for the PC (SAS v 9.2 Inc., Cary, NC, USA). Exposure data (e.g. total number concentration as measured by different instruments) fit the lognormal distribution better than the normal distribution. The natural logarithms of the measured exposure data were used for all analyses, and the geometric mean and geometric standard deviation were used to describe such distributions. Similarly, all biomarker data, except for inflammatory cells, were found to be log-normally or approximately log-normally distributed and all subsequent analyses were performed on log-transformed data. Inflammatory cell data (PMN), which were expressed as percent difference from controls, were normally distributed. All (exposure and biomarker) data below the LOD were substituted by LOD/$\sqrt{2}$. Paired t-tests were performed on log transformed measurements to test for the differences in cytokines, total protein and *8-OH-dG* between background morning (NL_{AM}, U_{AM}) and afternoon (NL_{PM}, U_{PM}) samples. If the biomarker PM value was found to be statistically different from the AM value, this was reported as average percentage difference from the background (AM) concentration. Unpaired t-test was performed for the differences between background and photocopy environment exposures.

Because of the repeated measurement design on exposure days, mixed models with a random intercept and compound symmetry covariance structure were used to test for changes in cytokines, total protein and *8-OH-dG* levels between (NL_0, U_0) and (NL_6, U_6), 24 h (NL_{24}, U_{24}) and 30 h (NL_{30}, U_{30}). We considered a p-value of <0.05 to be significant. Lowess smooth R (project version 2.12.2) was used for the Lowess smoothing of data (60–80% smoothing parameter) to examine the relationship between the mean daily particle concentration and log measurements of cytokines, total proteins and *8-OH-dG*. The Lowess model does not account for the multiple observations; it is an exploratory technique and the repeat measurements do not affect the curve.

Results

Exposure assessment

Volunteers in the photocopy environment were exposed almost exclusively to NPs with a broad size distribution and peak maxima in the 20–40 nm range, as illustrated in Figure 2-A2). No significant emissions were measured in the 0.5–20 μm range (APS instrument, data omitted). Size distributions would vary only slightly between different exposure days, but exposure intensities and their profiles (number, frequency and intensity of peaks, for example) were far more variable. Figure 2 -A3 illustrates four distinct exposure profiles. The total number concentration increased during the day to several folds above background (measured overnight and before 7 AM when the photocopy centre opened), as illustrated in Figure 2-A1 for one exposure day. The total number concentration measured by FMPS and CPC were highly correlated (rho 0.96) and the reported statistics on number concentration are based on the CPC for which we had complete data for every day. The median (50th percentile) of the daily mean total particle concentration in the photocopy environment was 9500 particles/cm^3, (25th percentile, 5040; 75th percentile 20,200; maximum, 26,500 particles/cm^3). The highest peak number concentration was 189,500 particles/cm^3. In contrast, background size distributions had maximum in the 50–70 nm range and varied little between different days (data omitted). The median of the daily mean total particle number concentration for background days was 1540 particles/cm^3, (25th percentile, 1410; 75th percentile, 1870; maximum, 10,480 particles/cm^3). The highest peak number concentration recorded for background days was 18,600 particles/cm^3. Exposures in the photocopy centre were statistically significantly higher than those of background (t-test, $p = 0.002$).

Concentrations of other markers (ozone, VOCs, CO and CO2) in the photocopy environment were not significantly different from background (t-test, $p > 0.1$ for all). Ozone levels were low; range of daily means, 8–13 ppbv; peak value during exposure days was 25 ppbv. The total VOCs were always below 0.1 ppm. The CO levels were consistently non-detectable (0.0 ppm) and the CO_2 levels varied within a range of 600–750 ppm.

The chemical composition of all airborne particulate matter fractions, including the ultrafine fraction, was found to be complex and contained all major elements and classes of analytes found in the toners, including metals, SVOCs, as well as organic and elemental carbon, as described in the companion publication (Bello et al. 2012). The airborne ultrafine fraction contained 50% organic carbon, 0.1% elemental carbon, and ~50% inorganic material. The most abundant elements in the inorganic fraction were sulphur (S, 5.7%), iron (Fe, 0.42%) and silicon (Si, 0.6%), as well as smaller amounts (1% or less) of zinc (Zn, 0.22%), aluminium (Al, 0.12%), titanium (Ti, (0.05%), tin (Sn, 0.01%), manganese (Mn, 100 ppm), phosphorus (P, 560 ppm), and magnesium (Mg, 650 ppm), all of which were present in the toner formulation. The water-soluble fraction of metals in the airborne PM was metal- and size-fraction dependent. Generally, water solubility was the highest for the ultrafine fraction and smallest for the coarse fraction. Iron, Ti and Al for example were poorly water-soluble, <10% in the ultrafine fraction. Calcium, Na, K, Zn and S were highly water soluble, 85–100%, whereas Mn was 50%. Single particle elemental analysis revealed presence of NPs of several metals oxides, especially iron oxide and titanium dioxide, as well as other NPs of more complex elemental composition, most notable being those which contained Fe, Ni, Cr and Mn (Bello et al. 2012). Several long chain alkanes (C24, or tetracosane to C40, tetracontane) were found in all fractions of airborne particulate matter (as well as in the toner), the most abundant being tetracontane, octatriacontane and hexatriacontane in the ultrafine fraction.

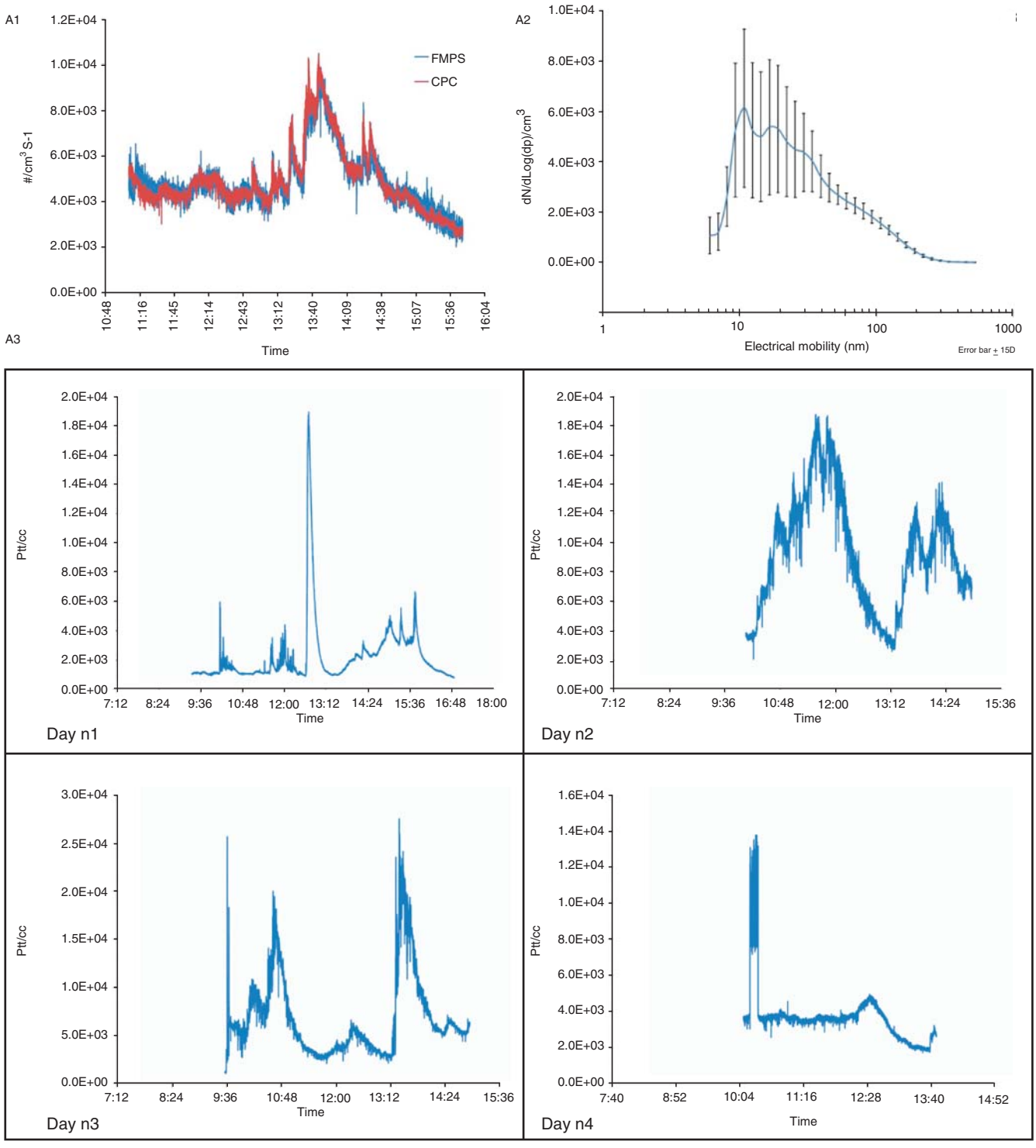

Figure 2. Illustration of volunteer's exposures for 1 day. (A1) Total number concentration over the whole exposure period as measured by the fast mobility particle sizer (FMPS) and condensation particle counter; (A2) Mean particle size distribution from the FMPS with error bars indicating one standard deviation; (A3) Examples of four distinct exposure profiles at the copy centre during exposure days.

Diurnal variations in NL markers and 8-OH-dG in urine

In order to account for any normal daily variations (AM vs. PM) in the NL cytokines or urinary *8-OH-dG* levels in exposed individuals, NLs from volunteers who remained in the control office environment were analysed ($n = 9$). Additionally, data were collected from another set of eight volunteers, recruited as part of a pilot case–control study of chronically exposed individuals, using the same methods and protocols, which were pooled to yield a total of 17 subjects. No repeat measurements were performed for background sampling. Comparisons were performed using paired t-tests on the log-transformed data, except for the %PMNs, which were not log-transformed. The mean levels of cytokines, total proteins, % PMNs and *8-OH-dG* are shown in Table I. No significant change was observed between AM and PM values for *8-OH-dG*, total protein, inflammatory cell numbers and levels of 10

Table I. Data on the biomarker levels in the nasal lavage (cytokines, PMN, total proteins) and urine of 9 healthy volunteers on background and exposure days. Data represent the geometric mean concentration (μg/mL for total proteins and pg/mL for cytokines and chemokines) and 95% confidence interval of the mean.

	Biomarker Levels, Geometric Mean (95% CI)					
	Background[a]		Exposure[b]			
BIOMARKER	NL_{AM}	NL_{PM}	NL_0	NL_6	NL_{24}	NL_{30}
Total Proteins	424.11 (330.30, 550.04)	411.58 (311.06, 550.04)	464.05 (376.15, 566.80)	1043.15 (943.88, 1164.45)	862.64 (765.09, 972.63)	620.17 (533.79, 720.54)
EGF	93.69 (61.56, 144.03)	85.63 (55.70, 132.95)	90.02 (63.43, 125.21)	160.77 (90.92, 281.46)	184.93 (121.51, 281.46)	146.94 (97.51, 221.41)
G-CSF	17.12 (9.49, 30.88)	9.03 (4.71, 17.46)	10.70 (6.82, 16.78)	104.58 (48.91, 221.41)	65.37 (24.78, 174.16)	29.08 (16.12, 52.98)
GM-CSF	0.52 (0.25, 1.07)	0.85 (0.30, 2.44)	1.43 (0.82, 2.48)	1.13 (0.64, 1.99)	1.40 (0.68, 2.92)	2.46 (1.14, 5.31)
Fractalkine	70.11 (49.90, 98.49)	60.34 (42.10, 87.36)	56.26 (42.10, 75.19)	196.37 (112.17, 343.78)	135.64 (91.84, 200.34)	88.59 (54.60, 142.59)
IL-1a	44.26 (29.08, 67.36)	52.46 (32.79, 85.63)	58.56 (40.45, 85.63)	71.52 (49.90, 102.51)	82.27 (62.80, 108.85)	80.64 (64.07, 100.48)
IL-1b	2.18 (1.36, 3.46)	2.46 (1.97, 3.06)	2.23 (1.62, 3.00)	5.21 (3.49, 7.77)	3.42 (2.61, 4.48)	2.83 (2.23, 3.56)
IL-6	1.05 (0.57, 1.95)	1.21 (0.40, 1.70)	1.30 (0.66, 2.53)	6.75 (4.53, 10.18)	3.86 (2.51, 5.99)	2.72 (1.58, 4.66)
IL-8	262.43 (139.77, 497.70)	287.15 (175.91, 464.05)	278.66 (174.16, 445.86)	2392.27 (1540.71, 3714.50)	1495.18 (871.31, 2565.73)	862.64 (473.43, 1571.84)
IL-10	0.37 (0.20, 0.68)	2.39 (0.25, 2.45)	0.69 (0.44, 1.09)	3.67 (1.97, 6.75)	1.72 (0.83, 3.63)	0.66 (0.31, 1.42)
TNF-α	0.37 (0.23, 0.60)	0.36 (0.21, 0.60)	0.67 (0.37, 1.23)	1.90 (1.51, 2.41)	1.20 (0.73, 1.95)	0.65 (0.46, 0.92)
IFN-γ	0.14 (0.06, 0.30)	0.13 (0.06, 0.28)	0.43 (0.22, 0.84)	0.44 (0.13, 1.46)	0.14 (0.07, 0.25)	0.11 (0.08, 0.16)
MCP-1	57.40 (36.60, 83.10)	55.15 (34.12, 90.02)	79.84 (54.60, 119.10)	304.90 (200.34, 464.05)	190.57 (122.73, 298.87)	117.92 (85.63, 160.77)
VEGF	102.51 (67.36, 157.59)	83.10 (45.15, 159.17)	80.64 (54.60, 120.30)	464.05 (259.82, 828.82)	354.25 (223.63, 561.16)	181.27 (115.58, 281.46)
%PMN	12.43 (8.68,16.17)	12.07 (9.09,15.05)	14.94 (10.62,19.24)	39.72 (29.75,49.69)	28.77 (21.21,36.32)	22.67 (16.00,29.35)
URINE	U_{AM}	U_{PM}	U_0	U_6	U_{24}	U_{30}
8-OH-dG	6.17 (4.39, 8.58)	6.42 (4.62, 8.94)	6.69 (4.71, 9.58)	13.46 (10.07, 17.99)	10.18 (6.55, 15.64)	8.17 (5.37, 12.43)

[a]Biomarker data in the morning (NL_{AM}, U_{AM}) and afternoon (NLPM, UM) on a non-exposure day.
[b]Biomarker data on an exposure day at different time points: pre-exposure (NL0), immediately after (NL6), next day morning (NL_{24}) and afternoon (NL_{30}).

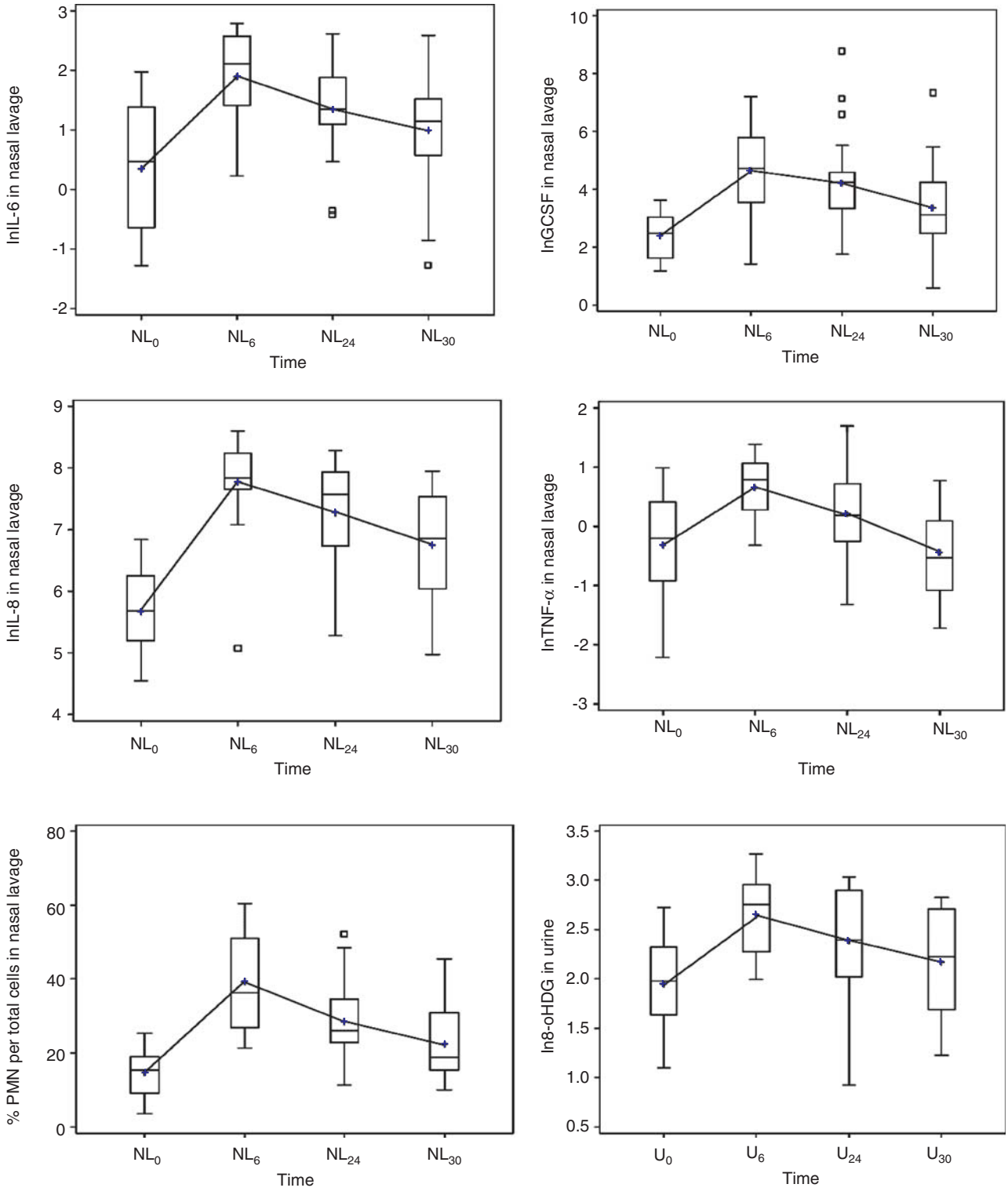

Figure 3. Box plot distribution of select representative biomarkers in nasal lavage and urine in relation to time on exposure days. The Y-axis represents the natural log values (Ln(Biomarker)). The horizontal lines in the box plot represent the median with 25 and 75% of the values being inside the box. Whiskers correspond to 5th and 95th % of all the values. Open squares represent outliers. Lines connect the mean values.

inflammatory cytokines, including EGF, G-CSF, GM-CSF, IL-10, IL-6, IL-8, IL-1α, IFNγ, MCP-1 and TNF-α. By comparison, the mean levels of fractalkine and VEGF were significantly lower (16.4% and 31.2%, respectively) in the background afternoon as compared with morning, whereas the mean level of IL-1β was 12.5% higher in the background afternoon samples. For reporting data from subsequent analyses, we conservatively used the higher background values for these cytokines (i.e., PM values for fractalkine and VEGF) to compare them with their corresponding values on exposure days.

Inflammatory markers in the nasal lavage

The percentage of PMNs per total cells identified in each NL was calculated. The mean PMNs in NL_0, NL_6, NL_{24} and NL_{30}

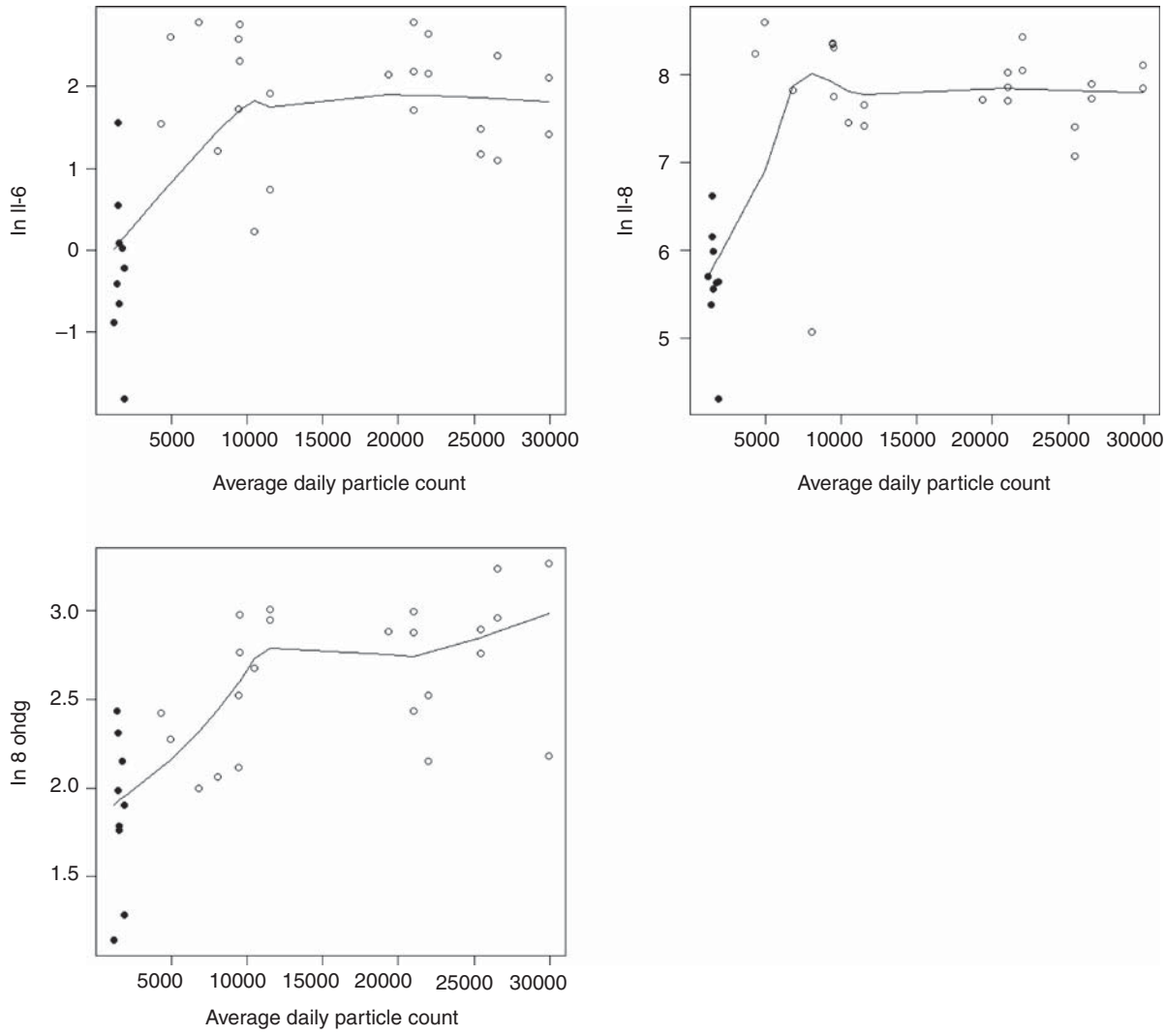

Figure 4. Correlation between particle number concentrations measured by the CPC 3007 (X-axis) and inflammatory and oxidative stress markers (Y-axis, Ln (value)).

were 14.9%, 39.7%, 28.8% and 22.7%, respectively (Table I). Significant elevations in PMNs occurred immediately post-exposure ($p < 0.001$), followed by subsequent reduction; however, levels failed to return to baseline even after 24 h post-exposure (Figure 3).

The concentration of cytokines, namely IL-6, IL-8 TNF, IL-1β, G-CSF, EGF, IL-10, MCP1, fractalkine and VEGF, were significantly higher ($p < 0.001$) immediately post-exposure (NL_6) than pre-exposure (NL_0) concentrations (Table I, Figure 3). The ratio of the (post-exposure/pre-exposure) geometric mean values (NL_6 to NL_0) ranged from 2 to 10, depending on the type of cytokine. As examples, G-CSF showed the highest increase, 9.77 times higher than pre-exposure (NL_0) concentration. Similarly, the ratio of the (NL_6/NL_0) geometric mean concentration values of other cytokines were as follows IL-8, 8.58; VEGF, 5.75; IL-6, 5.19; IL-10, 5.31; MCP1, 3.81; fractalkine, 3.49; TNF-α, 2.83; EGF, 1.78; finally IL-1β, 2.33. There was no significant difference in the pre- and post-exposure concentrations of GM-CSF, IFNγ and IL-1α. At each follow-up period after exposure (NL_{24} and NL_{30}), the concentration of the cytokines IL-6, IL-8, TNF, IL-1, G-CSF, EGF, IL-10, MCP1, fractalkine and VEGF decreased in a time-dependent manner. Interestingly, the NL_{30} concentration of cytokines TNFα, IL-1β, G-CSF, IL-10, MCP1 and VEGF reached baseline levels, whereas the levels of the IL-6, IL-8, EGF and fractalkine remained significantly higher than the pre-exposure levels ($p < 0.05$, Figure S2).

The mean total protein concentration in NL_0 was 464.05 μg/mL. The mean concentrations in NL_6, NL_{24} and NL_{30} were 1043.15, 862.64 and 620.17 μg/mL, respectively. Significant increases ($p < 0.001$) in total protein concentrations were observed after 6 h of a single exposure and the levels remained significantly higher even after 24 h of exposure.

NL cytokine/chemokine values were not normalised to the total protein content because total protein also increases in response to exposures in a similar fashion to cytokines/chemokines. In addition, our optimised NL sampling protocol resulted in improved reproducibility and high recovery of the sprayed saline solution (>90%). Normalisation to total protein in this case would have artificially masked exposure-related changes in NL biomarkers.

Significant increase in 8-OH-dG in urine after acute NP exposure

The concentration of *8-OH-dG* increased significantly ($p < 0.001$) after acute exposure (Figure 3); the mean natural log values (Ln) were 2 times higher as compared with baseline levels and remained significantly higher up to 24 h after exposure (Figure S2).

Association between particle number concentration and biological response

The relationship between the daily average total number concentration and biological responses (cytokines and total protein) was linear at the low end of the curve (up to ~10,000 particle cm^{-3}) and flattened out at higher average exposure levels (10,000–30,000 particles/cm^3) as illustrated graphically in Figure 4 (A, B) for IL-6 and IL-8. The concentration of 8-*OH-dG* increased more linearly with the average particle number concentration over the whole range (Figure 4C).

Discussion

Significant emissions of NPs from photocopiers raise a legitimate concern over potential health effects of such exposures to workers and consumers. An investigation of the relationships of exposure-health effects for nanomaterials in general and engineered nanomaterials and nanotechnologies in particular presents several major methodological challenges. These challenges include a lack of validated NP monitoring instrumentation for personal exposure assessment, the unsettled debate about the more suitable exposure and dose metrics (number concentration, surface area, surface activity or mass), the ever-present background NPs from air pollution and other activities, and the complex and partially understood chemistry of these exposures. Additionally, there is also great interest in developing robust methodology for epidemiological studies of engineered NPs. Challenges in this regard have to do with difficulties in accessing and recruiting large and stable cohorts of workers, since a large fraction of current nanomanufacturing occurs at the research and development stage, involves close reactors, processing is intermittent in nature, and many different types of NPs are utilised. Due to the rapid development and commercialisation of nanotechnologies, there is a strong motivation to identify potentially hazardous exposures early on in the developmental process. There is also a genuine need for developing robust and non-invasive methods that rely on meaningful early-effect biomarkers to judge the toxicological potency of such exposures in realistic settings under realistic exposure scenarios and with small cohorts of exposed people.

In this study, we assessed a panel of clinical biomarkers in the NL and urine samples of young healthy subjects in response to acute exposure to NPs from photocopiers. The methodology specifically targeted non-invasive sampling techniques, short-term timeframes, analyses of clearance kinetics, and the use of sensitive and meaningful biomarkers. Our present study demonstrated that short-term exposures to NPs emitted during photocopying induced biomarkers of upper airway inflammation and systemic oxidative stress, thereby providing useful insights into toxicological potency, the type of immune responses elicited by these particles and the clearance kinetics of these effects. In this regard, the present study is important methodologically in that it demonstrates the feasibility of simple non-invasive techniques such as NL combined with meaningful biomarkers and careful designs (e.g., subjects being used as their own controls, repeated sampling and detailed chemical exposure characterisation) to probe potential toxicological effects of realistic NP exposure scenarios using small groups of people, short-term exposures and low cost. Such features are highly desirable and we believe they will increasingly play an important role in the molecular epidemiology of engineered NPs.

Upon exposure to NPs, a complex combination of inflammatory mediators was induced and most of the cytokines induced were pro-inflammatory in nature; these included IL-6, IL-8, TNFα and IL-1β. Additional host proteins that act as growth and/or differentiation factors were also induced, including G-CSF, the primary mediator of PMN production and release in the bone marrow and EGF, which induces cell proliferation and differentiation (Laskin 2007; Frampton 2007; Frampton et al. 2006). Additionally, two chemokines that control the key regulatory mechanisms of cell trafficking at sites of inflammation, MCP-1 and fractalkine, and a key regulator of vascular permeability, VEGF, were also regulated upon NP exposure (Driscoll et al. 1997; Laskin 2007). Therefore, environments that contain NPs may cause the blood vessels to become leaky, thereby drawing more fluid and inflammatory cells to sites of inflammation. This would explain the increase in total protein concentration and PMNs in NL post-exposure. Interestingly, elevated levels of IL-10, a primarily anti-inflammatory cytokine, were also observed, indicating activation of a protective/repair feedback mechanism (Quinn et al. 2000). Our findings are consistent with the hypothesis that a network of cytokines is involved in NP-induced inflammation response (Li et al. 2010; Oberdorster 2001).

The inflammatory effects observed in the present study are similar to those induced by other NP exposures, including exposures to ambient air pollution, cigarette smoke and diesel exhaust, thereby suggesting shared mechanisms of observed biological effects across many different NP types and chemistries (Baeza-Squiban et al. 1999; Ghio et al. 2012; Takizawa et al. 2000). Chronic exposure to such NPs have already been reported to play a critical role in various clinical conditions such as loss of pulmonary function, increased bronchial hyper-responsiveness, chronic obstructive pulmonary disease (COPD), emphysema, fibrosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease and cancers (Sangani & Ghio 2011; Boczkowski & Hoet 2010; Gwinn & Vallyathan 2006). Many past studies have reported higher levels of leucocyte count and inflammatory cytokines such as IL-8, IL-6 and TNF-α in patient with COPD, acute sinusitis and other respiratory diseases. Hurst et al. (2005) used nasal wash and induced sputum and compared the levels of inflammatory cytokines between COPD patients and healthy controls and reported significantly higher IL-8 levels in nasal wash in COPD patients, 156.1 pg/mL as compared with control subjects, 58.9 pg/mL. Additionally,

they reported that the nasal IL-8 concentration correlated positively with that in the sputum, suggesting similar inflammatory effects in upper and lower airways. In another study, Ramirez et al. (2002) reported significantly higher total proteins, TNF-α and IL-8 concentrations in NL fluids of subjects with acute sinusitis subjects. Similarly, increased levels of urinary *8-OH-dG* have been associated with other exposures such as air pollution and exposure to diesel exhaust. Wei et al. (2009) observed three times higher concentration of *8-OH-dG* after 8 h shift (mean post-shift, 6.92/pre-shift, 1.83 μmol/1 mol of creatinine), in the urine samples of two individuals (security guards) exposed to air pollution. Moreover, in a similar study, Lee et al. (2010) reported significantly higher urinary *8-OH-dG* concentration in diesel exhaust emission inspectors (mean 14.05 ± 12.71 μg/g creatinine) as compared with controls (6.58 ± 4.39 μg/g creatinine). All these studies provide a strong argument that various inflammatory and oxidative stress pathways are activated in a similar fashion in different NP exposure scenarios, consistent with our findings. The reported levels of various biomarkers in the past studies are comparable to our current study. Although in the present study the biomarker responses returned to baseline level approximately 24–30 h post-exposure, and by themselves they do not constitute a health risk, one could hypothesise based on the clearance kinetics and mechanisms of action, that repeated, chronic exposures to NPs emitted from photocopy equipment (beyond a certain threshold) could lead to various health conditions or exacerbation of existing ones.

The chemical composition of airborne NPs emitted from photocopiers is complex. They represent a clear case of mixed NP exposures, including SVOCs, several metal oxides, carbon black and other components, such as salts and organics. On a number concentration basis, the vast majority of them seem to be SVOCs originating from condensation processes. They include long chain alkanes, well documented in this case (Bello et al. 2012), which are of little interest toxicologically. However, alkanes can only account for a small percentage of the total organic carbon mass (<2% of the 50% organic carbon mass in the nano fraction) and from some preliminary Fourier transform infrared spectroscopy analysis of the nano fraction it appears that its organic carbon fraction is far more complex. These organic NPs are likely to be important in the observed biological responses. However, there are no other published toxicological data on the actual NPs from photocopiers against which to compare our observations. The second important group of NPs is metal oxides, especially iron oxide, titanium dioxide and fumed silica. Several metals were also found in ppm level (Zn and Mn) and their exact phase distribution (e.g., organometallic salts or metal oxides) in the toner is not well understood. Elemental carbon (EC) was found in small amounts (0.01% of the total nano fraction mass) and most of it likely originates from the carbon black in toners.

Chronic exposures to carbon black may lead to declined lung function and may have deleterious effects on respiratory morbidity (Gardiner et al. 2001). Additionally, carbon black is classified by the International Agency for Research on Cancer as a Group 2 B carcinogen (possibly carcinogenic to humans) based on 'sufficient evidence' in animals and 'inadequate evidence' in humans (Baan 2007). Engineered nano-TiO_2 and Fe_2O_3 are known to cause acute lung injury, increases microvascular permeability, neutrophil influx, macrophage accumulation and pulmonary emphysema in mice (Zhu et al. 2008; Wang et al. 2007). Studies of human lung cells exposed to nano-TiO_2 reported cell death over a wide range of concentrations (3.6–2000 μg/mL) over 1–48 h exposures (Sayes et al. 2006). In mice studies, intratracheal instillation of nano-TiO_2 was shown to induce chronic inflammation (Park et al. 2009). Additionally, nano-TiO_2 (0.05% as Ti) has been classified as a suspect human carcinogen by NIOSH and some studies have reported inflammatory responses in lung cells upon exposure to nano-TiO_2 (Singh et al. 2007). Intratracheal instillation of nano-ZnO in mice induced significant pulmonary inflammation and marked body weight loss accompanied with anaemia (Liu et al. 2008) and nano-ZnO is also known to cause acute toxicity under *in vitro* conditions (Lanone et al. 2009). Similarly, animal studies done using nano-amorphous and nano-crystalline silica showed inflammation, granuloma formation and emphysema in lungs (Napierska et al. 2010). Although some of these ingredients are in small quantities, they equate millions of NPs deposited in the airways. In addition, it is important to consider potential synergistic effects between different components of the mixture, especially the organic fraction and metal oxides as such effects have been observed in the past. For example, Guo et al. (2009) showed that co-exposure to carbon black and Fe_2O_3 particles caused a synergistic oxidative effect which was significantly greater than the additive effects of exposures to either particle type alone. Additionally, they suggested that intracellular redox reaction between carbon black and Fe^{3+} was likely responsible for the observed synergistic oxidative effect.

In the present investigation, significantly higher *8-OH-dG* concentrations in post-exposure urine samples were also observed, suggesting that exposures to NPs from photocopiers may contribute to an increase in systemic oxidative stress in the body. However, the elevated *8-OH-dG* data may also result from other processes, in particular increased white blood cell turnover which may contribute to increase in reactive oxygen species, thereby increasing oxidative stress. NPs are the likely cause of the observed effects, given that they were the only agent to change significantly from the surrounding background environment. In addition, *in vitro* cellular toxicity evaluations by our group with collected NPs have resulted in increased levels of several of these cytokines in THP-1 cell line and human primary respiratory epithelial cells (Khatri et al. unpublished observations). Nevertheless, one cannot rule out a possible synergistic effect of different NPs with themselves and with other airborne pollutants, especially ozone and other VOCs, despite the finding that levels were very low and were not significantly different from that found in the office environment.

The relationship of inflammatory biomarkers (especially cytokines) with daily average particle number concentration appears to be non-linear, as seen by particle effect at higher concentrations. Although this observation is difficult to explain, it raises several interesting possibilities. In this natural experiment, the mean particle number concentration

reached 30,000 particles cm^{-3}, which is an order of magnitude higher than background. It is also possible that the observed inflammatory responses may follow a switch-like response, meaning that once a certain target dose (deposited NPs in the upper airways) is achieved, inflammatory pathways/processes are fully activated, but the rate of cytokine production is controlled by other rate-limiting processes and not deposited NPs. In addition, the average airborne particle number concentration may be a good surrogate metric of the deposited target dose in the airways, but peak exposures and their frequencies may be more important. Further explorations of alternative exposure metrics, such as peak number concentration and frequency, lag time to first peak exposure, etc. will be of interest. The ICRP (International Commission on Radiological Protection) lung deposition models applied to the daily average size distributions predicted ~6% deposition of the total number concentration in the nose and upper airways, ~10% in the tracheo-bronchial region and ~32% in the alveolar region. Several billion particles end up in the nose region during a 6 h period at the exposure levels measured in this study. It is therefore logical to hypothesise that similar inflammatory responses occur in the deep airways and that additional biomarkers of deep airway inflammation and in the circulatory system should be incorporated in future investigations. Significantly higher levels of *8-OH-dG* in urine post-exposure likely reflect the fact that the estimated deep airway deposition is five times higher than nose-upper airway deposition and calls for further investigations of other systemic effects.

One of the strengths of the present study was that we used the nasal aspiration technique to collect the NL samples, which results in improved recoveries (90% of sprayed saline solution), reduced variability and a high rate of retention of recruited subjects, compared with traditional irrigation techniques (2–10 mL/nostril of 0.9% NaCl solution) (Howarth et al. 2005). Although supplementing NL with analysis of BAL samples would provide a more comprehensive picture of the status of the airways (upper and lower airways), BAL is a highly invasive and risky procedure (Prakash 2005). Given the size distribution and chemical composition of NPs from photocopiers, observed effects in the upper airways in volunteers and the inflammation induced *in vitro* in human primary bronchial cell lines (unpublished data), we hypothesise that inflammation of the lower airways is also occurring, a possibility that is currently under investigation.

Finally, this study exploits real-world exposures in determining the toxicological potency of NPs. This is in sharp contrast to past human studies in these environments that lacked exposure information (Balakrishnan & Das 2010; Gadhia et al. 2005; Goud et al. 2004) or studies that focused on exposures to toners, which are quite different in size and chemistry from the emitted NPs themselves. Additionally, subjects here were used as their own control, increasing study power and minimising confounding factors such as differences in individual characteristics. In this study, we have investigated the methodological utility of inflammatory markers in NL and urine to study early effect biomarkers for engineered NPs, which may provide valuable information in identifying potentially problematic exposure scenarios and guide further mechanistic investigations and timely interventions across a diverse and fast-changing nanotechnology sector.

Conclusions

Our study provides for the first time evidence that short-term exposure to NPs from photocopiers induce upper airway inflammation and oxidative stress in healthy individuals. In the current scenario, these responses return to baseline approximately 24 h post-exposure and it is reasonable to assume that these temporal system perturbations (inflammation followed by recovery) are unlikely to represent any significant adverse effects in healthy individuals. However, toner formulations change considerably across industry, and NP exposures from other photocopiers may be more (or less) potent. Exposure levels may also be much higher. The observed biological responses raise a series of questions about potential health implications of chronic exposures to employees of these centres or exposures of individuals (children included) with respiratory disorders (e.g., asthma) or other chronic diseases. Such repeated exposures can logically lead to chronic inflammation, which may serve as the gateway to a variety of additional conditions. Of note, our preliminary data on the same biomarkers from workers chronically exposed to these photocopy environments suggest that these workers are in a status of chronic upper airway inflammation. Further in-depth clinical, toxicological and epidemiological investigations on such exposures to workers and consumers are clearly warranted.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Abder-Rahman HA, Nusair S. 2007. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) as a short-term predictor of regional and occupational health problems. J UOEH 29(3):247–258.

Armbruster C, Dekan G, Hovorka A. 1996. Granulomatous pneumonitis and mediastinal lymphadenopathy due to photocopier toner dust. Lancet 348(9028):690.

Baan RA. 2007. Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. Inhal Toxicol 19(Suppl 1):213–228.

Baeza-Squiban A, Bonvallot V, Boland S, Marano F. 1999. Airborne particles evoke an inflammatory response in human airway epithelium. Activation of transcription factors. Cell Biol Toxicol 15(6):375–380.

Balakrishnan M, Das A. 2010. Chromosomal aberration of workers occupationally exposed to photocopy machines in sular, south india. Int J Pharm Bio Sci 1(4):B304–B307.
Bar-Sela S, Shoenfeld Y. 2008. Photocopy machines and occupational antiphospholipid syndrome. Isr Med Assoc J 10(1):52–54.
Barthel M, Pedan V, Hahn O, Rothhardt M, Bresch H, Jann O, et al. 2011. XRF-analysis of fine and ultrafine particles emitted from laser printing devices. Environ Sci Technol 45(18):7819–7825.
Bello D, Martin J, Sun Q, Santeufemio C, Shafer M, Demokritou P. 2012. Physicochemical and morphological characterization of nanoparticles from photocopiers: implications for environmental health. Nanotoxicology (Posted online on May 2, 2012. doi:10.3109/17435390.2012.689883).
Boczkowski J, Hoet P. 2010. What's new in nanotoxicology? Implications for public health from a brief review of the 2008 literature. Nanotoxicology 4(1):1–14.
Bonner JC. 2010. Nanoparticles as a potential cause of pleural and interstitial lung disease. Proc Am Thorac Soc 7(2):138–141.
Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. 2006. Biomarkers of oxidative damage in human disease. Clin Chem 52(4):601–623.
Donaldson K, Tran L, Jimenez L, Duffin R, Newby D, Mills N, et al. 2005. Combustion-derived nanoparticles: A review of their toxicology following inhalation exposure. Particle Fibre Toxicol 2(1):10.
Driscoll KE, Carter JM, Hassenbein DG, Howard B. 1997. Cytokines and particle-induced inflammatory cell recruitment. Environ Health Perspect 105(Suppl 5):1159–1164.
Eisen EA, Costello S, Chevrier J, Picciotto S. 2011. Epidemiologic challenges for studies of occupational exposure to engineered nanoparticles; a commentary. J Occup Environ Med 53(6 Suppl):S57–S61.
Frampton MW. 2007. Does inhalation of ultrafine particles cause pulmonary vascular effects in humans? Inhal Toxicol 19 (Suppl 1):75–79.
Frampton MW, Stewart JC, Oberdorster G, Morrow PE, Chalupa D, Pietropaoli AP, et al. 2006. Inhalation of ultrafine particles alters blood leukocyte expression of adhesion molecules in humans. Environ Health Perspect 114(1):51–58.
Fujimoto C, Kido H, Sawabuchi T, Mizuno D, Hayama M, Yanagawa H, et al. 2009. Evaluation of nasal IgA secretion in normal subjects by nasal spray and aspiration. Auris Nasus Larynx 36(3):300–304.
Gadhia PK, Patel D, Solanki KB, Tamakuwala DN, Pithawala MN. 2005. A preliminary cytogenetic and hematological study of photocopying machine operators. Indian J Occup Environ Med 9(1):22–25.
Gardiner K, van Tongeren M, Harrington M. 2001. Respiratory health effects from exposure to carbon black: results of the phase 2 and 3 cross sectional studies in the European carbon black manufacturing industry. Occup Environ Med 58(8):496–503.
Ghio AJ, Smith CB, Madden MC. 2012. Diesel exhaust particles and airway inflammation. Curr Opin Pulm Med 18(2):144–150.
Goud KI, Hasan Q, Balakrishna N, Rao KP, Ahuja YR. 2004. Genotoxicity evaluation of individuals working with photocopying machines. Mutat Res 563(2):151–158.
Guo B, Zebda R, Drake SJ, Sayes CM. 2009. Synergistic effect of co-exposure to carbon black and Fe2O3 nanoparticles on oxidative stress in cultured lung epithelial cells. Part Fibre Toxicol 6:4.
Gwinn MR, Vallyathan V. 2006. Nanoparticles: health effects–pros and cons. Environ Health Perspect 114(12):1818–1825.
He C, Morawska L, Taplin L. 2007. Particle emission characteristics of office printers. Environ Sci Technol 41(17):6039–6045.
Howarth PH, Persson CG, Meltzer EO, Jacobson MR, Durham SR, Silkoff PE. 2005. Objective monitoring of nasal airway inflammation in rhinitis. J Allergy Clin Immunol 115(3 Suppl 1):S414–S441.
Hurst JR, Wilkinson TM, Perera WR, Donaldson GC, Wedzicha JA. 2005. Relationships among bacteria, upper airway, lower airway, and systemic inflammation in COPD. Chest 127(4):1219–1226.
Lanone S, Rogerieux F, Geys J, Dupont A, Maillot-Marechal E, Boczkowski J, et al. 2009. Comparative toxicity of 24 manufactured nanoparticles in human alveolar epithelial and macrophage cell lines. Part Fibre Toxicol 6:14.
Laskin SV, Laumbach RJ, Kipen HM. 2007. Inflammatory cytokines and lung toxicity. In: Descotes RV, Ha J, editors. Cytokines in human health- immunotoxicology, pathology, and therapeutic applications.
Lee C-W , Hsu D-J. 2007. Measurements of fine and ultrafine particles formation in photocopy centers in Taiwan. Atmos Environ 41:6598–6609.
Lee MW, Chen ML, Lung SC, Tsai CJ, Yin XJ, Mao IF. 2010. Exposure assessment of PM2.5 and urinary 8-OHdG for diesel exhaust emission inspector. Sci Total Environ 408(3):505–510.
Li JJ, Muralikrishnan S, Ng CT, Yung LY, Bay BH. 2010. Nanoparticle-induced pulmonary toxicity. Exp Biol Med (Maywood) 235(9):1025–1033.
Li N, Nel AE. 2011. Feasibility of biomarker studies for engineered nanoparticles: what can be learned from air pollution research. J Occup Environ Med 53(6 Suppl):S74–S79.
Li N, Xia T, Nel AE. 2008. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. Free Radic Biol Med 44(9):1689–1699.
Liu WX, Wang HF, Wang TC, Gu YQ, Yan L. 2008. Actute toxicity of nano-sized Zn oxide in ICR mice via intratracheal instillation. J Environ Occup Med 25(4):360–364.
Loft S, Fischer-Nielsen A, Jeding IB, Vistisen K, Poulsen HE. 1993. 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. J Toxicol Environ Health 40(2–3):391–404.
Manikantan P, Balachandar V, Sasikala S, Mohanadevi S, Lakshmankumar B. 2010. DNA Damage in workers occupationally exposed to photocopying machines in Coimbatore south India, using comet assay. Internet J Toxicol 7:2.
McDougall CM, Blaylock MG, Douglas JG, Brooker RJ, Helms PJ, Walsh GM. 2008. Nasal epithelial cells as surrogates for bronchial epithelial cells in airway inflammation studies. Am J Respir Cell Mol Biol 39(5):560–568.
Morawska L, He C, Johnson G, Jayaratne R, Salthammer T, Wang H, et al. 2009. An investigation into the characteristics and formation mechanisms of particles originating from the operation of laser printers. Environ Sci Technol 43(4):1015–1022.
Napierska D, Thomassen LC, Lison D, Martens JA, Hoet PH. 2010. The nanosilica hazard: another variable entity. Part Fibre Toxicol 7(1):39.
Nel A, Xia T, Madler L, Li N. 2006. Toxic potential of materials at the nanolevel. Science 311(5761):622–627.
Oberdorster G. 2001. Pulmonary effects of inhaled ultrafine particles. Int Arch Occup Environ Health 74(1):1–8.
Oberdorster G, Oberdorster E, Oberdorster J. 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823–839.
Park EJ, Yoon J, Choi K, Yi J, Park K. 2009. Induction of chronic inflammation in mice treated with titanium dioxide nanoparticles by intratracheal instillation. Toxicology 260(1–3):37–46.
Payne AN, Cheng JB. 1990. PMNs and airway inflammation/hyperreactivity. Agents Actions 29(3-4):181–183.
Pilger A, Rudiger HW. 2006. 8-Hydroxy-2′-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. Int Arch Occup Environ Health 80(1):1–15.
Pitrez PM, Brennan S, Turner S, Sly PD. 2005. Nasal wash as an alternative to bronchoalveolar lavage in detecting early pulmonary inflammation in children with cystic fibrosis. Respirology 10(2):177–182.
Prakash UBS. 2005. Bronchoscopy. In: Mason RJ, Murray J, Broaddus VC, Nadel J, editors. Textbook of respiratory medicine. Philadelphia, PA: Saunders Elsevier.
Prat J, Xaubet A, Mullol J, Plaza V, Maso M, Lleonart R, et al. 1993. Immunocytologic analysis of nasal cells obtained by nasal lavage: a comparative study with a standard method of cell identification. Allergy 48(8):587–591.
Quinn TJ, Taylor S, Wohlford-Lenane CL, Schwartz DA. 2000. IL-10 reduces grain dust-induced airway inflammation and airway hyperreactivity. J Appl Physiol 88(1):173–179.
Repka-Ramirez S, Naranch K, Park YJ, Clauw D, Baraniuk JN. 2002. Cytokines in nasal lavage fluids from acute sinusitis, allergic rhinitis, and chronic fatigue syndrome subjects. Allergy Asthma Proc 23(3):185–190.
Rushton EK, Jiang J, Leonard SS, Eberly S, Castranova V, Biswas P, et al. 2010. Concept of assessing nanoparticle hazards considering nanoparticle dosemetric and chemical/biological response metrics. J Toxicol Environ Health A 73(5):445–461.
Rybicki BA, Amend KL, Maliarik MJ, Iannuzzi MC. 2004. Photocopier exposure and risk of sarcoidosis in African-American sibs. Sarcoidosis Vasc Diffuse Lung Dis 21(1):49–55.
Sangani RG, Ghio AJ. 2011. Lung injury after cigarette smoking is particle related. Int J Chron Obstruct Pulmon Dis 6:191–198.
Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, et al. 2006. Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. Toxicol Sci 92(1):174–185.
Shigenaga MK, Ames BN. 1991. Assays for 8-hydroxy-2'-deoxyguanosine: a biomarker of in vivo oxidative DNA damage. Free Radic Biol Med 10(3-4):211–216.

Singh S, Shi T, Duffin R, Albrecht C, van Berlo D, Hohr D, et al. 2007. Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO2: role of the specific surface area and of surface methylation of the particles. Toxicol Appl Pharmacol 222(2):141–151.

Song S. 2010. Number Concentration of ambient ultrafine particles and oxidative DNA damage in schoolchildren in Korea. In: ISEE 22nd Annual Conference; 28 August-1 September 2010; Seoul, Korea.

Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, et al. 2000. Increased expression of inflammatory mediators in small-airway epithelium from tobacco smokers. Am J Physiol Lung Cell Mol Physiol 278(5):L906–L913.

Theegarten D, Boukercha S, Philippou S, Anhenn O. 2010. Submesothelial deposition of carbon nanoparticles after toner exposition: case report. Diagn Pathol 5(1):77.

Wang J, Zhou G, Chen C, Yu H, Wang T, Ma Y, et al. 2007. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett 168(2):176–185.

Wei Y, Han IK, Shao M, Hu M, Zhang OJ, Tang X. 2009. PM2.5 constituents and oxidative DNA damage in humans. Environ Sci Technol 43(13):4757–4762.

Wensing M, Schripp T, Uhde E, Salthammer T. 2008. Ultra-fine particles release from hardcopy devices: sources, real-room measurements and efficiency of filter accessories. Sci Total Environ 407(1):418–427.

Wu LL, Chiou CC, Chang PY, Wu JT. 2004. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 339(1–2):1–9.

Xia T, Li N, Nel AE. 2009. Potential health impact of nanoparticles. Annu Rev Public Health 30:137–150.

Yang W, Peters JI, Williams RO 3rd. 2008. Inhaled nanoparticles–a current review. Int J Pharm 356(1-2):239–247.

Zhu MT, Feng WY, Wang B, Wang TC, Gu YQ, Wang M, et al. 2008. Comparative study of pulmonary responses to nano- and submicron-sized ferric oxide in rats. Toxicology 247(2–3):102–111.

Supplementary material available online

Supplementary Figures S1 and S2.