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Combustion particles emitted during church services: Implications for human respiratory health

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ABSTRACT

Burning candles and incense generate particulate matter (PM) that produces poor indoor air quality and may cause human pulmonary problems. This study physically characterised combustion particles collected in a church during services. In addition, the emissions from five types of candles and two types of incense were investigated using a combustion chamber. The plasmid scission assay was used to determine the oxidative capacities of these church particles. The corresponding risk factor (CRf) was derived from the emission factor (Ef) and the oxidative DNA damage, and used to evaluate the relative respiratory exposure risks. Real-time PM measurements in the church during candle–incense burning services showed that the levels (91.6 μg/m^3 for PM_{10}; 38.9 μg/m^3 for $PM_{2.5}$) exceeded the European Union (EU) air quality guidelines. The combustion chamber testing, using the same environmental conditions, showed that the incense Ef for both PM_{10} (490.6–587.9 mg/g) and $PM_{2.5}$ (290.1–417.2 mg/g) exceeded that of candles; particularly the $PM_{2.5}$ emissions. These CRf results suggested that the exposure to significant amounts of incense PM could result in a higher risk of oxidative DNA adducts (27.4–32.8 times) than tobacco PM. The generation and subsequent inhalation of PM during church activities may therefore pose significant risks in terms of respiratory health effects.

1. Introduction

Particulate matter (PM), generated during combustion processes and occurring in fine and ultrafine size fractions, is a common component of indoor air (BéruBé et al., 2004). Human respiratory exposure to these microscopic particles has been linked to the formation of oxidative stress, DNA adducts and respiratory diseases (Bérubé et al., 2007). Many epidemiological studies have linked outdoor air pollution to mortality and morbidity (Dominici et al., 2006), however, people spend approximately 87% of their time indoors (BéruBé et al., 2004) in both private and public buildings. Clinical studies have demonstrated that exposure to high PM concentrations causes irreversible damage in the respiratory system (Stinn et al., 2005). Human behaviour involving combustion, such as smoking, candle and incense burning, results in the rapid generation of significant amounts of ultrafine particles (UFPs), which can pass through to the distal respiratory system with resultant initiation of cardiopulmonary diseases (Steinvil et al., 2008).

The dominant religion in the UK, comprising approximately 53% of the adult population, is Christianity (Tearfund, 2007). It is reported that on average 4.9 million people make weekly visits to church, and additionally, 3.9 million and 5.7 million attended church during Easter and Christmas, respectively (Tearfund, 2007). Candles and incense are commonly used during church services, generating PM and resulting in poor indoor air quality (Loupa et al., 2010). This is especially the case for the ultrafine sized fractions (Pagels et al., 2009).

Combustion chambers, with controlled humidity and temperature, have been used for the quantification and collection of PM (Pagels et al., 2009). The results have shown that particle emissions from candles and incense varied, and they depended both on their pre-combustion composition and the combustion conditions (Pagels et al., 2009; Yang et al., 2007). The main PM type released from candles was nano-soot particles, whereas incense particles consisted of nano-soot, micro-soot, mineral and organic particles (Chuang et al., in press; Pagels et al., 2009). The primary soot nanoparticles had a tendency to agglomerate into larger soot nano-structured particles with chain and cluster morphologies; controlled by parameters such as particle concentrations, humidity and temperature (Liu et al., 2003). In addition, other components of candles and incense, such as wicks, colour pigments and fragrant smelling chemicals, contained metals and organics that were able to condense onto the surfaces of the primary particles by nucleation (Tissari et al., 2007, 2008).

The capacity of PM to cause oxidative DNA damage by free radicals was assessed. The acellular plasmid scission assay (PSA) is an established method of analysing different types of PM such as indoor PM (Shao et al., 2007). The principle of this assay is that free radicals generated from particles can convert the supercoiled, undamaged, isoform plasmids to relaxed and linear isoforms (Shao et al., 2007).

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This change in quaternary structure alters their electrophoretic mobility, thus enabling separation and quantitation on a gel.

Influenced by the physicochemical characteristics, free radical generation by combustion-derived PM is the primary source of oxidative stress, potentially resulting in cell dysfunction, inflammation and PM-related diseases (Donaldson et al., 2005). DNA is believed to be a critical target for PM-related reactive oxygen species (ROS), and potentially associated with the development of inflammatory lung diseases, including cancer (Danielsen et al., 2009).

Our previous study determined that physicochemistry was related to bioreactivity, leading to oxidative DNA damage (Shao et al., 2007). Moreover, few studies have assessed their respiratory exposure risk during church activities. An electrical low pressure impactor (ELPI) was used for particle collection and gravimetric measurements. Field emission scanning electron microscopy (FE-SEM) was used to investigate the morphology of particles collected from ELPI substrates and swabbed from the church walls. To determine oxidative DNA damage caused by particulate bioreactivity, the PSA was employed. For both the church and test chamber, an exposure assessment was undertaken, and subsequently linked to their oxidative capacities.

2. Materials and methods

2.1. Measurement site

Particle collection and measurement were conducted during the Easter holiday (3rd–6th April, 2010) in a church located approximately 8 km north-west of Cardiff city centre (Wales, UK): the background in this area was suburban and had minor vehicle traffic activities. The corresponding outdoor PM data during this sampling period was obtained from the Department for the UK Air Quality Archive (Defra, 2010).

The church used for sample collection (total volume of 885 m^3; Fig. 1) comprised an entrance room, one congregation area, two altar areas, one preparation room and one storage room. During the sampling campaign, the preparation and storage rooms were closed with a 1 cm gap under the door. All the church windows were locked. The ventilation system used in this church was positioned on the roof (5.4 m in height from the ground). The ELPI used for PM measurements and collection (Dekati Ltd, Finland) was set up in the storage room and connected with an extended tube to the congregation area (approximately 1 m above ground level, and 7 m to the main altar area). Most of the burning activities were performed in the altar areas. All church activities during the study periods, such as cleaning and the switching on/off of the heater, were logged by the church staff.

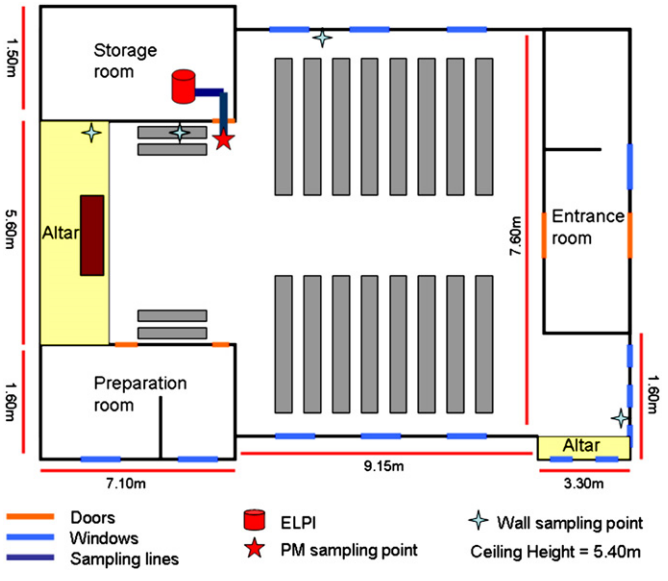

Fig. 1. Illustration of the sampling locations in the church. One entrance room, one congregation area, two altar areas, one preparation room and one storage room for a total volume of 885 m^3. The ELPI was positioned in the storage room with an extended tube to the congregation areas (red star). The wall wiped samples were taken from the main area and the two altar areas at 1.5 m above ground level (blue star). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Chamber measurements

The burning of four types of candles (C1–C4), one oil candle (O1), one charcoal base (for incense burning; CH1) and two types of incense (0.1 g of resins; I1–I2) was performed inside a polyvinyl chloride combustion chamber (volume 0.0495 m^3; Fig. S1) in a dedicated fume cupboard located in a laboratory at the School of Biosciences (Cardiff University). The incoming air, average temperature and humidity of 22 °C and 53% respectively, was passed through a high efficiency particulate air (HEPA) filter, and directed into the chamber at a constant flow rate of 6 l/min. The resultant smoke was mixed by a fan inside the chamber and was introduced into the secondary dilution chamber, mixing with 24 l/min of HEPA-filtered air. The ELPI was used to measure and collect the diluted particulate smoke.

2.3. ELPI

The ELPI was used to size PM ranging between 0.007 μm and 10 μm mean diameter by 12 inertial-based cascade impactors (Price et al., 2010). The particles were collected onto 25 mm aluminium foil substrates at a consistent flow rate of 30 l/min after zeroing. A fast mobility particle sizer (FMPS; Mode 3091, TSI Inc., USA) and an aerodynamic particle sizer (APS; Mode 3321, TSI Inc., USA) were both used to calibrate the ELPI ranging 0.0056–0.56 μm and 0.5–20 μm, respectively. The measurement mode was set at 400,000 fA current range with a particle density of 1 g/cm^3 (Sander et al., 2011). Grease was not coated onto the foil substrates (the recommended method of eliminating particle 'bounce-off') in order to reduce contamination. The organic particles produced by candle–incense burning were 'sticky' and the amount of 'bounce-off' during the collection is believed to be insignificant. All samples were kept at 4 °C prior to further analyses.

2.4. PM sampling from the church walls

Particles adhering to the church walls were swabbed onto 47 mm glass microfibre filters (2 μm pores; Whatman, UK). The sampling spots were chosen in the main congregation area and the two altar areas at 1.5 m above ground level (respiratory zones; Fig. 1).

2.5. FE-SEM

FE-SEM (Philips Electron Optics, NL) was undertaken on the ELPI and wall-swabbed samples using the methods previously described (BéruBé et al., 1999). Briefly, the filters were mounted onto aluminium SEM stubs (13 mm; Agar, UK), then imaged by FE-SEM at an accelerating voltage of 25 kV, spot size 3.

2.6. Determination of oxidative DNA damage

The PSA has been commonly used as an *in vitro* marker for the oxidative capacities of PM (Shao et al., 2007). PM samples extracted from the ELPI substrates were diluted with molecular grade water (Sigma-Aldrich, UK) to 250 μg/ml (n = 9). The PSA is described in detail in previous studies (Shao et al., 2007). The samples were run with negative controls (molecular water; 5% damage) and positive controls (a restriction enzyme, Pst I; 100% damage).

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2.7. Data analysis

The differences in oxidative DNA damage by the samples analysed by PSA were evaluated using the Student *t*-test. Particle concentrations for the coarse PM (size range of 2.5 μm to 10 μm; $PM_{2.5-10}$) were transformed to nature log to investigate particle variation over a time interval when the church heating system was switched on and off. All statistical analyses in this study used SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). The level of significance for all statistical analyses was $p<0.05$.

3. Results

3.1. Particle emissions and human activities

The time profile of particle concentrations in church during the Easter holiday (3rd–6th April, 2010) confirmed that particle levels were associated with church activities (Fig. 2). The average particle concentrations were 102.7 μg/m^3 for PM_{10} and 53.4 μg/m^3 for $PM_{2.5}$ on the first two days (Saturday and Easter Sunday), and then the levels significantly decreased to 80.4 μg/m^3 (PM_{10}) and 24.4 μg/m^3 ($PM_{2.5}$) over the last two days (Easter Monday and Tuesday). During this period the outdoor PM_{10} concentration in this area was 12.8 μg/m^3. The recorded time of increase in PM concentrations corresponded exactly with candle/incense burning and cleaning, with negligible influence from outdoor PM. Before the church opened to the public on 3rd April (Saturday), there was a small peak that resulted from the cleaning at 19:16 h. On the same day between 20:00 and 21:00 h, PM_{10} and $PM_{2.5}$ concentrations reached their highest levels, at 1318.6 μg/m^3 and 1065.8 μg/m^3 respectively, generated from the burning of 36 candles and 1 incense censer with a charcoal base. Afterwards, a minor peak was found due to candles having been extinguished following church closure to the public. Following closure, the levels of $PM_{2.5}$ dropped continually, whereas that of the PM_{10} remained at a steady level. The morning Easter Mass (4th April) carried out between 10:00 and 12:00 h, caused PM concentrations to climb to 221.4 μg/m^3 with the burning of 110 candles. In addition to these two main events, there were also services that involved burning fewer candles, between 2 and 17, which saw PM levels increase in the coarse fractions.

The effects of particulate agglomeration in $PM_{2.5-10}$ were investigated following the church post-service activities (Fig. S2). This size fraction was chosen since the majority of combustion-derived particles were $PM_{2.5}$ following their aggregation in the air into the coarse size range; thereby the variation of $PM_{2.5-10}$ could be used as an indicator for that phenomenon. There was a significant peak of $PM_{2.5-10}$ observed each time just after the heater was switched off. The $PM_{2.5-10}$ range was dependent on the number of candle/incense used.

3.2. Church particle behaviour

A linear regression model was used to correlate particle emissions and number of lit candles ($N_{candles}$) to estimate the PM_{10} (C_{PM10}; $R^2=0.72$, $p<0.05$) and $PM_{2.5}$ ($C_{PM2.5}$; $R^2=0.93$, $p<0.05$) concentrations in the church (Fig. S3). The equations were as follows (Eqs. (1) and (2)):

$$C_{PM10} = 0.76(N_{candles}) + 134.2 \quad (1)$$

$$C_{PM2.5} = 0.89(N_{candles}) + 51.3. \quad (2)$$

It was determined that 0.76 μg/m^3 of PM_{10} and 0.89 μg/m^3 of $PM_{2.5}$ per candle contributed to the church's ambient background levels. These particles remained indoors and were released outdoors or sedimented on the church wall at a very slow rate, taking several hours for airborne levels to fall back to background levels. For example, 3 candles were burnt on Easter Monday, and for approximately 7 h the PM concentrations were observed to exceed church and outdoor backgrounds both in PM_{10} and $PM_{2.5}$ (Fig. S4). This phenomenon occurred for every combustive activity and was dependent on the number of products (candles and incense) combusted.

3.3. Particle morphology

FE-SEM revealed that the majority of particles collected from the ELPI and wall samples (Fig. 3) were spherical particles. Although there were a few irregular-shaped particles (Fig. 3b, white circled) observed on the glass microfibre filters (background; Fig. 3b, white arrowed).

3.4. Ef of church combustion products

Particle Ef was used to characterise the amount of particulate pollutant discharged into the ambient air by a specific source, and is expressed as micrograms of emitted particles per gram of material (mg/g) (Charles et al., 2008; Daisey et al., 1998). The Ef of the candle samples (C1–4) ranged between 6.4 and 27.6 mg/g for PM_{10} and 1.1 and 20.9 mg/g for $PM_{2.5}$, whereas the PM_{10} and $PM_{2.5}$ Efs of the oil candle (O1) were only 0.8 mg/g and 0.3 mg/g, respectively (Table 1).

For the incense burning a charcoal base was needed for combustion. This charcoal base had an Ef of 30.6 mg/g (PM_{10}) and 23.0 mg/g ($PM_{2.5}$). To distinguish the Ef for incense only from incense and charcoal co-combustion, a non-linear regression model was used to estimate the concentrations of charcoal PM_{10} ($R^2=0.97$, $p<0.05$) and $PM_{2.5}$ ($R^2=0.98$, $p<0.05$) (Fig. S5). After 20 min of burning the charcoal had progressed from the early glowing red stage to the white 'ash' stage; incense was then added resulting in emissions of 490.6–587.9 mg/g for PM_{10} and 290.1–417.2 mg/g for $PM_{2.5}$, in addition to the charcoal base emissions (Table 1).

The Ef ratios of $PM_{2.5}$ to PM_{10} ($PM_{2.5}/PM_{10}$) in the church emissions were divided into two groups: high $PM_{2.5}$ emission (≥0.5) and low $PM_{2.5}$ emission (<0.5). The former consisted of both candle and incense samples (Table 1), ranging from 0.59 (I2) to 0.84 (C2). The latter contained only candles (C3, C4 and O1) in the range of 0.18 and 0.33.

3.5. Particulate oxidative capacity

The relative oxidative capacity of particles, as determined by PSA (Table 1), revealed that DNA damaged at 250 μg/ml PM_{10} from candle, charcoal and incense

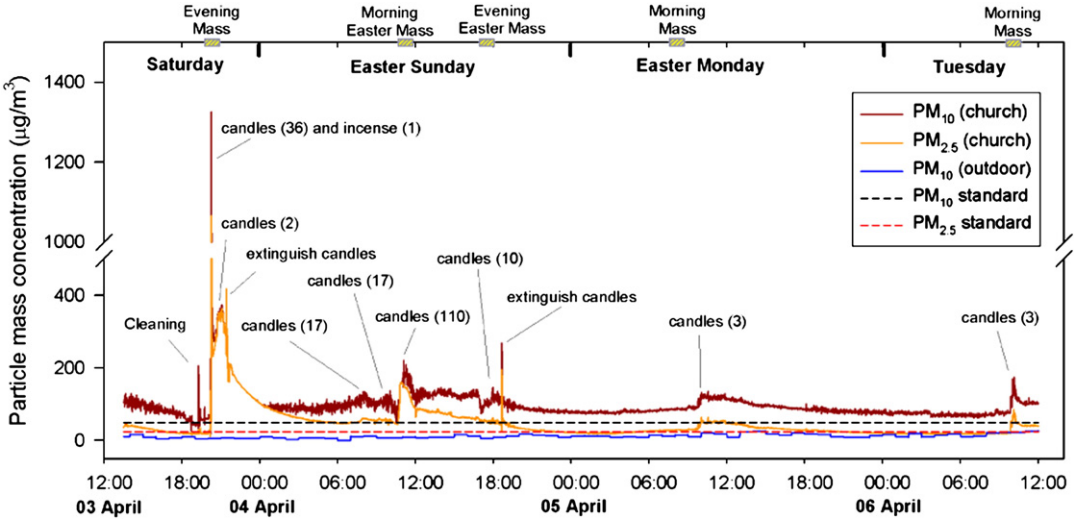

Fig. 2. The profiles of mass concentrations in $PM_{2.5}$ and PM_{10} during Easter (from 3rd to 6th April, 2010). In addition, outdoor PM_{10} data in this area was obtained from the UK Air Quality Archive (blue line). Black and red dot lines present the EU guidelines for PM_{10} and $PM_{2.5}$, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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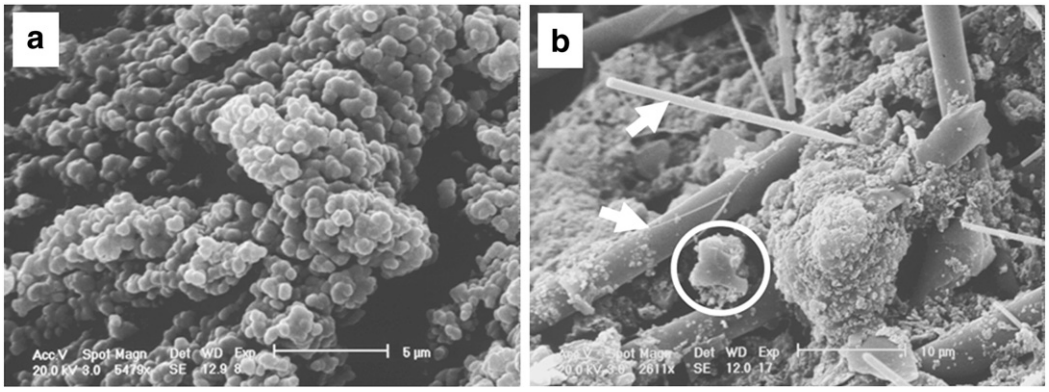

Fig. 3. FE-SEM images of the particles collected from ELPI (a; scale bar: 5 μm) and church walls (b; scale bar: 10 μm). The ELPI collection was dominated by spherical soot particles. Soot and a few irregular-shaped particles (white circled) were adhered on the walls (background glass microfibers; white arrowed).

averaged 46.1% (36.8–50.8%), whereas church ambient particles caused 32.6% damage. The 12 stages of ELPI substrates were divided into three size fractions, including NPs (0.007–0.094 μm), fine (0.094–2.4 μm) and coarse particles (2.4–10 μm). A size-dependent response of oxidative DNA damage was observed from the three size fractions at 250 μg/ml (Fig. 4; $p<0.05$).

3.6. Relative risk of church-related PM

A corresponding risk factor (CRf), incorporating both the Ef and oxidative DNA damage, was used to assess respiratory PM exposure by linking PM_{10} Ef to oxidative DNA damage (CRf = Ef × oxidative DNA damage). A product with higher CRf was considered as having higher risk for respiratory ROS formation. The CRf of incense burnt with a charcoal base (I1–2) ranged from 216.6 to 222.1, whereas the CRf was 14.4 for charcoal combustion alone (CH1; Table 1). The CRf of candle samples ranged between 10.2 and 13.8, excluding C3 (2.9) and O1 (0.4).

4. Discussion

During the Easter holiday (3rd–6th April, 2010) numerous candles and incense were burnt in an urban (Cardiff, South Wales, UK) church, causing poor indoor air quality. The church particle concentrations for PM_{10} and $PM_{2.5}$ both exceeded the EU air quality guidelines of 50 μg/m^3 for 24-hour mean PM_{10} and 25 μg/m^3 for 1-year mean $PM_{2.5}$ (European Commission, 2011), either during services, such as Mass, or as the church's ambient background air. Human activities were identified as the main cause of the excess airborne PM in the church, especially incense burning. It was noted that a large amount of particles was generated in the few minutes after the extinguishing of combustion sources. A similar phenomenon has been observed in previous studies (Loupa et al., 2010). Non-combustion-related activities were also seen to influence PM concentrations, such as cleaning and heating (Samek et al., 2007). This study did not observe a significant contribution of PM from the heating system in the church.

Inside the church, the increase in particle concentrations, once the candles had been lit, was very quick. However, it took several hours for particle levels to return to the non-service background levels due to the limited ventilation. Therefore, smaller particles suspended in the church's air for extended periods after services appeared to coagulate into coarser agglomerative particles, due to the change of atmospheric conditions (*i.e.* temperature and humidity), once the heater had been switched off. The church's walls were a major site for particle deposition, reducing the number of suspended particles (Pagels et al., 2009), but also resulted in darkening and discoloration of the paintwork. SEM analysis demonstrated that the majority of the particles on the wall were soot, with a few larger irregular-shaped particles collected that may have been generated from the incense burning, more specifically, the charcoal-based burning.

We had to take into account that there were two major types of incense used for worship, classified according to whether they are directly- or indirectly-burnt. Most incense studies have been performed on direct combustion, such as joss sticks (Chuang et al., in press), but less information is available on the indirect-burning of incense using an additional heat supplement such as charcoal; as used by the church in this study. Recalculating the incense particle emissions *via* a non-linear regression model for charcoal-based emissions prevented an over-estimation in the amount of incense PM produced.

To facilitate the determination of the potential risk to human health following exposure to incense smoke, environmental tobacco smoke (ETS), a common combustion-derived indoor air pollutant

Table 1
Emission factors, oxidative DNA damage and corresponding risk factors of church-related particles and ETS, and their comparison with ETS by ratio (mean ± S.D.).

Type	Ef (mg/g)[a]					Oxidative DNA damage (%)		CRf[b] (mg/g)
	PM_{10}	$PM_{2.5}$	$PM_{2.5}/PM_{10}$	% ETS (PM_{10})	% ETS ($PM_{2.5}$)	PM_{10}	% ETS (PM_{10})	PM_{10}
C1	23.2 ± 0.5	16.5 ± 0.4	0.71	1.3	1.3	44.1 ± 1.3	0.4	10.2
C2	24.8 ± 3.8	20.9 ± 2.4	0.84	1.4	1.7	50.8 ± 11.9	0.5	12.6
C3	6.4 ± 2.1	1.1 ± 0.5	0.18	0.4	0.1	44.8 ± 0.8	0.5	2.9
C4	27.6 ± 6.4	9.0 ± 3.9	0.33	1.5	0.7	49.9 ± 3.7	0.5	13.8
O1	0.8 ± 0.1	0.3 ± 0.0	0.31	0.0	0.0	50.6 ± 2.4	0.5	0.4
CH1	30.6 ± 2.0	23.0 ± 2.7	0.75	1.7	1.9	47.0 ± 6.0	0.5	14.4
I1	587.9 ± 149.4	417.2 ± 72.9	0.71	32.8	33.6	36.8 ± 8.9	0.4	216.6
I2	490.6 ± 115.2	290.1 ± 94.3	0.59	27.4	23.4	45.3 ± 4.8	0.5	222.1
Church	–	–	–	–	–	32.6 ± 5.1	0.3	–
ETS	17.9 ± 2.0[c]	12.4 ± 1.3[d]	0.69	1	1	99.0 ± 1.0[e]	1	17.7

[a] Emission factor: microgram of particles emitted per gram of church-related products or per gram of cigarette.
[b] CRf: corresponding risk factor (CRf (mg/g) = Ef (mg/g) × oxidative DNA damage (%)).
[c] PM_{10} ETS data was from Charles et al. (2008).
[d] $PM_{2.5}$ ETS data was from Daisey et al. (1998).
[e] PM_{10} ETS data was from Shao et al. (2007).

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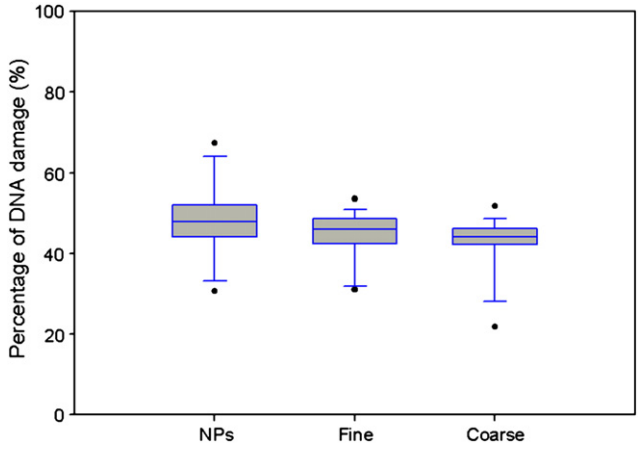

Fig. 4. Comparison of percentage DNA damage caused by three size fractions of the church-related particles, NPs (0.007–0.094 μm), fine (0.094–2.4 μm) and coarse particles (2.4–10 μm) all at 250 μg/ml (n = 9). A size-dependent response was observed by the Student's *t*-test ($p<0.05$). The middle line represents the median values; the black dots show the 5% and 95% percentiles.

known to be a risk factor for the development of cardiopulmonary diseases (WHO, 2009), we employed as a surrogate toxic emission to estimate risk. Table 1 lists a comparison of the particulate Ef of church-related products with that derived for ETS (Charles et al., 2008; Daisey et al., 1998). The results indicated that PM_{10} released from candles (except for C3 and O1) and incense burning had a higher risk factor than that generated from ETS for the same mass of combustive material. This comparison would apply to average PM levels in a room, and therefore, passive exposure to ETS rather than direct cigarette smoking. This particularly applied to the incense samples (27.4 and 32.8 times). The emissions of $PM_{2.5}$ were consistent with that of PM_{10}, excluding candle C4, with church-related products capable of generating more fine particles than ETS. $PM_{2.5}$ was the major fraction generated from combustion activities, such as ETS (Daisey et al., 1998). A similar outcome was found in the steady burning of candles and incense. However, it was noted that the majority of coarse particles were produced from particular types of candle (C3 and C4) and an oil candle (O1). There was no significant increase in $PM_{2.5}$ levels on ignition of the candles, which is in agreement with previous findings (Loupa et al., 2010).

As a means to translate the theoretical risk into a tangible biological effect, the oxidative capacity of candle–incense PM was measured. Overall, there was 32.6% DNA damage caused by the PM_{10} collected from the church air, which was slightly lower than the damage caused by the PM_{10} from candles and incense used in the test chamber (Table 1). This decrease probably resulted from the church PM being mixed with less bioreactive outdoor-sourced nature particles, *e.g.* road dust minerals. Particulate physicochemical characteristics such as size and surface area have long been recognised as determinants of ROS formation (Donaldson et al., 2005). The ROS measurements revealed that particles in the smaller size fractions (*i.e.* nano-sized and fine) were capable of inducing more DNA damage than coarser particles. The majority of spherical soot particles in the nano-sized fractions were produced from candle and incense combustion (Chuang et al., in press; Pagels et al., 2009), and previous studies have demonstrated that these low solubility nano-particles can generate ROS, both *in vitro* (Danielsen et al., 2009; Murphy et al., 1999) and *in vivo* (Evans et al., 2006; Rouse et al., 2008), to a greater extent than larger particles. In addition, additives from church-related products were able to condense onto the particle surfaces and may contribute to increased DNA damage (Shao et al., 2007; Tissari et al., 2007). ETS has been determined as having 99% DNA damage capacity at 250 μg/ml of PM_{10} by PSA (Shao et al., 2007), and the church-related PM_{10} was able to induce between 0.3 and 0.5 times the DNA damage of ETS (Table 1). The relationship between intracellular particle reactivity and DNA damage has been verified in human epidemiological and toxicological investigations (Danielsen et al., 2009; Navasumrit et al., 2008). For example, inhalation of incense smoke significantly increased the levels of leukocyte 8-hydroxy-2′-deoxyguanosine (8-OHdG; a well characterised biomarker of ROS-induced DNA damage) and DNA strand breaks in temple workers (Navasumrit et al., 2008). The bioreactivity was attributed to PM-containing ROS and led to bulky DNA damage, *i.e.* single- and double-strand DNA breaks (Danielsen et al., 2009). In particular, single-strand breaks are usually generated by ROS, resulting in a sequence of radical reactions within the DNA backbone, and subsequent breakage of the molecule (Bertram and Hass, 2008). Single-strand breaks also impede any ensuing transcription, replication and repair processes (Bertram and Hass, 2008).

Oxidative stress induced inflammation is a process associated with the initiation of a number of lung diseases. When inhaling PM into the lung environment, it is speculated that the intracellular oxidant levels increase as a result of direct generation of ROS from the surface of particles, soluble compounds, altered function of mitochondria or NADPH-oxidase, activation of inflammatory cells and generation of reactive nitrogen species (Risom et al., 2005). This cascade of such effects can stimulate an influx of inflammatory cells into the lung, leading to a second wave of ROS, since activated inflammatory cells generate and release large quantities of free radicals (Kelly, 2003). Consequently, deity worshipers and church workers should be identified as an "at risk" group, given their burden of inhalation exposure to indoor air laden with incense, candles and charcoal-derived combustion products. Comparison of the CRfs showed that incense posed the highest risk, with a CRf of more than 12-fold that of ETS (Table 1). The results indicated that incense PM was the critical pollutant when compared to the same mass of the other church-related burning sources and ETS. The other products, *i.e.* candles and charcoal, had relatively lower CRfs when compared to ETS.

The particle concentrations in the church environments and CRf were based on the assumption that the pollutant concentration in the building was homogeneous. In reality, the particle levels inhaled by worshipers and workers may depend on the areas where the church activities were being held. The altar area, for example, was the main location for combustion, and people proximal to this area may be exposed to higher than average PM levels. The particulate pollutants emitted by the candles and incense were in excess of EU guidelines, and although people stay in church for only a relatively short-time, the exposure to excessively high masses of PM has been shown to induce irreversible health effects (Stinn et al., 2005). There is also the potential for adverse health effects to develop in the church workers and regular church attendees due to continuous long-term exposures to PM. The persistent deposition of nano-soot or ultrafine carbonaceous particles that deliver oxidative stress to the lungs has long been shown to cause activation of the oxidative stress-responsive nuclear factor (NF)-κB (Shukla et al., 2000), stimulation of early response proto-oncogenes involved in cell proliferation and injury and apoptosis-related gene expression (Timblin et al., 2002), that in concert, initiates a cascade of deleterious events leading to airway inflammation and disease (Cho et al., 2010).

For risk assessment, it is important to have information about structure–activity relationships, and by using the PSA, we were able to relate the different DNA isoforms to a given toxic mode, *i.e.* concentration of PM-induced ROS. It should be noted that the PSA cannot be used as a measurement of the potential genotoxicity of PM, since the assay used naked DNA isolated from bacteria, which is a very different DNA compartmentalised within the nucleus of a eukaryotic cell. Furthermore, many cellular mechanisms, such as particle up-take, metabolism, and antioxidant defence system are not taken into account. The PSA is simply an *in vitro* toxicology tool used as a preliminary assessment of the potential *in vivo* translation of particle bioreactivity, as

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measured by ROS formation (Koshy et al., 2007; Seaton et al., 2005; Shao et al., 2007). Therefore, the oxidative DNA damage observed in this study may be interpreted as being a biomarker of early biological effects following PM exposure. Additional investigations involving human cell-based systems (BéruBé, 2011) and long-term monitoring of exposure and biomarker levels will be required to better confirm incense smoke exposure as a risk factor for human disease. Nevertheless, *in vitro* elucidation of the putative mechanisms of action for church pollutants allows for improved risk assessment for church visitors and employees, especially those with pre-existing cardiopulmonary disease, given their increased susceptibility to the effects of indoor air pollutants (Cho et al., 2010). In the interim, good ventilation and a reduction in combustion sources would be the basic mitigating requirements to achieve safer indoor air quality in the church environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.envint.2011.07.009.

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