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5	Corresponding Author	Family Name	**BéruBé**
6		Particle	
7		Given Name	**Kelly**
8		Suffix	
9		Organization	Cardiff University
10		Division	School of Biosciences
11		Address	Museum Avenue, Cardiff CF10 3AX, Wales, UK
12		e-mail	Berube@cardiff.ac.uk
13	Author	Family Name	**Chuang**
14		Particle	
15		Given Name	**Hsiao-Chi**
16		Suffix	
17		Organization	Cardiff University
18		Division	School of Biosciences
19		Address	Museum Avenue, Cardiff CF10 3AX, Wales, UK
20		e-mail	
21	Author	Family Name	**Jones**
22		Particle	
23		Given Name	**Tim**
24		Suffix	
25		Organization	Cardiff University
26		Division	School of Earth and Ocean Sciences
27		Address	Main Building, Park Place, Cardiff CF10 3YE, Wales, UK
28		e-mail	
29	Author	Family Name	**Chen**

30		Particle	
31		Given Name	**Yang**
32		Suffix	
33		Organization	University College Cork
34		Division	Department of Chemistry and Environmental Research Institute
35		Address	Cork , Ireland
36		e-mail	
37		Family Name	**Bell**
38		Particle	
39		Given Name	**Jennifer**
40		Suffix	
41	Author	Organization	University College Cork
42		Division	Department of Chemistry and Environmental Research Institute
43		Address	Cork , Ireland
44		e-mail	
45		Family Name	**Wenger**
46		Particle	
47		Given Name	**John**
48		Suffix	
49	Author	Organization	University College Cork
50		Division	Department of Chemistry and Environmental Research Institute
51		Address	Cork , Ireland
52		e-mail	

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56 Abstract

Airborne particles generated from the burning of incense have been characterized in order to gain an insight into the possible implications for human respiratory health. Physical characterization performed using field emission-scanning electron microscopy showed incense particulate smoke mainly consisted of soot particles with fine and ultrafine fractions in various aggregated forms. A range of organic compounds present in incense smoke have been identified using derivatisation reactions coupled with gas chromatography-mass spectrometry analysis. A total of 19 polar organic compounds were positively identified in the samples, including the biomass burning markers levoglucosan, mannosan and galactosan, as well as a number of aromatic acids and phenols. Formaldehyde was among 12 carbonyl compounds detected and predominantly associated with the gas phase, whereas six different quinones were also identified in the incense

		particulate smoke. The nano-structured incense soot particles intermixed with organics (e.g. formaldehyde and quinones) could increase the oxidative capacity. When considering the worldwide prevalence of incense burning and resulting high respiratory exposures, the oxygenated organics identified in this study have significant human health implications, especially for susceptible populations.
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ORIGINAL PAPER

Characterisation of airborne particles and associated organic components produced from incense burning

Hsiao-Chi Chuang · Tim Jones · Yang Chen · Jennifer Bell · John Wenger · Kelly BéruBé

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Abstract Airborne particles generated from the burning of incense have been characterized in order to gain an insight into the possible implications for human respiratory health. Physical characterization performed using field emission-scanning electron microscopy showed incense particulate smoke mainly consisted of soot particles with fine and ultrafine fractions in various aggregated forms. A range of organic compounds present in incense smoke have been identified using derivatisation reactions coupled with gas chromatography-mass spectrometry analysis. A total of 19 polar organic compounds were positively identified in the samples, including the biomass burning markers levoglucosan, mannosan and galactosan, as well as a number of aromatic acids and phenols. Formaldehyde was among 12 carbonyl compounds detected and predominantly associated with the gas phase, whereas six different quinones were also identified in the incense particulate smoke. The nano-structured incense soot particles intermixed with organics (e.g. formaldehyde and quinones) could increase the oxidative capacity. When considering the worldwide prevalence of incense burning and resulting high respiratory exposures, the oxygenated organics identified in this study have significant human health implications, especially for susceptible populations.

Keywords Carbonyl · Combustion · Incense · Joss sticks · Polar organic · Quinone

Introduction

Particulate matter (PM) generated during combustion, and occurring in fine and ultrafine size fractions, is a common component of ambient air. Epidemiological studies have linked PM to adverse human health effects, such as cardiopulmonary diseases [1]. The respirable PM (i.e. $PM_{2.5}$; less than 2.5 μm in aerodynamic diameter) has been implicated in causing adverse pulmonary effects in humans, given its ability to interact with the distal lung (e.g. alveoli) environment following inhalation [2]. During burning, in addition to the PM emission, organic compounds are released as gases, and the combination or synergistic effects of the two different phases may increase the risk of pulmonary disease [1]. Recently, combustion-related indoor activities have been the focus of inhalation toxicological and exposure assessments due to people spending the majority of their time indoors; these include incense and biomass burning, all of which can contribute to poor indoor air quality and become a public health concern [3]. The health end-points associated with exposure to air pollution are considered to be driven by the production of reactive oxygen species (ROS) initially in respiratory environment, causing inflammatory reactions and concomitant lung injury, as well as down-stream

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H.-C. Chuang · K. BéruBé (✉)
School of Biosciences, Cardiff University,
Museum Avenue,
Cardiff CF10 3AX Wales, UK
e-mail: Berube@cardiff.ac.uk

T. Jones
School of Earth and Ocean Sciences, Cardiff University,
Main Building, Park Place,
Cardiff CF10 3YE Wales, UK

Y. Chen · J. Bell · J. Wenger
Department of Chemistry and Environmental Research Institute,
University College Cork,
Cork, Ireland

systemic disease (e.g. cardiopulmonary morbidity and mortality) [4].

Incense is commonly used during religious services and often in significant quantities. For example, in Kao-Hsiong city (Taiwan) in 2003, the Taiwan Environmental Protection Agency (EPA) calculated that approximately 28.7 metric tonnes of incense were burnt in temples [5]. In addition, an indication of the worldwide market for the incense trade between Asian countries (e.g. Taiwan) and the USA has been estimated to cost 4.6 million US dollars [6]. When considering incense burning in enclosed environments, the use of incense worldwide poses a serious public health issue, as currently identified by the World Health Organisation (WHO) [7].

Studies on the composition of PM from organic matter combustion have shown that a significant fraction of the total particulate emissions could be attributed to organic constituents [8]. Polar organic compounds, including acids, alkanols, aldehydes and sugar derivatives, have been identified in fine-sized PM from various sources [9]. These organics, typically, have been found to contribute to more than 50% of the identified compounds and could comprise up to 80% of the solvent extractable organic chemicals in PM [9]. Carbonyls, many of which have been listed as air toxins, are usually found at higher concentration indoors when compared to outdoors, and are mainly emitted from combustion and building materials [8]. Most studies on incense PM organic fractions have been qualitative with some quantitative measurements of polycyclic aromatic hydrocarbon (PAH) [10], and hence, there is a paucity of studies on incense combustion-derived polar fractions such as oxidized derivatives of aromatic compounds (i.e. quinones) [11].

A range of oxygenated organic compounds in whole incense smoke have been investigated in previous studies using two-dimensional gas chromatography (GC×GC) [12–14], to show that the organic content of an aerosol may constitute a significant proportion of both particulate and gas phases triggering differential health effects post-exposure [15]. Consequently, when conducting an air toxins study, denuder-filter sampling techniques can be employed for the rapid and simultaneous capture of PM and gas phase oxygenated compounds (e.g. carbonyl) [16]. Following oxygenated compound collection, derivatisation has commonly been used for robust GC analysis. For example, *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) has been used to react with carbonyls with the following advantages: (1) better chromatographic separation, (2) higher sensitivity and (3) unknown compound identification [16].

Combustion-derived oxygenated organics in both particle and gas phases are of concern because of their high reactivity in the atmosphere and human lung environments [17]. The level of knowledge about incense-derived combustion products contrasts with our knowledge of other combustion-derived indoor particles, such as cigarette smoke, which have been intensely researched [18]. To achieve the objectives in this study, incense smoke was physicochemically characterized by field emission-scanning electron microscopy (FE-SEM) and GC/mass spectrometry (MS) to assess its potential implications for respiratory human health.

Materials and methods

Sample collection

Incense in the form of joss sticks, which are commonly used for religious ceremonies and to perfume air, were selected for this study. The samples consisted of four brands manufactured in Taiwan and three brands manufactured in China (Table 1, A–G). This study grouped these incense sticks by their main fragrant woods, including agarwood (Types A and B), sandalwood (Type C and D) and unknown (no information on the stem materials; Types E–G). The colour of the incense itself ranged from yellow to black (Table 1); however, there was no information on the organic components that made up the incense. The lengths, shapes and weights of the joss sticks are also given in Table 1.

All the incense burning (n=3) was performed in a polyethylene terephthalate tube (0.0032 m^3; length, 0.41 m; diameter, 0.1 m) mimicking a typical indoor burning

t1.1 **Table 1** Characteristics of the seven brands of joss sticks (n=6; mean±SD)

t1.2 Incense (n=6)	Length of whole stick (cm)	Length of combustion part (cm)	Weight of whole stick (g)	Colour	Main material	Manufacture
t1.3 A	39.48±0.26	28.63±0.20	1.66±0.13	Dark yellow	Agarwood	Taiwan
t1.4 B	39.38±0.15	28.38±0.16	1.68±0.12	Dark yellow	Agarwood	Taiwan
t1.5 C	39.48±0.18	28.20±0.11	1.83±0.17	Black	Sandalwood	Taiwan
t1.6 D	39.58±0.08	28.83±0.12	1.42±0.08	Dark yellow	Sandalwood	Taiwan
t1.7 E	33.33±1.50	25.10±4.54	1.39±0.16	Yellow	Unknown	China
t1.8 F	34.00±0.06	24.35±0.19	1.32±0.16	Yellow	Unknown	China
t1.9 G	33.23±0.30	23.83±0.29	0.97±0.17	Black	Unknown	China

Q1

environment at an average of 25 °C and 55% relative humidity (Fig. 1). The incoming clean air was conditioned and purified by an absorption dryer (Ecodry KA-MT; Parker, Charlotte, NC, USA), and was directed into the combustion tube at a constant flow rate of 12 $L min^{-1}$ to maintain the natural burning rate and to reduce heat accumulation inside the tube. The incense stick was ignited, and the resultant smoke was then passed through a five-channel annular denuder (University Research Glassware, Chapel Hill, NC, USA) coated with XAD-4 resin, followed by a 47-mm quartz filter at a consistent flow rate of 10 $L min^{-1}$ for the entirety of the 30-min burn. The denuder was used to capture the gaseous organics; the filter was for particle collection. After sampling, the denuder and filter were extracted with appropriate solvents and subject to derivatisation and GC/MS analysis. All the chemicals used in this study were obtained from Sigma Aldrich Chemical Co. at the highest purity available and used without further purification.

Field emission-scanning electron microscopy

A PM_{10} selective-inlet head (horizontal elutriator; C30 Classifier; Thermo, UK) was used to collect PM onto a 47-mm diameter polycarbonate filter (0.67 μm pores; Millipore, UK) with a consistent flow rate at 30 $L min^{-1}$ [19]. Source-specific particles were collected directly for 5 min. FE-SEM (Philips Electron Optics, Eindhoven, Netherlands) was undertaken on the samples using the methods of BéruBé et al. [19] using filters mounted onto aluminium SEM stubs (13 mm; Agar, UK), then imaged by FE-SEM at an accelerating voltage of 25 kV, spot size 3.

Q2

BSTFA derivatisation

Polar organic compounds containing carboxyl and hydroxyl groups were converted to their trimethylsilyl (TMS) derivatives using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) following the method of Kourtchev et al. [20]. The contents of denuder and a quarter of the filter were extracted three times with 20 ml dichloromethane:methanol (80:20 *v*/*v*) under ultrasonic agitation for 30 min. Following reduction to approximately 1 ml by rotary evaporation, the samples were transferred to 2-ml amber vials and blown until dry using a pure nitrogen stream. The residues were derivatised by the addition of 60 μl of BSTFA containing 1% trimethylchlorosilane (BSTFA+1% TMCS) and 30 μl pyridine at 80 °C for 1 h [21]. The derivative samples were cooled to room temperature, filtered and analyzed by GC/MS.

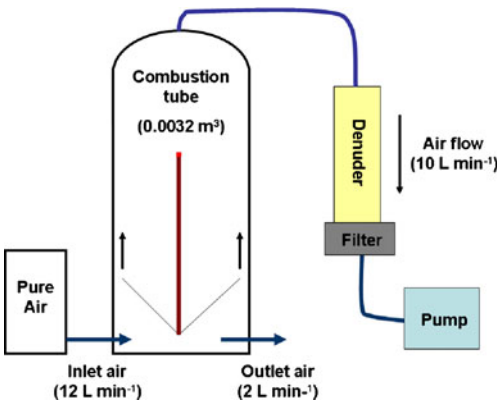

Fig. 1 Schematic diagram of the experimental set-up for collection of the gas and particle phase emissions from burning incense sticks

PFBHA derivatisation

Carbonyls were converted to their oxime derivatives via reaction with PFBHA [16, 22]. The contents of the denuder and a quarter of the filter were extracted three times with 20 ml methanol and sonicated for 15 min. The samples were subsequently derivatised with 10 mg PFBHA for 24 h in the dark at room temperature. Of each sample, 0.1 ml was dried under a nitrogen stream, and the residue was then re-dissolved in 100 μl hexane in amber vials prior to filtration for GC/MS analysis.

Derivatisation of quinones

Quinones were converted to their diacetyl derivatives by reaction with acetic anhydride following the method of Cho et al. [23]. The denuder and filter samples were sonicated in dichloromethane for 15 min, reduced to 1 ml by rotary evaporation and then reacted with 200 μl acetic anhydride and 100 mg zinc as a catalyst at 80 °C for 15 min followed by cooling to room temperature. The heating was repeated for 15 min after adding a further 100 mg of zinc. The reaction was again cooled to room temperature and quenched by addition of 0.5 ml distilled water and 3 ml *n*-pentane. Following centrifugation at 2,000 rpm for 10 min, the *n*-pentane layer was filtered using PTFE membrane filters (0.45 μm pore size) and transferred to clean amber vials. The sample was evaporated until dry under a nitrogen stream and then re-dissolved in 100 μl acetonitrile.

GC/MS analysis

All the samples were analysed after derivatisation using a GC/MS instrument comprising a split/splitless injector (Varian 1079; Varian Inc., Palo Alto, CA, USA) interfaced to an ion trap MS (Saturn 200; Varian Inc., Palo Alto, CA, USA). The GC was equipped with a Rtx® 5MS fused silica capillary column (Crossbond® 5% diphenyl, 95% dimethylpolysiloxane, 0.25 μm film thickness, 30 m in length and 0.25 mm i.d., Restek Corp., Bellefonte, PA, USA). Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. The MS was operated at an electron energy of 70 eV and

Q3

Q4

temperature of 200 °C using electron ionization (EI) operated in full scan mode over the mass range 50–650 *m/z* with a scan time of 1 s. Identification of the quinone derivatives was also facilitated by operating the instrument in single ion monitoring (SIM) mode (*m/z* 110 for 1,4-benzoquinone, *m/z* 124 for methyl-1,4-benzoquinone, *m/z* 222 for 2,6-di-tert-butyl-1,4-benzoquinone, *m/z* 160 for 1,2-naphthoquinone, *m/z* 174 for methyl-1,4-naphthoquinone and *m/z* 210 for 9,10-anthraquinone). The sample injection volume was 1 µl. The GC oven temperature programme was identical to that reported in the literature for the different groups of derivatives [16, 20, 22, 23]. Denuder and filter blanks were prepared and analysed in the same manner to determine the possible presence of contaminants. The majority of compounds present in the denuder and filter samples were identified by comparison with chromatographic retention times and mass spectra of authentic standards, which were also prepared using the derivatisation procedures described above. Some compounds were also identified by comparisons with a mass spectral library (National Institute of Standards and Technology 05 mass spectra database).

Results and discussion

FE-SEM

PM generated by incense burning poses an important risk for disease development due to its physicochemistry, including its morphology that can be observed under electron microscopy. The FE-SEM images show that soot particles dominated the fine and ultrafine fractions, with the individual soot nanospheres aggregated to form clusters (Fig. 2a) or chains (Fig. 2b). A smaller number of larger spherical soot particles were also observed in micro-sized fractions (Fig. 2c). The second most abundant particle types

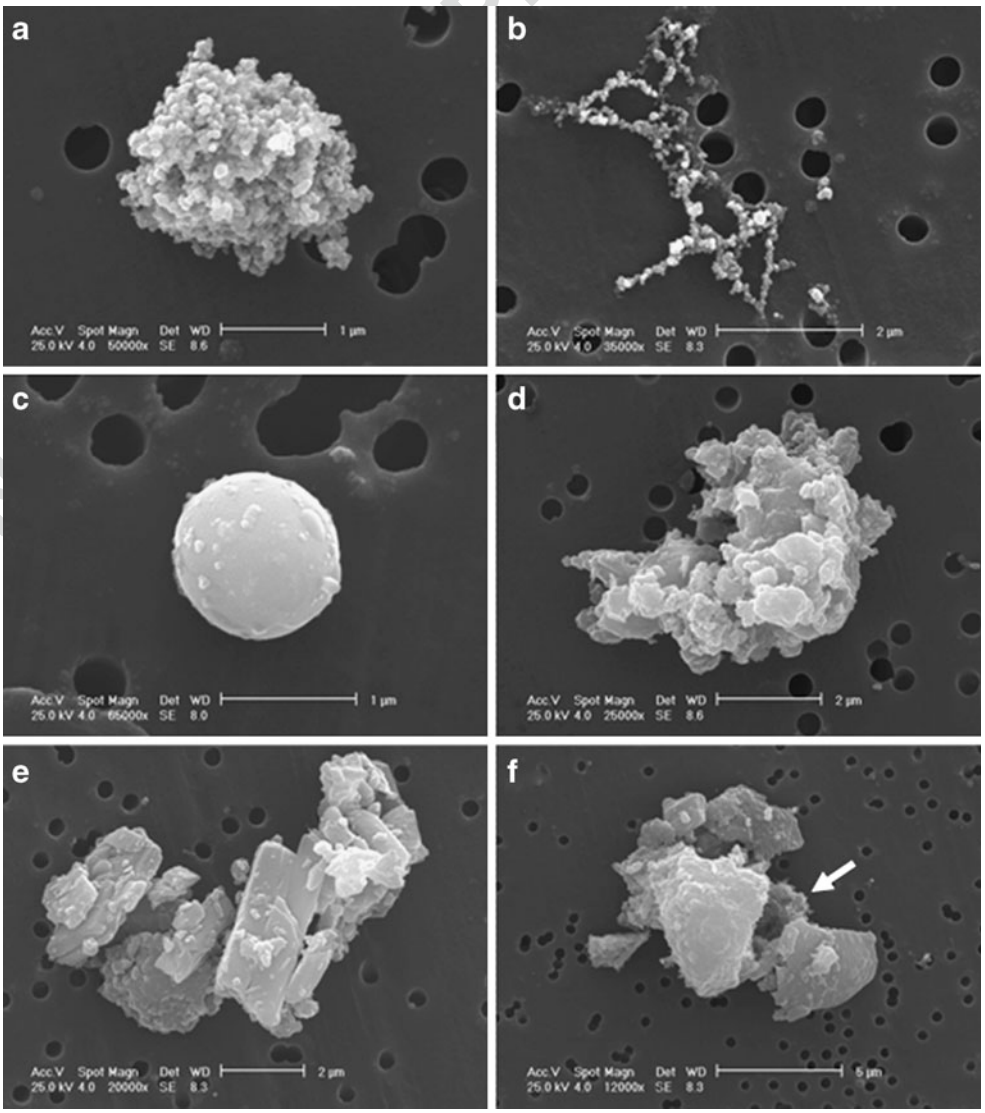

Fig. 2 FE-SEM images of particles produced from incense burning. **a** Soot aggregate, developing from a chain into cluster. **b** Chain soot particles. **c** Larger spherical soot-liked particle. **d** Irregular-shaped particle (clay). **e** Irregular-shaped particle aggregates. **f** Irregular-shaped particle aggregates and soot aggregates (*arrow*)

were irregular-shaped PM clusters with a 'platy' morphology (Fig. 2d) or more 'blocky' shapes (Fig. 2e). These may be attributed to unburnt incense components such as organic materials (e.g. powdered charcoal) or inorganic 'filler' minerals (e.g. finely powdered limestone). Composite particles were also commonly identified, often with irregular-shaped particles acting as substrates for the soot aggregates, which adhered to their surfaces (Fig. 2f, arrow).

Human respiratory disease is driven by the culmination of acute and chronic exposure to intra- and extra-cellular ROS generation [24]. There are various extra-cellular ROS sources that can provoke directly or indirectly oxidative stress. Many individual air pollutants that comprise an ambient mixture are free radicals or have the ability to drive free radical reactions. For instance, the oxidative stress caused by PM and organics may arise from mixed sources, involving (1) direct generation of ROS from the surface of particles or the particle itself, (2) soluble compounds (e.g. organic and inorganic compounds), (3) altered function of mitochondria or NADPH-oxidase and (4) activation of inflammatory cells capable of generating ROS and reactive nitrogen species [24]. Of the identified particle types, soot is of the greatest health concern because it can generate large quantities of free radicals as a function of their small size and large surface areas [25]. Biological ROS generation may also be correlated to PM type or morphology [26] with spherical and irregular-shaped PM providing a platform for inter-mixing or intercalating with organics that cause redox cycling leading to apoptosis [24, 25].

BSTFA derivatives

A total of 19 polar organic compounds (9 acids and 10 hydroxyls) was positively identified using BSTFA derivatisation (Table 2). The relative abundance of these compounds in the gas and particle phases varied slightly from sample to sample (Fig. 3). No consistency in the ratio of gas to PM (G/P) abundance from incense burning was observed for either acids or hydroxyls. In terms of the groups of incense-fragrant wood, 3-hydroxyprop-2-enoic acid, benzoic acid, 4-hydroxybutanoic acid, vanillic acid, 1,4-cyclohexanediol, glycerine, 4-hydroxybenzaldehyde, galactosan, mannosan and levoglucosan were determined in both particle and gas phases among agarwood, sandalwood and the unknown group (Table 2).

Polar organics have been widely used in atmospheric research for tracing and identifying specific sources in the ambient air. Levoglucosan, mannosan and galactosan, for example, are primary combustion products of cellulose and hemicellulose and thus commonly used as tracers for biomass burning [27]. Joss sticks are made of a wooden

Table 2 Polar organic compounds (acids and hydroxyls) identified in the gas (G) and particle (P) phase components of incense smoke (n=3)

Compound	G/P[a]	Incense types					
		Agarwood		Sandalwood		Unknown	
		G	P	G	P	G	P
Acid							
6-Hydroxyhexanoic acid	1.4	V	–	V	V	V	V
Propanoic acid	1.2	V	–	V	–	V	V
3-Hydroxyprop-2-enoic acid	5.8	V	V	V	V	V	V
Benzoic acid	7.2	V	V	V	V	V	V
Glyceric acid	1.5	V	–	V	V	V	V
4-Hydroxybutanoic acid	0.2	V	V	V	V	V	V
Cinnamic acid	0.7	V	V	–	V	–	–
3-Hydroxybenzoic acid	0.0	–	V	–	V	–	V
Vanillic acid	0.2	V	V	V	V	V	V
Hydroxyl							
Butanol	1.8	V	–	V	–	V	V
1,4-Cyclohexanediol	0.4	V	V	V	V	V	V
2,2′-Oxybisethanol	7.9	V		V	V	V	V
Glycerin	1.1	V	V	V	V	V	V
4-Hydroxybenzaldehyde	0.6	V	V	V	V	V	V
m-Cresol	1.9	V	–	V	–	V	V
Resorcinol	0.0	V	V	–	V		V
Galactosan	0.1	V	V	V	V	V	V
Mannosan	0.1	V	V	V	V	V	V
Levoglucosan	0.2	V	V	V	V	V	V

The incense has been classified into three types, agarwood, sandalwood and unknown, corresponding to the wood material used in the sticks

"–" compound not found in the sample, "V" compound found in the sample

[a] Relative abundance in the gas and particle phases

Q9

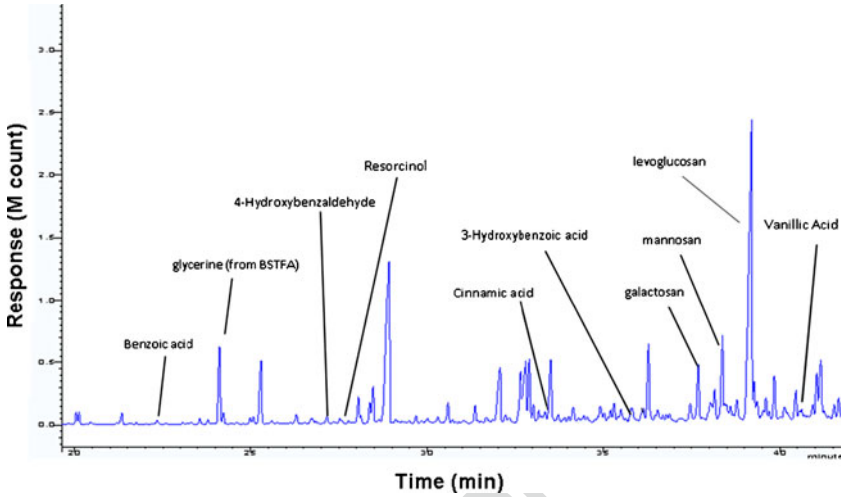

Fig. 3 Total ion chromatogram showing BSTFA derivatives of particulate phase polar organic compounds (filter sample) produced from burning incense

stem coated with a range of incense materials including aromatic wood, bark, herbs, seeds and flowers [28]. The biomass-derived chemo-markers were detected as major species in incense smoke [14]. The other polar organics identified in this study are mainly oxygenated aromatic compounds; a number of them have been observed in incense smoke emissions and aromatic resins [12, 13, 29, 30]. Although it is difficult to assess the health implications of exposure to the polar organics due to the paucity of experimental information on the toxicology of these compounds, some of these polar organic compounds could potentially be used as chemo-markers of incense burning or biomarkers following exposure [31].

PFBHA derivatives

The chromatograms of PFBHA derivatives obtained from burning the different types of incense were very similar and featured the same major peaks. There were 12 carbonyl compounds identified in the samples (Table 3). The

t3.1 **Table 3** Carbonyls and quinones identified in the gas (G) and particle (P) phase components of incense smoke (n=3)

Compound	G/P[a]	Incense types					
		Agarwood		Sandalwood		Unknown	
		G	P	G	P	G	P
Carbonyl							
Formaldehyde	10.4	V	V	V	V	V	V
Acetaldehyde	5.4	V	V	–	V	–	V
Acetone	9.7	V	V	V	V	V	V
2-Butanone	3.4	V	V	V	V	V	V
3-Methyl-2-butanone	5.8	V	V	V	V	V	V
3-Pentanone	16.9	V	–	V	V	V	–
2-Heptanone	–	V	–	V	–	V	–
2-Butenal	–	V	–	V	–	V	–
3(2H)-Pyridazinone	–	V	–	V	–	V	–
Benzaldehyde	–	V	–	–	–	–	–
Glyoxal	119.1	V	V	V	V	V	V
Methylglyoxal	85.3	V	V	V	V	V	V
Quinone							
1,4-BQ	0.7	V	V	V	V	V	V
MBQ	0.1	V	V	V	V	V	V
DTBQ	0.2	V	V	V	V	V	V
1,2-NQ	0.2	V	V	–	–	–	–
MNQ	0.1	V	V	V	V	V	V
9,10-AQ	0.0	V	V	V	V	V	V

The incense has been classified into three types, agarwood, sandalwood and unknown, corresponding to the wood material used in the sticks

"–" compound not found in the sample, "V" compound found in the sample

[a] Relative abundance in the gas and particle phases

Q1

intensity of the peaks in the denuder samples was significantly larger than in the filter samples (3.4–119.1 times), indicating that the carbonyls were mainly present in the gas phase (Table 3). For instance, some, such as 2-heptanone, 2-butenal, 3(2H)-pyridazinone and benzaldehyde, were only found in the gas phase. However, several carbonyls were observed in both phases from the three groups of incense: formaldehyde, acetone, 2-butanone, 3-methyl-2-butanone, glyoxal and methylglyoxal (Table 3).

A note in previous indoor research [8], incense burning is an important source for human exposure to carbonyl pollutants, which can cause poor indoor air quality and increase the health concern of disease development. One of the carbonyls in incense smoke, for example, has been firmly recognized as a toxic substance by the US EPA: formaldehyde [32]. Formaldehyde is recognised as a level B1 probable human carcinogen, which is based on limited evidence of carcinogenicity in humans. This chemical can cause irritation to the skin, eyes and respiratory systems, and reproductive diseases [32]. Furthermore, the high reactivity leads carbonyls to generate free radicals in biological environments and causes oxidative stress, DNA adducts and inflammatory responses, such as formaldehyde [33].

Quinone derivatives

Six quinone standards were pre-selected for their important properties and scanned for using GC/MS in the SIM mode: 1,4-benzoquinone (1,4-BQ), methyl-1,4-benzoquinone (MBQ), 2,6-di-tert-butyl-1,4-benzoquinone (DTBQ), 1,2-naphthoquinone (1,2-NQ), methyl-1,4-naphthoquinone (MNQ) and 9,10-anthraquinone (9,10-AQ). Five of which were found predominantly in the incense particle phase, including 1,4-BQ, MBQ, 1,2-NQ and 9,10-AQ, as the most abundant (Table 3).

Although quinones have previously been observed in emissions arising from fuel combustion [23], this is the first study in which these compounds have been identified in incense smoke. Particulate quinones are highly reactive and can bind covalently with nucleophilic regions of proteins and DNA [31] and increase ROS formation by redox cycling [34]. The macromolecule damage driven by quinones initialled cell defence mechanism causing apoptosis [34].

Our previous study determined that incense PM was able to increase acellular ROS formation and oxidative DNA damage [35], which could be driven by their organic fractions (e.g. carbonyls and quinones) and their physical characteristics (e.g. spherical soot). Therefore, particulate quinones generated from incense burning are of particular concern, as they may be able to penetrate into the distal lung environments due to PM's respirable size and cause adverse health effects.

Conclusions

A comprehensive characterisation of oxygenated organic compounds generated by incense burning and their potential adverse health effects was undertaken. This study identified a broad range of oxygenates, including polar organics, carbonyls and quinones, in incense smoke that are known critical indoor air pollutants. A number of the compounds are known to be toxic, and information on their relative abundance in the gas and particle phases has been obtained. This study demonstrated that incense smoke may pose a significant adverse risk to human health, and further toxicology studies based on these organic compounds should be the subject of extensive investigations.

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