

## Genotoxicity, inflammation and physico-chemical properties of fine particle samples from an incineration energy plant and urban air

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Received 9 October 2006; received in revised form 19 February 2007; accepted 7 May 2007

Available online 26 June 2007

### Abstract

Airborne particulate matter (PM) was sampled by use of an electrostatic sampler in an oven hall and a receiving hall in a waste-incineration energy plant, and from urban air in a heavy-traffic street and from background air in Copenhagen. PM was sampled for 1–2 weeks, four samples at each site. The samples were extracted and examined for mutagenicity in *Salmonella typhimurium* strains TA98, YG1041 and YG5161, for content of inorganic elements and for the presence of eight polycyclic aromatic hydrocarbons. The induction of *IL-6* and *IL-8* mRNA expression and the presence of DNA damage – tested by the comet assay – were determined after 24-h incubations with human A549 lung epithelial cells.

The PM<sub>2.5</sub> concentration was about twofold greater in the oven hall than in the receiving hall. The particle size distribution in the receiving hall was similar to that in street air (maximum mode at about 25 nm), but the distribution was completely different in the oven hall (maximum mode at about 150 nm). Also chemically, the samples from the oven hall were highly different from the other samples.

PM extracts from the receiving hall, street and background air were more mutagenic than the PM extracts from the oven hall. PM from all four sites caused similar levels of DNA damage in A549 cells; only the oven hall samples gave results that were statistically significantly different from those obtained with street-air samples.

The receiving hall and the urban air samples were similarly inflammatory (relative *IL-8* mRNA expression), whereas the oven hall did not cause a statistically significant increase in *IL-8* mRNA expression. A principal component analysis separated the oven hall and the receiving hall by the first principal component. These two sites were separated from street and background air with the second principal component. Several clusters of constituents were identified. One cluster consisted of all the polycyclic aromatic hydrocarbons (PAH), several groups of metals and one group of the biological endpoints (DNA damage, *IL-6* and *IL-8* mRNA expression). The PAH and the inorganic content of the air in the receiving hall may be due to vehicle emissions and suspended waste particles. The inorganic content in the street and background air may have been influenced by break wear, road emissions and long-range transport. The results from a partial least-square regression analysis predicted that both PAHs and a group of metals including

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Fe and Mn contributed to *IL-6* and *IL-8* induction. Only Mn and Sr were predicted to influence DNA damage statistically significantly. Among the PAHs only chrysene had influence on DNA damage.

The PM from the oven hall was markedly different from the PM at other locations in particle size distribution, chemical composition and the resulting biological effects when A549 cells were incubated with the PM. These characteristics and observations in the oven hall indicated that the PM source was oven exhaust, which was well combusted.

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**Keywords:** Urban air; Incineration energy plant; Genotoxicity; Inflammation; Chemical composition; Particle size distribution

## 1. Introduction

Urban particulate air pollution has a major impact on the quality of life and human health [1,2]. Especially, there is concern over suspended fine and ultrafine particulate matter in urban areas, to which traffic contributes as a major source. Even though the problem has been intensively studied in the last decade, scientists are still struggling to understand the mechanisms behind the adverse health effects of modern urban particulate air pollution. However, it is not only in urban areas that people are exposed to particulate air pollution. In some occupational environments, the exposure levels are much higher than in urban air and the composition will be very different.

Incineration plants in industrialised countries are of great importance since waste is being generated in ever-increasing amounts and new waste-recycling facilities are being built. In Denmark, a total of 13.3 millions tonnes of waste was produced in 2004, of which 65% was recycled in 32 incineration recycling plants. In the period 1994–2004 the total amount of waste increased by 20% [3]. Exposures to bio-aerosols, dust concentrations and volatile and odorous compounds have been investigated [4–6]. Bacterial mutagenicity of samples from municipal and hospital-waste combustion emissions [7] and from the emission gas inside a municipal waste incinerator [8] has been investigated. Pani et al. [9] investigated the mutagenic activity of airborne particulates (total particulate matter and the respirable fraction) collected inside the working area of a municipal incinerator. However, not much attention has been focused on particle-size distributions and the *in vitro* toxicity of airborne fine particulate matter at incineration plant facilities.

The toxicity of urban particulate matter has been investigated in many *in vitro* studies (e.g. [10–14]). The aim of this study was to gain knowledge about fine particle concentrations, particle-size distributions, chemistry and *in vitro* toxicity at two sites in a waste-incineration recycling energy plant and compare the results with those obtained with samples collected in urban street and background air.

## 2. Materials and methods

### 2.1. Sampling sites

Samples were collected at four sites, which are described in Table 1. Four samples were collected from each site.

### 2.2. Particle collection and sample preparation

Particles were collected with a newly developed sampler that collects fine particulate matter [15]. Briefly, the sampler is based on a commercial electrostatic office-air cleaner with an air velocity of  $175 \text{ m}^3 \text{ h}^{-1}$ . The collection efficiency window varies from 25 to 70% between 30 nm and  $2.5 \text{ }\mu\text{m}$ , with a maximum (60–70%) between 0.2 and  $0.8 \text{ }\mu\text{m}$ . Particle recovery tests showed that the recovery rate in the aqueous solution was >98%, varying between 98.2 and 99.8 for the four different sites. For dry particulate matter, testing of NIST SRM 2975 showed that the recovery rate was >80%; details of the particle-extraction method are given in Ref. [15].

### 2.3. $\text{PM}_{2.5}$ measurements and particle size distribution

At the two sites in the incineration plant we determined  $\text{PM}_{2.5}$  concentrations during 7–10 days, with a sampling time of 2–3 days, using a Triplex cyclone (BGI, MA, USA), 37-mm Teflon filters (Millipore, Denmark, cat. 0\*: FALP03700) and a pump (Aircon 2, Gilian, IL, USA) with an airflow of 1.5 l/min.  $\text{PM}_{2.5}$  levels were not determined in street air and background air.

The particle-size distributions at the incineration plant were unknown and, therefore, we determined the distributions at both sites in the size range  $0.0098\text{--}10 \text{ }\mu\text{m}$  with a SMPS (Model 5.400, Grimm, Germany) and an Aerosol Particle Sizer (APS-3321; TSI Inc., USA) during 3–4 days during the same period in which particle samples were collected. The distributions were compared with distributions from the urban street site, which were determined with the same instruments during 15 days in October–November 2004.

### 2.4. Particle chemistry

#### 2.4.1. Metal analysis

Elements were determined with ICP-MS (PE Elan 6100DRC ICP-MS with PE auto-sampler AS-91, Perkin-Elmer, Hvidovre, Denmark) at the Geological Survey of

Table 1  
Description of the sampling sites

Site	Sampling time and sampling period	Description
Urban street air (Street air <sup>a</sup> )	10–13 days, 19/04-02/06, 2005	Jagtvej is a street in a street canyon in Copenhagen. High-traffic street with 26,000 vehicles passing daily (National Environmental Research Institute's Monitoring Station)
Urban background air (Background air <sup>a</sup> )	13-16 days, 19/4-15/06, 2005	Roof of the National Research Centre for the Working Environment. Representative of urban background air in Copenhagen. On the roof a three-storey building
Oven hall (Oven hall <sup>a</sup> )	7–8 days, 15/03-13/04, 2005	Incineration plant: indoors in a very large hall (63,000 m <sup>3</sup> ) with an oven (combustion chamber, capacity > 20 tonnes/h) and recirculation channels. Sampling was done at quota 27.5 near the recirculation pre-heater
Receiving hall (Receiving hall <sup>a</sup> )	7–8 days, 15/03-13/04, 2005	Incineration plant: a very large hall (64,000 m <sup>3</sup> ) where the waste is delivered. About 700 vehicles passing every weekday and about 150 in weekends. The sampler and particle instruments were placed at ground level close to where the vehicles stop to unload the waste

<sup>a</sup> Abbreviation for the site.

Denmark and Greenland, Copenhagen-Denmark. About 5 mg of sample was dissolved in 5 ml of a mixture of 2/3 concentrated HCl and 1/3 concentrated HNO<sub>3</sub> and evaporated at about 100 °C to dryness. The residue was mixed with 50 ml 0.7% HNO<sub>3</sub> and 5 ml was used for injection by the auto-sampler. All chemicals were of Suprapur grade from Merck. A multi-element standard containing about 100 ng/ml of each element was used for calibration. The following elements were detected by quantitative analysis: Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sr, Te, TL, U, V and Zn. The following elements were detected by semi-quantitative analysis: Au, Ce, Cs, Dy, Er, Eu, Gd, Ge, Hf, Hg, Ho, In, Ir, La, Lu, Nb, Mod, Os, P, Pd, Pr, Pt, Re, Rh, Ru, Sb, Sc, Si, Sm, Sn, Ta, Tb, Th, Ti, Tm, W, Y, Yb and Zr.

## 2.5. PAH analysis

The particle-bound PAHs (benzo[*a*]anthracene, chrysene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*g,h,i*]perylene, benzo[*k*]fluoranthene, dibenzo[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene) were determined by means of HPLC with fluorescence detection. In stored samples these PAHs are the most robust to quantify [16]. The samples were collected during long sampling times (7–16 days) and the samples were concentrated under evaporation. Low molecular weight PAHs with two to four rings would be expected to evaporate during sampling and extraction. The samples were extracted by automated pressurised liquid extraction. About 1–3 mg sample was placed in a 5-ml extraction cell with Ottawa sand and a top-filter. The procedure started with a 7-min thermal equilibration during which the cells were heated to 150 °C, filled with acetonitrile and pressurised to 2000 psi. The extraction was continued under static conditions for 10 min. After the static extraction the samples were flushed with ca. 2.8 ml fresh acetonitrile. Finally, the cells were purged with nitrogen gas for 60 s at 150 psi. After extraction the samples were stored

in vials at –20 °C until they were processed further. The samples were concentrated from ~6.5 ml to ~150–260 µl with nitrogen at room temperature. The samples (10 µl) were analysed by HPLC on a Waters<sup>®</sup> Alliance system (Waters Limited, Ontario, Canada) with UV ( $\lambda = 254$  nm) and by fluorescence detection ( $\lambda_{\text{ex}} = 224$  nm;  $\lambda_{\text{em}} = 330$  nm). The column was a Waters<sup>®</sup> reversed phase C18 PAH column (250 mm × 4.6 mm, 5-µm particles). The mobile phase was a linear gradient of 50–100% acetonitrile in water during 0–5 min, and 100% acetonitrile from 5 to 33 min. The PAHs of interest eluted before 24 min. Calibration curves for the eight individual PAHs were determined with a 16-PAH mix solution (10 µg/ml; 47940-U, Supelco, Sigma–Aldrich, Ontario, Canada); a blank sample (HPLC grade acetonitrile) was included as a negative control.

## 2.6. Incubations with A549 cells

Along with the field samples, four samples of cell medium (0.25 mg NaCl/ml) were also tested. These test samples were used as controls for the extraction and lyophilisation process, and processed identical to the field samples. All particle samples, including the NaCl controls were sterilised by  $\gamma$ -irradiation at 35 kGy to avoid activity from bacteria and spores. The samples were suspended in culture medium at 0.5 mg/ml and sonicated (with a “disruptor horn”, Branson digital sonifier, Branson Ultrasonics Corporation, CT, USA) with 10-s pulses between 10-s pauses during 4 min. Pilot experiments to assess DNA damage and interleukin responses with different concentrations of sample from all four sites revealed that the concentration 0.25 mg/ml resulted in responses that were proportional to the amount of test material added. The DNA damage was also below the response of the positive control (30 µM H<sub>2</sub>O<sub>2</sub>) for all measurements. Therefore, only one concentration (0.25 mg/ml) was prepared for the bioassays along with the control (0 mg/ml). Half a million A549 human lung epithelial cells (American type tissue collection, Rockville,

MD, USA) were seeded in 24-well plates (Nunc 146485). After reaching about 80–100% confluence, they were treated with particle samples in 1 ml culture medium (89% Ham's F12, 1% penicillin–streptomycin and 10% FBS, all from Invitrogen, Denmark) at 37 °C for 24 h. A 549 cells incubated without or with H<sub>2</sub>O<sub>2</sub> (30 µM) for 30 min served as controls in the comet assay.

For the LDH determination, 1 ml of the supernatant from each well (three wells per sample) was transferred to an Eppendorf tube. The tubes was stored at 4 °C and analysed the next day. Samples for *IL-6* and *IL-8* mRNA were collected in triplicate. The wells were washed twice with 1 ml PBS, and 300 µl of RLT buffer (Quiagen, Denmark) were added to each well. The solution was agitated until the cells were lysed, the cells from two wells (one sample) were pooled, transferred to Eppendorf tubes and stored at –80 °C. The toxicity on the basis of LDH release was determined with controls with medium from cell-free incubations, incubations with the NaCl control, and from replicate incubations in which the cells were lysed with 1% Triton-X.

For the comet assay the cells were harvested by washing the wells twice with 1 ml PBS. Freezing medium (750 µl culture medium containing 10% DMSO) was added to each well and the cells were removed by scraping with a rubber policeman and pipetting three times. One hundred µl was transferred to Eppendorf tubes, which were stored at –80 °C for up to three weeks.

### 2.7. DNA damage and cytokine mRNA

DNA damage was analysed with the comet assay as described [17], except that the cell-agarose mixtures were cast onto a 100 mm × 85 mm GelBond film (Cambrex Bio Science, Rockland, ME, USA) with a polyethylene moulding form (100 mm × 75 mm × 10 mm) with eight holes ( $d = 19.5$  mm). Each data point (four samples from each site) was the average of four different analyses resulting in four independent data from each site.

RNA was purified directly from the RLT extract using the vacuum protocol for NucleoSpin 96 RNA (Macherey-Nagel, Düren, Germany) according to manufacturer's protocol, except that RNA was eluted with 2 µl × 50 µl RNase-free H<sub>2</sub>O. The concentration of RNA was determined spectrophotometrically. Because the recovery was low, the mRNA was pooled from four replicate incubations for each determination. The samples were stored at –80 °C. cDNA was prepared from the samples using TaqMan reverse-transcription reagents (Applied Biosystems, Nærum, Denmark) as described by the manufacturer. A negative control sample (without reverse transcriptase) was included in all cDNA syntheses.

*IL-6* and *IL-8* gene expression was determined using real-time RT-PCR with human β-actin as a reference. Pre-developed TaqMan assay reagents (*IL-6*, Hs00174131.m1; *IL-8*, Hs99999034.m1; β-actin, 4310881E) and Universal PCR Master Mix (4326708) were used for both determinations. All reagents were purchased from Applied Biosystems (Nærum,

Denmark). The primer/probe was validated and shown to be quantitative over a range of 128-fold for *IL-6* and 64-fold for *IL-8*. Messenger RNA measurements were excluded if the reporter value fell outside the range in which the PCR was found to be quantitative.

Fifteen µl of each sample was run in triplicate on an ABI-PRISM 7700 sequence detector (PE Biosystems, Foster City, CA, USA) under standard thermal cycler conditions. Target and β-actin levels were quantified in separate wells. The target genes were normalized to β-actin by subtracting the cycle threshold-value of β-actin RNA (Ct reference value) from the Ct value of the gene of interest (Ct-target), i.e. is  $\Delta Ct = Ct\text{-target} - Ct\text{-ref}$ . The relative expression of the target gene was calculated by the comparative method  $2^{-\Delta Ct}$  [18]. The standard deviation on triplicates was always below 1. The standard deviation on repeated measurements of the same control sample in separate experiments was always below 20%, indicating the maximum day-to day variation of the assay. A negative control in which RNA was not converted to cDNA (without reverse transcriptase) and a “no-template” control were included in each real-time PCR.

### 2.8. Cytotoxicity

Release of lactate dehydrogenase (LDH) was determined as a measure of cytotoxicity. The concentration of LDH was quantified with the Cytotoxicity Detection Kit (LDH) (Roche A/S, Hvidovre, Denmark) as recommended by the manufacturer.

### 2.9. Salmonella mutagenicity assay

#### 2.9.1. Sample extraction and fractionation

All chemicals used for the extraction and fractionation procedures were of analytical grade. The particle samples were extracted by pressurised liquid extraction (ASE 200, Dionex, CA, USA). For all four sites the four independent samples were pooled to obtain enough sample material for the assay. Sample amounts between 200 and 365 mg were used for extraction. The sample was mixed with 5 g of anhydrous sodium sulphate in an 11-ml extraction cell. To produce the crude extracts, hexane:acetone (1:1, v/v) was used as the extraction solvent. Extraction conditions were as follows: 150 °C and 14 MPa and 7 min heating time followed by 3 min × 5 min static extraction. About 80% (w/w) of the crude extracts were transferred to new vials to be used for fractionation. The rest of the crude extracts were also transferred to new vials, 1 ml of DMSO was added and the extracts were evaporated under a gentle stream of nitrogen at 30 °C (Tubovap II, Zymark, MA, USA).

Sample extracts were fractionated by using 15-mm inner diameter columns packed with 5 g of silica gel (deactivated with 10% (w/w) water in methanol). The samples were applied and the columns were eluted with 5 ml of hexane followed by 15 ml of hexane:dichloromethane (3:1, v/v) and 30 ml of methanol. The three fractions were collected in separate vials. The first fraction contained non-polar *n*-alkanes. The second fraction contained moderately polar PAHs, alkyl-PAHs, *O*- and

S-heterocyclics. The third fraction was the polar fraction and contained N-heterocyclics, nitro-, amino- and oxy-PAHs [19]. All three fractions were evaporated like the crude extracts.

A test was also conducted to determine the amount of extractable organic matter in the crude fraction. About 25–60 mg of the sample from the four sites were extracted as described above. The crude extracts were placed in pre-weighed vials and dried under a gentle stream of nitrogen at 30 °C. The vials were weighed and the extractable organic matter was determined. A test was conducted to determine the accuracy of the balance on which the same vial was weighed ten times. The variation was in the order of 0.1%.

### 2.9.2. Microsuspension assay

Mutagenicity testing was done using *Salmonella* frame-shift tester strains with S9 (from Moltox Inc., NC, USA) and without metabolic activation.

The mutagenicity was tested with the microsuspension method as described by Kado et al. [20,21] with minor modifications. As a reference, NIST SRM 1649a (NIST, USA) was also fractionated and tested. The fractionated samples were tested at five concentrations between 0.1 and 1.5 mg/plate in addition to the negative control (0 mg/plate). Due to limitations in the amount of sample, the crude fractions were tested at four concentrations between 0.15 and 0.9 mg/plate. 2-Aminoanthracene (2AA) and 2-nitrofluorene (2NF) were used as positive controls in the following concentrations: TA98 + S9: 0.125 µg 2AA/plate; TA98 – S9: 0.335 µg 2NF/plate; YG5161 + S9: 0.125 µg 2AA/plate; YG5161 – S9: 0.335 µg 2NF/plate; YG1041 + S9: 0.0625 µg 2AA/plate; YG1041 – S9: 0.0835 µg 2NF/plate. The strains used in the assay were (1) TA98, which is a standard strain for detection of frame-shift mutations [22], (2) YG1041, derived from TA98, but more sensitive to nitro-arenes and aromatic amines [23,24] and (3) YG5161, derived from TA98, but more sensitive to PAHs, e.g., benzo[*a*]pyrene [25,26].

### 2.10. Statistics

The comet assay data, relative *IL-8*, *IL-6* mRNA expression and LDH data were analysed by one-way ANOVA with Tukey's Studentized range test for pair-wise comparisons. To fulfil the criteria for variance homogeneity, *IL-8* and *IL-6* mRNA expression were logarithmically transformed. The results of the microsuspension assay were expressed as induced revertants per mg of sample, calculated by linear regression analysis of the linear part of the dose–response curve. Apart from the linear dose–response relationship, the criterion for a positive response was that at least two concentrations gave at least a two-fold response above the control value [22]. To compare slopes between sites, analysis of covariance was used with Bonferroni correction where the significance level was  $p < 0.01$ . Concentrations of inorganic elements and PAH data from the four sites were compared by one-way ANOVA with Tukey's Studentized range test for pair-wise comparisons with logarithmically transformed data. A multivariate analysis was

performed consisting of principal component analysis (PCA) and partial least-square regression (PLS). PCA and PLS analyses were done on centred data with full cross-validation using the software Unscrambler version 7.6 (Camo A/S, Oslo, Norway). PCA was used to describe the variance of the data set and included the inorganic elements, PAHs, comet assay data, LDH data, *IL-6* and *IL-8* data, which gave 822 observations. PLS was used for prediction of variables on biological endpoints and included the 16 samples and the variables inorganic elements and the PAHs. All 742 observations were included in the prediction of comet assay results, *IL-8* and *IL-6* results. The significant regression coefficients were calculated by the uncertainty test option in the software [27]. Metal data, PAH data, comet data, *IL-6* and *IL-8* mRNA data were included in the analysis. Results of the *Salmonella* mutagenicity assay were not included because the four samples from each site were pooled.

## 3. Results

### 3.1. Particle data and size distributions

Particle recovery data for the PM samples and the temperature and relative humidity (RH) during sampling are given in Table 2. The largest amounts of particles were collected in the oven hall. The oven hall had higher levels of PM<sub>2.5</sub> ( $110 \pm 17 \mu\text{g}/\text{m}^3$ ,  $n=4$ ) compared with the receiving hall ( $59 \pm 17 \mu\text{g}/\text{m}^3$ ,  $n=4$ ). In the oven hall, the temperature and RH% were relatively constant during the sampling period, whereas in the receiving hall the temperature and RH% reflected outdoor conditions.

Particle-size distribution (Fig. 1) in the receiving hall was similar to that in street air, with the main mode about 25 nm in the mobility-diameter range and 0.6–0.8 µm in the aerodynamic diameter range. However, the concentrations were higher in the receiving hall. The size distribution in the oven hall was completely different from that in the receiving hall and in street air, with the main mode at about 0.15 µm in the mobility-diameter range.

### 3.2. Particle chemistry

#### 3.2.1. Inorganic elements

The elemental composition of the samples is given in Table 3. The reason for the high silver content was that during sampling with the ESP corona ions most likely oxidise the silver coating on the collection plates, some of which will be retrieved in the samples [15].

Generally, the oven hall samples differed from those taken at the other three sites: the oven hall had higher concentrations of K, Pb, Cd, Rb, Cs and lower concen-

Table 2  
Net recovery amounts of particle samples and meteorological data

	Street air	Background air	Oven hall	Receiving hall
Sample 1				
Recovered mass (mg)	136.3	111.3 <sup>a</sup>	502.0	109.8
Temperature ± S.D. (°C)	8.4 ± 3.0	10.9 ± 4.4	26.1 ± 1.5	1.5 ± 2.3
RH ± S.D. (%)	57.7 ± 14.5	61.0 ± 15.3	21.0 ± 8.3	57.2 ± 15.8
Sample 2				
Recovered mass (mg)	130.3	88.1 <sup>a</sup>	860.2	178.6
Temperature ± S.D. (°C)	11.7 ± 3.8	13.9 ± 4.8	27.6 ± 1.3	6.1 ± 2.1
RH ± S.D. (%)	62.1 ± 14.1	54.4 ± 16.4	22.6 ± 4.4	74.3 ± 15.3
Sample 3				
Recovered mass (mg)	59.4	72 <sup>a</sup>	660.4	306.0
Temperature ± S.D. (°C)	13.1 ± 3.0	18.8 ± 5.3	27.4 ± 4.3	7.9 ± 4.5
RH ± S.D. (%)	53.0 ± 12.3	57.0 ± 16.0	19.7 ± 3.5	55.6 ± 14.7
Sample 4				
Recovered mass (mg)	150.5	75.1 <sup>a</sup>	559.2	139.4
Temperature ± S.D. (°C)	16.1 ± 4.0	16.6 ± 4.2	28.2 ± 1.5	9.7 ± 3.2
RH ± S.D. (%)	62.0 ± 17.6	62.0 ± 17.0	20.7 ± 4.0	64.0 ± 11.4

<sup>a</sup> At this site two samplers were used for collection and the mass is the sum of recovered particles from both samplers. Urban street air, sampling periods; sample 1: 19/04-29/04; sample 2: 29/04-10/05; sample 3: 11/05-20/05; sample 4: 20/05-02/06. Urban background air, sampling periods; sample 1: 19/04-04/05; sample 2: 04/05-20/05; sample 3: 20/05-02/06; sample 4: 02/06-15/06. Incineration plant oven hall and receiving hall, sampling periods; sample 1: 15/03-22/03; sample 2: 22/03-29/03; sample 3: 29/03-06/04; sample 4: 06/04-13/04.

trations of Ni, Mg, and V. The concentration of Ni in oven hall samples was only 0.5–3% that in samples from the other sites.

The receiving hall had higher concentrations of Ca, Sr and Y compared with the other sites and lower concentrations of Cu than street and background air. The street air and background air had relatively similar concentrations except that in background air there were statistically significantly lower concentrations of Sn, Bi, Mo, Nb and Zr.

### 3.2.2. PAH analysis

Table 4 shows the results of the individual PAHs and the sum of the concentrations of the eight compounds.

When expressed as the sum of the PAH ( $\Sigma$ PAH) at each site, the receiving hall samples contained significantly higher PAH amounts ( $p < 0.02$ ) compared with the oven hall samples. The receiving hall sample contained more of most of the individual PAHs than the oven hall sample. Of the PAHs, benzo[a]pyrene was most abundant at all four sites.

### 3.3. Inflammatory cytokines and cytotoxicity

None of the samples were cytotoxic at the concentrations used for incubations with A549 cells, as determined by the LDH release (controls:  $13.4 \pm 1.7\%$ , oven hall:  $10.6 \pm 4.1\%$ , receiving hall:  $9.5 \pm 10.4\%$ , street air:

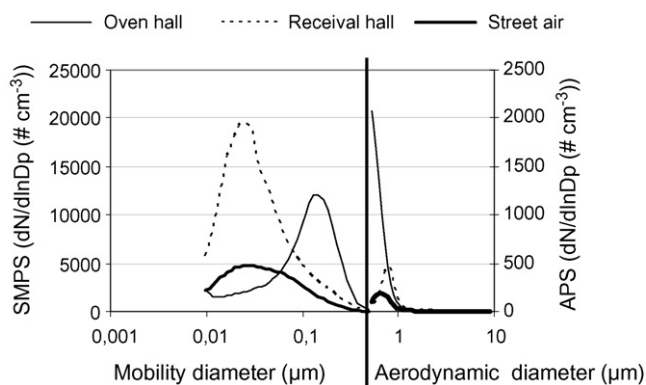


Fig. 1. Particle-size distributions determined by SMPS and APS in the oven hall, receiving hall and street air.

Table 3  
Average concentrations (ppm) of inorganic elements in particle samples

Element <sup>a</sup>	Oven hall	Receiving Hall	Street air	Background air
<b>Major</b>				
Ca	12,773 ± 5,960	<b>42,640 ± 23,133</b>	13,376 ± 5,701	8,635 ± 988
Na	10,193 ± 2,005	22,283 ± 14,647	22,826 ± 8,478	36,386 ± 17,306
Fe	3,446 ± 1,729	23,812 ± 32,064	18,505 ± 4,915	8,692 ± 3,339
Ni	<b>99 ± 31</b>	3,146 ± 2,960	8,648 ± 8,234	19,879 ± 12,491
Al	4,018 ± 2683	7,963 ± 3,081	5,946 ± 2,974	15,295 ± 11,555
Ag	4,655 ± 2,081	11,463 ± 2,815	11,202 ± 2,889	13,528 ± 4,757
<b>Minor and trace</b>				
K	<b>9,598 ± 1,385</b>	5,482 ± 1,113	4,296 ± 533	5,629 ± 298
Zn	5,199 ± 1,423	2,406 ± 366	2,882 ± 989	4,542 ± 1,750
Mg	<b>1,071 ± 577</b>	3,735 ± 1,267	2,829 ± 521	4,236 ± 1,857
Cu	427 ± 230	533 ± 73	1,687 ± 174	1,568 ± 693
Pb	<b>1437 ± 494</b>	473 ± 174	320 ± 196	372 ± 93
Ba	256 ± 142	918 ± 373	703 ± 250	540 ± 137
Si	436 ± 85	346 ± 35	529 ± 198	650 ± 248
Cr	74 ± 71	116 ± 37	271 ± 342	645 ± 736
P	628 ± 503	324 ± 107	271 ± 120	247 ± 120
Mn	136 ± 80	438 ± 386	295 ± 60	198 ± 44
V	<b>31 ± 22</b>	142 ± 50	316 ± 47	428 ± 189
Ti	371 ± 235	240 ± 136	144 ± 51	137 ± 62
Sn	233 ± 136	36 ± 10	126 ± 35	46 ± 12
Sr	41 ± 19	<b>206 ± 120</b>	71 ± 22	60 ± 14
Sb	151 ± 106	23 ± 5	123 ± 32	40 ± 10
Mo	15.7 ± 8.3	24.3 ± 7.4	91.6 ± 26.3	41.5 ± 15.7
Cd	<b>53.5 ± 13.1</b>	7.7 ± 3.8	7.1 ± 5.8	3.6 ± 1.9
As	52.3 ± 28.6	41.7 ± 11.7	27.5 ± 5.4	38 ± 8.9
Se	22.7 ± 17.6	6.5 ± 6.1	32.9 ± 12	36.8 ± 16.2
Zr	9.3 ± 3.3	16.9 ± 4.2	<b>42 ± 11.5</b>	23.3 ± 4.6
Rb	<b>32.7 ± 6.5</b>	13.7 ± 1.7	9.6 ± 3.4	11.2 ± 2.1
B	23.4 ± 9.3	17.8 ± 6.6	18.5 ± 9.7	29.9 ± 11.1
Co	3.3 ± 2.1	9.3 ± 3.9	13 ± 5.8	20.8 ± 4.4
Y	1.5 ± 1.2	<b>14 ± 5.5</b>	1.4 ± 0.8	0.6 ± 0.2
Bi	7.7 ± 3.9	2.4 ± 0.8	13.1 ± 3.7	3.0 ± 1.7
Ce	5.3 ± 3.9	7.9 ± 4.9	8.5 ± 3.5	4.5 ± 1.0
La	2.8 ± 1.9	5.3 ± 3.1	4.1 ± 1.2	3.1 ± 0.8
W	0.4 ± 0.6	1.1 ± 0.3	2.6 ± 3.5	2.7 ± 2.5
Cs	<b>2.4 ± 0.7</b>	0.7 ± 0.1	0.5 ± 0.2	0.7 ± 0.4
Nb	0.4 ± 0.2	1.2 ± 0.7	1.2 ± 0.5	0.4 ± 0.1

<sup>a</sup> Data are given as the mean ± S.D. ( $n=4$ ). Concentrations in bold indicate statistically significant differences compared to the three other sites. Only those elements were included where concentrations were above the detection limit.

12.9 ± 6.7%, background air: 2.7 ± 5.7%). No correlation was detected between LDH and DNA damage, *IL-6* or *IL-8* mRNA expression.

In Fig. 2, the relative mRNA expression of *IL-8* is illustrated. The PM from the receiving hall, the street air and the background air, but not from the oven hall increased the relative *IL-8* mRNA expression ( $p < 0.001$ ). These three sites were also statistically significantly different from PM from the oven hall (receiving hall,  $p < 0.001$ ; street air,  $p < 0.001$ ; background air,  $p < 0.05$ ).

The relative *IL-6* mRNA expression levels in the control incubations were below the detection limit. However, the mRNA expression in samples from the four

sites could be quantified: a comparison showed that *IL-6* mRNA expression agreed well with the *IL-8* expression, with the samples from receiving hall and street air being significantly more inflammogenic than the background air ( $p < 0.05$ ) and the oven hall ( $p < 0.001$ ). However, PM from the background air site was not statistically different from that in the oven hall.

### 3.4. Genotoxicity

#### 3.4.1. DNA damage (comet assay)

The PM from all four sites induced DNA damage as determined by the increased percentage of DNA-in-

Table 4  
PAH concentrations of particle samples in ng/mg sample

	Oven hall (ng/mg)	Receiving hall (ng/mg)	Street air (ng/mg)	Background air (ng/mg)
BaA	0.14 ± 0.21	10.1 ± 6.5	3.2 ± 0.58	2.5 ± 4.0
Chry	0.14 ± 0.28	5.6 ± 4.6	1.8 ± 1.9	5.0 ± 8.4
BaF	1.3 ± 0.9	15.7 ± 8.7	5.1 ± 1.5	2.4 ± 1.8
BkF	0.4 ± 0.3	9.7 ± 5.8	3.1 ± 0.77	2.8 ± 3.6
BaP	2.9 ± 2.0	47 ± 39	10.9 ± 2.1	14.3 ± 21
Di( <i>ah</i> )A	1.4 ± 1.5	11.4 ± 9.7	4.5 ± 1.5	4.5 ± 5.8
B( <i>ghi</i> )P	1.6 ± 1.6	14.8 ± 8.9	8.0 ± 3.9	4.9 ± 4.1
I(123)P	0.78 ± 0.82	9.8 ± 7.0	3.2 ± 0.50	2.5 ± 1.9
ΣPAH	8.7 ± 3.2	124 ± 44	40 ± 5.4	39 ± 25

Data are give as the mean ± S.D.,  $n = 4$ . BaA: benzo[*a*]anhracen; BaP: benzo[*a*]pyrene; BbF: benzo[*b*]fluoranthene; B(*ghi*)Pe: benzo[*ghi*]perylene; BkF: benzo[*k*]fluoranthene; Chry: chrysene; Di(*ah*)A: dibenzo[*a,h*]anthracene; I(123)P: indeno[1,2,3-*cd*]pyrene.

tail in the comet assay ( $p < 0.001$ ; Fig. 3). Of the four sites, the street air samples were statistically significantly more DNA-damaging than oven hall sample ( $p < 0.05$ ), but there was no difference between the street air, receiving hall and background-air samples. The NaCl control did not induce any response compared with the control or the negative control ( $0 \mu\text{M H}_2\text{O}_2$ ).

### 3.4.2. Mutagenicity

The microsuspension assay was used to detect the mutagenic activity of the samples (Table 5). Due to lack of material, the PM samples from each site were pooled for this analysis.

There was no mutagenic activity in the NaCl control sample or in the non-polar fraction from any of the four sites in any of the three *Salmonella* strains.

### 3.4.3. Fractions

Of the moderately polar fractions, all but the oven hall sample were mutagenic (Table 5), but only with

S9. For all four sites, the moderately polar fraction was almost always less mutagenic than the polar fraction. The crude fraction of the sample from the receiving hall was more mutagenic than the moderately polar fraction and polar fraction with the strains TA98 and YG1041. The oven hall sample was not mutagenic in strain TA98 (–S9), and it was less mutagenic than street air and receiving hall samples in YG1041 (–S9) ( $p < 0.001$ ).

In the polar fraction and the crude fraction, higher activity was observed without metabolic activation (–S9), suggesting a direct mutagenic effect. In the moderately polar fraction the mutagenic response was only observed with metabolic activation (+S9). Generally, the sum of mutagenic activity of the moderately polar and polar fraction roughly equalled that in the crude fraction. The mutagenicity of the crude fraction of the receiving hall was stronger than the sum of the moderately polar and polar fractions, except with strain YG5161.

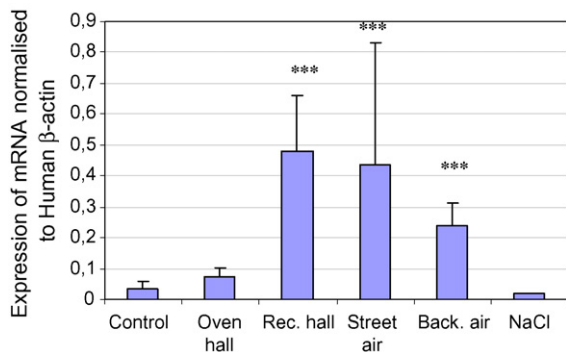


Fig. 2. *IL-8* mRNA levels in A549 cells incubated with  $25 \mu\text{g/ml}$  of air-pollution materials sampled at different sites. The data are given as the mean ± S.D.,  $n = 4$ . To fulfil the criteria for variance homogeneity data were log-transformed. \*\*\*Significant difference compared with the control,  $p < 0.001$ .

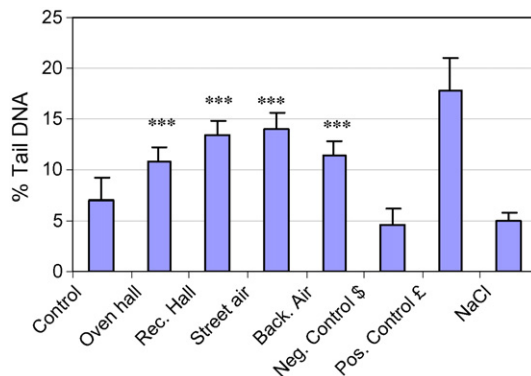


Fig. 3. DNA damage in A549 cells incubated with  $25 \mu\text{g/ml}$  of air-pollution materials sampled at different sites. The data are given as % tail DNA, mean ± S.D.,  $n = 4$ . \*\*\*Significant difference compared with the control,  $p < 0.001$ ; §  $0 \mu\text{M H}_2\text{O}_2$ ; £  $30 \mu\text{M H}_2\text{O}_2$ .

Table 5  
Mutagenicity of air pollution samples

Sample	TA98		YG5161		YG1041	
	–S9	+S9	–S9	+S9	–S9	+S9
Positive controls	925 ± 260	295 ± 66	1151 ± 282	404 ± 75	698 ± 315	275 ± 70
Rev/plate						
Oven hall						
Crude	0 <sup>a</sup>	117 ± 10	69 ± 7	ND	188 ± 21	0 <sup>a</sup>
Polar fraction	94 ± 6	101 ± 7	87 ± 5	52 ± 4	233 ± 18	178 ± 30
Mod. polar fraction	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
Receiving hall						
Crude	406 ± 9	230 ± 13	78 ± 8	95 ± 8	595 ± 36	307 ± 14
Polar fraction	124 ± 5	67 ± 4	114 ± 6	72 ± 3	333 ± 17	177 ± 13
Mod. polar fraction	0 <sup>a</sup>	53 ± 17	0 <sup>a</sup>	61 ± 5	0 <sup>a</sup>	73 ± 5
Street air						
Crude	344 ± 12	154 ± 11	82 ± 6	0 <sup>a</sup>	435 ± 12	194 ± 12
Polar fraction	306 ± 15	128 ± 7	202 ± 11	64 ± 6	449 ± 22	232 ± 21
Mod. polar fraction	0 <sup>a</sup>	87 ± 10	0 <sup>a</sup>	87 ± 10	0 <sup>a</sup>	62 ± 8
Background air						
Crude	223 ± 20	0 <sup>a</sup>	161 ± 32	0 <sup>a</sup>	217 ± 19	133 ± 20
Polar fraction	157 ± 9	89 ± 5	105 ± 8	0 <sup>a</sup>	338 ± 12	115 ± 9
Mod. polar fraction	0 <sup>a</sup>	53 ± 7	0 <sup>a</sup>	73 ± 6	0 <sup>a</sup>	68 ± 9
NIST SRM 1649a						
Crude	383 ± 21	497 ± 45	226 ± 13	186 ± 9	1194 ± 48	370 ± 24
Polar fraction	322 ± 21	354 ± 21	246 ± 15	197 ± 9	821 ± 27	468 ± 32
Mod. polar fraction	ND	2.9 ± 0.3	ND	10.8 ± 1.4	ND	4.9 ± 0.9

The values are the slopes of the dose–response curves, expressed as revertants per mg sample ± S.E. ND: not determined.

<sup>a</sup> Below the detection limit. See Section 2.

#### 3.4.4. Tester strains

Of the *Salmonella* strains used, YG1041 gave higher responses than YG5161 and TA98 suggesting the importance of nitroarenes and aromatic amines for mutagenic activity. Generally YG5161 did not produce more mutants than TA98, suggesting that the unsubstituted PAHs did not contribute strongly to the mutagenic activity.

#### 3.4.5. Comparison of sites

The general picture from Table 5 is that the oven-hall sample was the least mutagenic. With respect to the polar fraction, the oven hall was least mutagenic especially without S9. In TA98 without S9 the oven-hall sample was less mutagenic than the samples from the three other sites ( $p < 0.01$ ) and in YG1041 without S9 a statistically significant difference was observed between the oven hall and the three other sites ( $p < 0.001$ ). With the YG5161 strain, only the street-air sample was more mutagenic than the oven hall ( $p < 0.001$ ). In most cases the field samples were less mutagenic than NIST SRM 1649a. The moder-

ately polar fraction with S9 was an exception, where NIST SRM 1649a was statistically less mutagenic than the field samples. This is most likely because there was a lower amount of the eight PAHs in NIST SRM 1649a. Of the eight PAHs determined in this study, there was a total amount of 124 ng/mg in the receiving hall, 40 ng/mg in the street air and 39 ng/mg in the background air samples. For the same eight PAHs in NIST SRM 1649a, the total concentration is reported to be 24 ng/mg [28].

The amount of extractable organic matter in the crude fraction was 1.9% in the oven-hall sample, 11.9% in the receiving hall, 10.0% in the street air, and 7.1% in the background air. When the mutagenic potency was expressed as revertants per mg EOM (extractable organic matter), the PM from the oven hall was most mutagenic if there was a positive response (TA98 + S9, YG5161 + S9, YG1041 – S9). The mutagenic activity of samples from the three other sites was comparable, except with the YG1041–S9 and YG1041 + S9, where the sample from the receiving hall was most mutagenic.

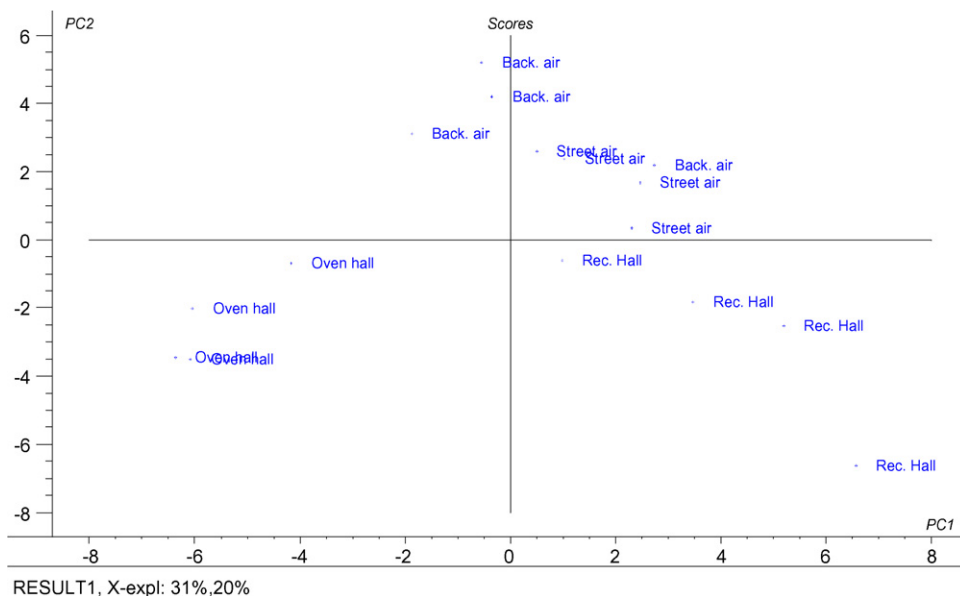


Fig. 4. Principal component analysis, score plot.

### 3.5. Principal component analysis (PCA)

Some metals and PAHs may originate from common sources, and it is to be expected, therefore, that within the data set there would be several groups of correlated metals and PAHs. The aim of the PCA analysis was to describe this underlying data structure [29]. Fig. 4 shows the score plot of the data for principal component 2 (PC2) versus principal component 1 (PC1). PC1 explains 31% of the variance of the data set and PC2 explains an additional 20%. PC1 separated the oven hall and the receiving hall very well. By applying PC2, the oven hall and the receiving hall were separated from the street and background air. The street air and background air were not separated, neither by PC1 nor PC2, and separation did not improve by applying PC3 or PC4. Thus, the score plot provided three groups: oven hall, receiving hall and the two urban sites – street air and background air – as one group.

Fig. 5 shows the loading plot for PC1 and PC2. All the PAHs and the total sum of all PAHs (PAHtot) were highly correlated and formed one group (group 1, Fig. 5). The loading of the following other variables was similar to that of the PAHs: Fe, Mn, Sr, Ca, Nb, La, Y, Ce, Ba and the biological endpoints DNA damage (comet assay results), *IL-6*, *IL-8*. These are also included in group 1. Opposite group 1 in the PC1 direction, a small group of metals (K, Cs, Cd, Pb, Rb) was found (group 2, Fig. 5). The variables in groups 1 and 2 are negatively correlated. With regard to PC2, the metals with the highest loadings

were Ni, Co, V, Cu, Na and Ag, Se and Si. Negative loadings were observed for Ti, Ca, (Y, Sr, Sc, Li, Rb, As, La, Ce). Of these, Cu, Co, V and Na formed a small group (group 3, Fig. 5) which was negatively correlated with Ti, Sc, Li, As and P (group 4). The variables (comet data, *IL6* and *IL-8*) were almost unambiguously described by PC1, which indicates that these response variables can be described by one principal component. The response (comet data, *IL6* and *IL8*) was positively correlated with the variables in group 1 and negatively correlated with variables in group 2 (Fig. 5).

### 3.6. Partial least-square regression

This analysis was performed to predict which metals or PAHs contributed to the biological endpoints. Regression coefficients with uncertainty limits corresponding to two times the standard deviations were calculated for all the variables [27]. The findings are summarised in Table 6.

For comet data, the model could explain 39% of the variance with one principal component. Mn and Sr had significantly positive regression coefficients. These two elements were in group 1 in the PCA (Fig. 5), suggesting that this group was significantly associated with DNA damage. Among the PAHs only chrysene contributed significantly. The variables that had significantly positive regression coefficients on *IL-6* and *IL-8* mRNA expression were almost identical. The metals were all from group 1 (Fig. 5). Some metals had statistically signifi-

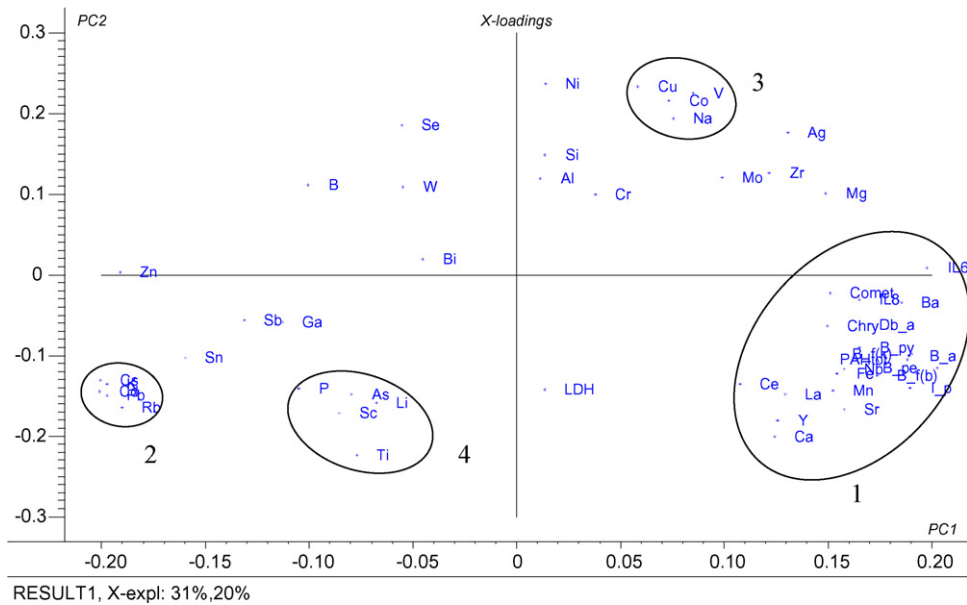


Fig. 5. Principal component analysis, loading plot. Four groups of variables (1–4) are indicated (see Section 2 for details).

Table 6  
Weighted regression coefficients from PLS analysis

	Group (Fig. 5)	Comet (DNA damage)	<i>IL-6</i>	<i>IL-8</i>
Ba	1		0.051 (0.018–0.087)	0.045 (0.004–0.084)
Ca	1		0.032 (0.003–0.061)	0.023 (0.004–0.041)
Cd	2		–0.052 (–0.016–0.090)	–0.036 (–0.008–0.0675)
Fe	1		0.033 (0.005–0.060)	0.024 (0.001–0.046)
K	2		–0.053 (–0.018–0.091)	–0.033 (–0.005–0.065)
Mg	-		0.033 (0.008–0.058)	
Mn	1	0.027 (0.003–0.051)	0.034 (0.008–0.061)	0.024 (0.003–0.046)
Pb	2		–0.047 (–0.017–0.079)	–0.037 (–0.005–0.069)
Rb	2		–0.050 (–0.013–0.088)	
Sr	1	0.028 (0.006–0.05)	0.036 (0.013–0.06)	0.023 (0.008–0.038)
Zn	-		–0.059 (–0.025–0.095)	–0.41 (–0.011–0.066)
Cs	2		–0.052 (–0.014–0.092)	–0.035 (–0.005–0.062)
Nb	1		0.048 (0.01–0.086)	
Y	1		0.033 (0.005–0.063)	
Benzo( <i>a</i> )anthracene	1		0.035 (0.008–0.0625)	0.039 (0.008–0.067)
Benzo( <i>b</i> )fluoranthene	1		0.041 (0.016–0.067)	0.040 (0.004–0.073)
Benzo( <i>g,h,i</i> )perylene	1		0.038 (0.009–0.062)	0.033 (0.011–0.052)
Benzo( <i>k</i> )fluoranthene	1		0.035 (0.009–0.060)	0.042 (0.009–0.073)
Chrysene	1	0.023 (0.003–0.041)		0.026 (0.013–0.035)
Dibenzo( <i>a,h</i> )anthracene	1			0.038 (0.005–0.06875)
Indeno(1,2,3- <i>cd</i> )pyrene	1		0.034 (0.008–0.059)	0.037 (0.013–0.057)
PAH <sub>tot</sub> (total sum)	1		0.032 (0.005–0.060)	0.037 (0.008–0.064)

Data are given with uncertainty limits in a partial least square regression (see Section 2 for details). All 742 observations were included in the prediction of comet assay results, *IL-8* and *IL-6* results. Only the variables that gave a statistically significant coefficient are presented. Positive values indicate a significant positive contribution in induction of the end-points. Negative values indicate a significant negative contribution in induction of the end-points.

cantly negative regression coefficients on *IL-6* and *IL-8* mRNA and most of these elements were from group 2 (Fig. 5). All of the PAHs, except benzo[a]pyrene, and the sum of all PAHs had statistically significantly positive regression coefficients on *IL-8* response. Most of the PAHs and the sum of all PAHs had statistically significantly positive regression coefficients on *IL-6* mRNA. This suggests that the particle-bound PAHs are responsible for increased *IL-6* and *IL-8* mRNA expression, or that the PAHs co-vary with other (unknown) constituent(s) that determine the response. The *IL-8* model included one extreme value (a street-air sample) determined with the Dixon's outlier test; without this extreme value the model improved from 45 to 75% with one principal component. For *IL-6* data, the model could explain 71% of the variance with one principal component.

## 4. Discussion

### 4.1. Particle data and size distributions

In the oven hall at the incineration plant, the  $PM_{2.5}$  levels were about two-fold higher than in the receiving hall. Both incineration-plant sites had higher  $PM_{2.5}$  levels than the busy street in Copenhagen. The  $PM_{2.5}$  levels in the oven hall were about four to five times higher than  $PM_{2.5}$  levels determined in the same street in other studies [30,31]. The  $PM_{2.5}$  levels in the oven hall were about six-fold higher than  $PM_{2.5}$  levels determined in background air in a previous study [30]. In the receiving hall, the  $PM_{2.5}$  levels were two to threefold higher than in the busy street. The high levels at the oven-hall site were most probably the consequence of leakage in the combustion oven and recirculation channels. In the receiving hall, exhaust from diesel vehicles and the dust generated by the unloading of the waste were the primary sources of  $PM_{2.5}$ . Thus, the exposure to fine particles at the incineration plant sites was considerably higher than the exposure in a busy street in Copenhagen. Other researchers have also measured high particle concentrations at incineration plant sites: Rahkonen [32] measured in Finland a total PM level between 0.3 and 7.1  $mg/m^3$  in a bunker hall and 0.6  $mg/m^3$  inside the cabin of a crane. Maitre and co-workers [33] measured 300–460  $\mu g/m^3$  of respirable particulate matter at two different incineration plants in France. Between 0.3–3.3  $mg/m^3$  of total dust was determined by personal samplers in different areas of a waste-incineration plant in Turku, Finland [4].

The particle size distribution in the receiving hall and in street air was similar with a maximum mode in the SMPS range at about 25 nm. The size distribution at the same site in the street has been determined previously to

have a maximum mode between 20 and 30 nm [34], and this distribution is influenced by several urban sources (i.e. traffic and other combustion). Diesel vehicles periodically influenced the air in the receiving hall during the day and the size distribution indicated that the vehicles had a strong influence. It is also possible that the size distribution at this site is influenced by city background, which has a maximum mode of about 30–40 nm [34], because the plant is located only about 10 km from a city centre and there are heavy-traffic roads close by. In the oven hall, the size distribution was completely different from the air in the receiving hall and the street air, with a maximum mode near 150 nm. This dominant mode is clearly not traffic-related and most probably influenced by leakage from the combustion oven and the recirculation channels.

### 4.2. Particle chemistry

#### 4.2.1. Inorganic elements

The score plot and the loading plot indicated that the oven hall was characterised by the group containing K, Rb, Cs, Pb and Cd (group 2, Fig. 5) and by the elements P, As, Li, Sc, Ti, Sn, Sb and Ga. K and P were most likely produced by the combustion of organic waste containing biologically occurring K and P [35]. Pb and Cd are impurities in the waste and important contaminant tracers for solid-waste incineration [36]. Arsenic (As) is also associated with incineration-plant combustion [37]. Other elements like Ti, Sn and Sb may be due to impurities in the waste that were released during combustion.

The receiving hall could be characterised by the PAHs and the metals in group 1 (Fig. 5). The receiving hall was influenced by diesel vehicles that contributed with PAHs. Vehicles could also be the source of the transition metals Fe and Mn, which are emitted from road and brake wear. The high Ca concentrations in the receiving hall could be due to unloading of building materials, because these contain Ca. Sr and the rare-earth metals La, Y and Ce may have been present in the waste.

Street and background air was influenced by the elements in group 3 (Fig. 5) consisting of Cu, Co, V and Na. Cu is a tracer for brake emissions [38,39]. Co and V are associated with coal and oil combustion [39,40]. The source of Na could be crustal but also sea spray. Street and background air were also associated with other elements such as Ni, Cr, Mo and Zr, which may originate from brake wear [38]. Ni could also originate from oil combustion [41].

In summary, the receiving hall was characterised by vehicle exhaust, break-wear emissions and emissions during the unloading of waste. Street and background

air was characterised by brake-wear emissions, crustal sources and coal/oil combustion sources indicative of emissions from traffic and long-range transport. The oven-hall samples probably reflected emissions from the combustion oven and the recirculation channels and were characterised by elements like Cd and Pb that were most likely present as impurities in the waste.

#### 4.2.2. PAH analysis

The principal component analysis showed that the receiving hall was characterised by the PAHs (loading plot, Fig. 5). This site had the highest PAH levels, but the variation between the four samples in the total of the PAHs and the individual PAHs was too great for it to be statistically significantly above the street and background-air samples. However, the receiving hall had statistically significantly higher concentrations of total PAHs and of most of the individual PAHs compared with the oven hall. The low PAH content in the oven hall PM compared with the three other sites is probably due to efficient combustion in the oven. In the receiving hall the source of PAHs is most probably diesel-vehicle exhaust.

#### 4.2.3. Inflammatory response

The inflammatory response was analysed by determining the relative mRNA expression of *IL-6* and *IL-8*. The PM from the oven hall, street air and background-air sites were all strong inducers of *IL-8* mRNA production ( $p < 0.001$ ), whereas the PM from the oven hall was non-responsive at the concentration tested. Urban samples of  $PM_{2.5}$  have previously been shown to induce *IL-8* release in A549 cells. Nam and co-workers [42] observed an effect of urban  $PM_{2.5}$  samples at both the transcriptional and protein levels, and Seaton and co-workers [43] found that  $PM_{2.5}$  samples from the London underground stations stimulated *IL-8* protein production, which was attributed to soluble transition metals. Transition metals in inhaled particles are able to catalyse the formation of reactive oxygen species, which may play a crucial role in inducing inflammation [44]. Metals in the soluble extracts of particles have been suggested to cause pulmonary responses based on investigations both *in vitro* and *in vivo* (see, e.g. [45–49]).

The *IL-6* mRNA and *IL-8* mRNA responses were very similar, with the receiving hall and the street-air samples being most potent. Urban  $PM_{2.5}$  samples had also previously shown to induce *IL-6* in L132 and A549 cells, at both the transcriptional and protein levels [11,50].

It has been reported that among several elements tested, Fe, V, Ni were the principal determinants of cytokine production in residual oil fly-ash [49,51,52]

We found that the oven hall samples contained less of the transition metals Fe, V, Ni than the three other sites ( $p < 0.02$ ). The oven hall PM also contained the lowest amount of extractable organic matter (2%). This may suggest that soluble transition metals or the organic content, or both, play an important role in the *IL-8* and *IL-6* synthesis in A549 cells.

#### 4.3. Genotoxicity

##### 4.3.1. DNA damage

The single-cell gel electrophoresis assay (comet assay) has been widely used to evaluate the genotoxic potential of environmental contaminants [53]. In the last decade several papers have reported the use of comet assay for detection of DNA damage in authentic collected particulate matter [54]. A number of studies have demonstrated induction of DNA damage by the comet assay in the lung epithelial cell line A 549, with  $PM_{10}$ ,  $PM_{2.5}$  and size fractions of  $PM_{10}$  particles sampled from various urban sites [55–60]. We have demonstrated that samples from urban air and from the receiving hall, which are both influenced by vehicle emissions, caused DNA damage ( $p < 0.001$ , Fig. 3). The oven-hall samples, which were not influenced by traffic emissions, were statistically less DNA-damaging than the street samples ( $p < 0.05$ ). Our study therefore supports the notion that street-air particles may be more genotoxic than those from other combustion sources.

##### 4.3.2. Mutagenicity

PM from all sites was mutagenic. There are many data that demonstrate that  $PM_{2.5}$  from urban/background/industrial sites are mutagenic in the *Salmonella typhimurium* assay (see e.g. [61–64]). Pani et al. [9] investigated the mutagenic activity inside the working area of the municipal incinerator in Trieste, Italy. They investigated both airborne and settled dust as total particulate matter and the respirable fraction. Most samples were mutagenic in strain TA98 with or without metabolic activation, and the authors attributed the mutagenic activity to incompletely burnt material that heavily polluted the environment. In the present study the oven-hall samples were mutagenic. However, in general the oven-hall site was the least mutagenic compared with the three other sites. The samples collected in this study were less mutagenic than NIST SRM 1649a, except in the polar fraction where the mutagenicity of street-air samples was comparable with that of NIST SRM 1649a.

In the fractionated extracts, the highest mutagenic activity for all four sites was found in the polar fraction.

The mutagenic activity was higher without metabolic activation. This may be because polar compounds contain nitro-PAHs that do not require S9 metabolic activation [64]. The moderately polar fraction, which contains un-substituted PAHs, was only weakly mutagenic and only with metabolic activation. The findings in the present study are in accordance with a detailed study by Barale et al. [12], who fractionated PM<sub>10</sub> samples from 17 Italian towns into five fractions and tested the mutagenicity of each fraction with strain TA98. Consistently they found that the polar fraction that contained nitro compounds was most mutagenic. Based on a literature review, Claxton and co-workers also concluded [65] that the most mutagenic activity usually is associated with the moderately polar/highly polar fractions, which contain nitro-aromatic compounds, aromatic amines and ketones. Nitrogen-containing compounds are usually associated with combustion sources, but are also produced when PAHs react with nitrogen and sunlight [24,66].

Among the three strains used, YG1041 was the most potent. This strain is more sensitive to nitro-compounds (e.g., 2-nitrofluorene and 1-nitropyrene) than strain TA98 [24]. A higher mutagenic activity of airborne samples with YG1041 compared with TA98 has previously been demonstrated [23,67,68]. YG1041 is a useful strain for the monitoring of mutagenicity in complex mixtures such as urban air [24].

The findings of a higher activity with YG1041 strongly suggest that the mutagenic activity is associated with nitro-PAHs and not with the unsubstituted PAHs. In a study by Lofroth et al. [69], electrostatic sampling was used for collecting urban particles <15 µm. The mutagenic activity was caused by polar compounds containing nitro groups. Other evidence indicating an only weak effect of unsubstituted PAHs is that we detected as many or fewer revertants with YG5161 (PAH-sensitive strain) as with TA98.

#### 4.4. PLS analysis

The PLS analysis showed (Table 6) that the comet response was only influenced statistically significantly by one PAH, chrysene. PAHs can exert DNA damage via the formation of DNA adducts [54]. Another mechanism is by redox-active quinones, which can be generated during metabolism of several PAHs in the lungs. Quinones can produce reactive oxygen species (ROS) by redox cycling, and as a result ROS can lead to DNA damage [70,71]. Metals that had a statistically significant influence were from group 1 (Fig. 5), Mn and Sr. Urinary Mn concentrations and DNA damage in blood samples

of welding-fume workers have shown a positive correlation [72]. Transition metals, in particular Fe, can generate the hydroxyl radical ( $\cdot\text{OH}$ ) via the Fenton reaction [73]. The hydroxyl radical is one of the most strongly oxidising biological radicals, and it reacts rapidly with most biological molecules, it damages cells and causes DNA damage [74]. The ability of Sr to induce DNA damage has not been investigated to our knowledge. Sr co-varied with Mn, which may be the possible reason for the statistically significant influence on DNA damage. Thus, the DNA damage is most likely due to the activity of Mn. Only chrysene contributed to DNA damage and this may suggest that PAHs have a minor effect on this end point. Gutierrez-Castillo et al. [57] investigated fine and coarse PM samples from Mexico City and observed that metals contributed more strongly than PAHs to induction of DNA damage. However, in another study a positive correlation of DNA adducts and the interaction of the total amount of transition metals and total amount of PAHs of PM<sub>2.5</sub> samples was found, indicating that both metals and PAHs may contribute equally [64].

*IL-6* and *IL-8* mRNA expression were positively and statistically significantly influenced by almost the same metals, except that *IL-6* mRNA expression was also influenced by Mg, Nb and Y. Similar to what was found for DNA damage, the metals inducing inflammation were from group 1. Fe and Mn in PM samples have been reported to be involved in *IL-6* and *IL-8* response [11,43,46,75]. Fe and Mn can generate ROS (as described above) and ROS may be involved in inflammation and the generation of *IL-6* and *IL-8* [44]. The other metals that were statistically significantly associated with *IL-6* and *IL-8* responses in the present study co-varied with Mn and Fe. It is possible, therefore, that Fe and Mn are most important for the *IL-6* and *IL-8* response. V and Ni may also be responsible for cytokine production [49,51,52], but they had no effect in the present study. Some metals (Cd, K, Pb, Rb, Zn and Cs) were negatively associated with *IL-6* and *IL-8* responses. Gutierrez-Castillo et al. [57] reported that Cr, Mn and Zn and some PAHs were negatively correlated to DNA damage and they suggest that different components in PM and other complex mixtures may interact with each other to produce synergistic, antagonistic or additive effects.

In summary, air-pollution particles collected from all four sites investigated in this study were genotoxic and inflammogenic (except from the oven hall) in mammalian cells and their extracts were mutagenic in the *S. typhimurium* assay. The particulate samples from the receiving hall in the incineration plant were as toxic as particles from a high-traffic street in Copenhagen. We found relationships of the elemental and organic compo-

sition of PM at the incineration-plant sites and urban-air sites with inflammatory end-points and DNA damage in mammalian cells.

## Acknowledgements

This study is part of Anoop Kumar Sharma's PhD project, funded by the National Institute of Occupational Health (NIOH), Denmark and the Department of Environment, Social and Spatial Change, Roskilde University, Denmark. The study was also supported by The Danish Research Council (grant 2052-03-0016), Air pollution in a life time health perspective (Airpolife) and The European Union (grant FP6-012912, NEST), Particle Risk. K. Jensen (Roskilde University), L. Pedersen (NIOH), V.K. Sørensen (NIOH), P.A. Clausen (NIOH), M. Gagnon (Health Canada), R.M. Maertens (Health Canada) are gratefully acknowledged for technical assistance. G.R. Douglas (Health Canada) is gratefully acknowledged for providing facilities and technical assistance for the microsuspension assay. National Environmental Research Institute, Roskilde is acknowledged for access to their Jagtvej monitoring station.

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