



Characterization of chemical components and bioreactivity of fine particulate matter (PM_{2.5}) during incense burning[☆]



K.H. Lui^a, Benjamin A. Musa Bandowe^b, Steven Sai Hang Ho^{c,d}, Hsiao-Chi Chuang^{e,f,**}, Jun-Ji Cao^{c,g}, Kai-Jen Chuang^{h,i}, S.C. Lee^j, Di Hu^k, K.F. Ho^{a,c,l,*}

^a The Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong, China

^b Institute of Geography, University of Bern, Hallerstrasse 12, 3012 Bern, Switzerland

^c Key Laboratory of Aerosol Chemistry and Physics, SKLLQG, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, 710075, China

^d Division of Atmospheric Sciences, Desert Research Institute, Reno, NV 89512, USA

^e School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan, ROC

^f Division of Pulmonary Medicine, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan, ROC

^g Institute of Global Environmental Change, Xi'an Jiaotong University, Xi'an, China

^h School of Public Health, College of Public Health and Nutrition, Taipei Medical University, Taipei, Taiwan, ROC

ⁱ Department of Public Health, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, ROC

^j Department of Civil and Structural Engineering, Research Center of Urban Environmental Technology and Management, The Hong Kong Polytechnic University, China

^k Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Kowloon, Hong Kong, China

^l Shenzhen Municipal Key Laboratory for Health Risk Analysis, Shenzhen Research Institute of the Chinese University of Hong Kong, Shenzhen, China

ARTICLE INFO

Article history:

Received 8 December 2015

Received in revised form

24 February 2016

Accepted 26 February 2016

Keywords:

Incense

Carbonyls

PAHs

OPAHs

Oxidative stress

ABSTRACT

The chemical and bioreactivity properties of fine particulate matter (PM_{2.5}) emitted during controlled burning of different brands of incense were characterized. Incenses marketed as being environmentally friendly emitted lower mass of PM_{2.5} particulates than did traditional incenses. However, the environmentally friendly incenses produced higher total concentrations of non-volatile polycyclic aromatic hydrocarbons (PAHs) and some oxygenated polycyclic aromatic hydrocarbons (OPAHs). Human alveolar epithelial A549 cells were exposed to the collected PM_{2.5}, followed by determining oxidative stress and inflammation. There was moderate to strong positive correlation ($R > 0.60$, $p < 0.05$) between selected PAHs and OPAHs against oxidative-inflammatory responses. Strong positive correlation was observed between interleukin 6 (IL-6) and summation of total Group B2 PAHs/OPAHs ($\sum_7\text{PAHs}/\sum\text{OPAHs}$). The experimental data indicate that emissions from the environmentally friendly incenses contained higher concentrations of several PAH and OPAH compounds than did traditional incense. Moreover, these PAHs and OPAHs were strongly correlated with inflammatory responses. The findings suggest a need to revise existing regulation of such products.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Incense has been widely used in Asia for millennia, and features in the religious and spiritual ceremonies of many cultures. The use of incense is increasingly popular in Western countries, with

imports to the USA in 1999 estimated at \$12.4 million (Jetter et al., 2002). Incense sticks consist of a slender bamboo stick onto which a mixture of ingredients is bonded, which usually derive from fragrant plant materials such as tree bark, resins, roots, flowers and essential oils (Jetter et al., 2002); other common forms include joss sticks and coils. Incense burning generates particles that contribute to air pollution in many Asia countries.

Persistent air pollution problems in China have prompted Chinese Buddhist and Taoist figures to call for environmentally friendly ways to burn incense in a bid to tackle air pollution. A few temples in China recently started to dispense free, environmentally friendly incense and forbade visitors from burning their own incense. Incense emissions consist of particulate and gas phases. Past research

[☆] This paper has been recommended for acceptance by Elena Paoletti.

* Corresponding author. The Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong, China.

** Corresponding author. School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan, ROC.

E-mail addresses: chuanghc@tmu.edu.tw (H.-C. Chuang), kfho@cuhk.edu.hk (K.F. Ho).

found that burning these materials could produce large amounts of particulate matter (PM), with greater average emissions than cigarettes (Mannix et al., 1996). In addition to PM, the combustion process also generates nitrogen dioxide, sulfur dioxide, formaldehyde, benzene, polycyclic aromatic hydrocarbons (PAHs) and other volatile organic compounds, many of which are confirmed or potentially carcinogenic (Cohen et al., 2013; Masih et al., 2012).

Airborne PM is a global public health concern, due to a variety of observable adverse health effects; the prime concerns relate to the production of reactive oxygen species (ROS) in the human body. ROS comprise chemically reactive oxygen radicals or oxygen-derived species such as hydroxyl radical ($\bullet\text{OH}$) and hydrogen peroxide (HOOH). Oxidative stress is an important underlying mechanism by which exposure to PM may lead to adverse health effects when overproduction of oxidants (e.g., ROS and free radicals) counteracts anti-oxidative defenses (Charrier et al., 2014).

Regular inhalation of incense smoke containing PM represents a risk for cancers of the respiratory tract, as the smoke was shown to be mutagenic and genotoxic in Ames *Salmonella* test (Chen and Lee, 1996). Numerous epidemiological and toxicological studies demonstrated close relationships between adverse health effects and exposure to ambient PM. Fine particulate matter (aerodynamic diameter $< 2.5 \mu\text{m}$: $\text{PM}_{2.5}$) can deposit in the lung periphery and elicit adverse inflammatory responses (Bitterle et al., 2006). An inflammatory response was reported when interleukin-8 (IL-8) and cyclooxygenase-2 (COX-2) genes were exposed in vitro to Indian and Japanese incense particles re-suspended from filter collection at concentrations of $10 \mu\text{g}/\text{ml}$ (Matsumura et al., 2010).

$\text{PM}_{2.5}$ induces generation of radicals that provoke oxidative stress in the respiratory environment, leading to inflammatory reaction and concomitant lung damage, which can ultimately result in cardiopulmonary morbidity or mortality in humans (Ballester et al., 2008; Boldo et al., 2006; Clancy et al., 2002; Medina et al., 2004). Previous studies focused on characterizing the emission factors of burning different types of “traditional” incense, without quantifying human health impacts, particularly of more potent $\text{PM}_{2.5}$ (Lee et al., 2002; Lee and Wang, 2004).

This study evaluates the potential health effects of exposure to smoke from traditional and marketed environmentally friendly types of incense, deducing corresponding health outcomes. It identifies the chemical components of $\text{PM}_{2.5}$ and further characterizes the relationship between $\text{PM}_{2.5}$ chemical properties and bioreactivity.

2. Materials and methods

2.1. Selection of test specimens

Five types of incense were tested for emissions. All of the sample

brands were purchased from supermarkets or religious supply stores (incense properties are shown in Table 1). The samples were labeled A to E, with C* and D* marketed as “environmentally friendly” and the others classified as “traditional” incenses.

2.2. Collection and analysis of particulate matter

Incense emission tests were conducted in an all-enclosed, stainless steel environmental chamber of 19.1 m^3 ($3.05 \text{ m} \times 3.05 \text{ m} \times 2.05 \text{ m}$) designed for measuring indoor source emissions. The system was described in a previous study (Wang et al., 2006). Three mini-volume air samplers (one Teflon and two quartz-fiber filters) equipped with $\text{PM}_{2.5}$ impactors (Airmetrics, OR, USA) were used to collect $\text{PM}_{2.5}$ emitted during combustion. The sampling inlet was positioned 1.5 m above the floor of the chamber, level with the incense tips. The operational flow rate was adjusted to 5 L min^{-1} before each sampling session. $\text{PM}_{2.5}$ samples were collected immediately after ignition on Teflon membrane ($\Phi = 47 \text{ mm}$, Pall Corporation, USA) and two quartz microfiber filters ($\Phi = 47 \text{ mm}$, Whatman, UK). The samples were collected for 1 h after the incense was extinguished. A blank $\text{PM}_{2.5}$ sample ($< 5 \mu\text{g m}^{-3}$) filter was used to collect background samples after each incense combustion, and was repeated for the five types of incense. After sampling, the filters were sealed in Petri dishes and refrigerated ($20 \text{ }^\circ\text{C}$) before chemical and biological analysis. The quartz filters were pre-heated at $900 \text{ }^\circ\text{C}$ for 3 h to remove any organic vapors on the filters. All filters were pre-conditioned at $23 \pm 0.5 \text{ }^\circ\text{C}$ temperature and $50 \pm 5\%$ relative humidity (RH) for 48 h before and after weighing the samples. Each filter was weighed on a microbalance ($\pm 1 \mu\text{g}$ precision, Sartorius AG MC5, Germany) before and after $\text{PM}_{2.5}$ sample collection. The mass concentration of collected $\text{PM}_{2.5}$ sample filters was subtracted from that of the blank filters in order to eliminate gas adsorption artifacts.

2.3. Chemical analysis

2.3.1. Carbonyls analysis

The accumulated particulates were extracted from the filters with 20 ml ultrapure methanol (HPLC grade, Sigma–Aldrich Corporation, USA) in 50 ml Falcon tubes and were then ultrasonicated (Branson 5510E-DTH, 40 kHz) in a water bath at $25 \text{ }^\circ\text{C}$ for 20 min. The extractant was transferred to a round-bottom flask and evaporated by rotary evaporator (RV10 Basic Rotary Evaporators, IKA Works, VWR, USA) at $30 \text{ }^\circ\text{C}$ until 5 ml remained. The remaining sample was transferred to Eppendorf vials and purged with nitrogen at room temperature overnight. The dried aerosol extractant was stored at $-20 \text{ }^\circ\text{C}$ before analysis of carbonyls by reaction with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) followed by gas

Table 1
Physical characteristic of incense sticks ($n = 3$ for each type of incense).

Incense name	Generic name	Length (cm) (whole stick)	Length (cm) (coated part)	Diameter (mm) (coated part)	Diameter (mm) (base part)	Average weight (g) (entire stick)	Chemical composition (as listed on the packaging)	Color
A	Guanyin tribute sandalwood	16.9 ± 0.1	8.2 ± 0.4	2.2 ± 0.1	0.7 ± 0.1	1.8 ± 0.1	Sandalwood	Yellow
B	Official tibetan tribute	16.8 ± 0.2	8.2 ± 0.7	2.3 ± 0.2	0.7 ± 0.1	1.6 ± 0.1	Unknown	Red
C ^a	Ultra-thin top-class smokeless incense	17.6 ± 0.2	9.4 ± 0.5	1.8 ± 0.1	0.7 ± 0.1	1.3 ± 0.1	Unknown	Yellow
D ^a	Ultra-thin marriott sandalwood (smokeless)	16.7 ± 0.1	8.0 ± 0.1	1.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	Sandalwood	Dark yellow
E	Good luck tribute sandalwood	17.5 ± 0.1	9.4 ± 0.1	1.9 ± 0.1	0.7 ± 0.1	1.3 ± 0.1	Sandalwood	Dark yellow

^a Represents environmental friendly incense.

chromatography–mass spectrometry (GC–MS) analyses. In summary, the carbonyl compounds were dissolved in aqueous solution to a concentration of 1 mg/L. An excess amount of PFBHA in aqueous solution (e.g., 0.5 ml of 5 mg/ml solution) was added to 5 ml of the carbonyl solution. The PFBHA-derivatives solution was then acidified to pH 2 and stood at room temperature for 24 h. This solution was further extracted with 2 ml hexane, washed with 50 mg anhydrous Na₂SO₄, and separated with the aqueous layer. Finally, 1 µl of hexane analyte was transferred for GC–MS analysis. The detailed PFBHA–GC/MS analytical procedure was described previously (Yu et al., 1995). This procedure was repeated for all of the collected samples for GC–MS analysis. Further experimental details were described previously (Dai et al., 2012). The concentrations of carbonyls were determined in each filter sample, and individual carbonyl compounds are listed in Table S1 (Supplementary Material).

2.3.2. Polycyclic aromatic hydrocarbons (PAHs), oxygenated polycyclic aromatic hydrocarbons (OPAHs) and azaarenes (AZAs) analysis

The concentrations of PAHs and alkyl-PAHs, OPAHs, and azaarenes were determined for each filter sample. Filters (4.6 cm diameter) were cut into smaller pieces, transferred to 33 ml accelerated solvent extractor (ASE) extraction cell, and then spiked with 40 µl mixture of 7-deuterated PAHs (10 µg ml⁻¹ each of naphthalene-D8, acenaphthene-D8, phenanthrene-D10, pyrene-D10, chrysene-D12, and benzo[ghi]perylene-D12), 40 µl of 2-deuterated-OPAH (benzophenone-D5, 9,10-anthraquinone-D8: 20 µg ml⁻¹), and 40 µl carbazole-D8 (20 µg ml⁻¹) as internal standard for PAHs + alkyl-PAHs, OPAHs, and azaarenes, respectively. Extra spaces within each ASE cell were filled with an inert bulk sorbent (Isolute HMN, Biotage, Uppsala, Sweden). Each sample was then extracted twice by pressurized liquid extraction (ASE 200; Dionex, Sunnyvale, CA, USA), firstly with dichloromethane and secondly with acetone:dichloromethane (2:1 v/v). The instrumental conditions of the ASE were described previously (Bandowe and Wilcke, 2010; Bandowe et al., 2010, 2011). The two extracts from each sample were combined, spiked with approximately 0.5 ml toluene (as keeper), and concentrated to a volume of <1 ml in a TurboVap II concentration evaporator workstation (Biotage, Charlotte, NC, USA) operated at a bath temperature of 39 °C and N₂ gas pressure of 15 psi (~103 kPa). Concentrated samples were then spiked with 25 µl of fluoranthene-D10 (22 µg ml⁻¹) before being transferred to a 2 ml vial for measurement of polycyclic aromatic compounds (PACs) using a gas chromatograph (Agilent 7890 N) coupled to a mass spectrometer (Agilent 5975C). Samples, blanks, and calibration standards (1 µl) were injected into the GC inlet in splitless mode, then vaporized and transferred with He (as the carrier gas) into an HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm) where all the solutions were separated and transferred into the mass selective detector. Within the MS, the target compounds were ionized in the electron impact ionization mode, and each ionized compound was detected in the selected ion monitoring (SIM) mode. Further details of the instrumental conditions of the GC–MS system, target and qualifier ions for each compound were described previously (Bandowe and Wilcke, 2010; Bandowe et al., 2011, 2014; Lundstedt et al., 2014). Target compounds were quantified using a set of calibration standards measured together with the samples during the same sequence, using the procedure for internal standard quantification. GC–MS data were recorded and processed using Agilent MSD ChemStation software. Further details of the quality control procedures can be found in Text S1 (Supplementary Material).

2.4. Extraction of PM_{2.5} in incense for bioreactivity investigation

The incense PM_{2.5} on Teflon filter was removed using two-stage sonication in methanol, followed by drying in a nitrogen stream (Lee et al., 2014). The particles were then re-suspended in dimethyl sulfoxide (DMSO) [$<0.01\%$ vol. in phosphate-buffered saline (PBS)] for further analysis. The bioreactivity tests are described in Sections 2.4.1–2.4.3.

2.4.1. ROS analysis

Oxidative stress has been implicated in various degenerative diseases such as atherosclerosis. The compound 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) is a cell-permeable non-fluorescent probe and a good overall indicator of oxidative status (Wang and Joseph, 1999). ROS driven from the incense PM_{2.5} were determined via cell-free dichlorodihydrofluorescein (DCFH) assay as described previously (Chuang et al., 2013b). Activated DCFH reagent was exposed to the incense PM_{2.5} at concentrations of 0 (control), 25, and 50 µg/ml. The fluorescence intensity (AU) was measured by Cary Eclipse fluorimeter (Varian Instruments, CA, USA).

2.4.2. Cell culture

Human lung alveolar epithelial A549 cells (American Type Culture Collection, USA) were seeded according to the A549 cell treatment in Lee et al (Lee et al., 2014). The cells were exposed to incense PM_{2.5} at concentrations of 0 (control), 25, and 50 µg/ml for 4 h. Each experiment was conducted in quadruplicate. The concentrations of PM_{2.5} in incense emissions that were used to test for oxidative-inflammatory effects (>80% cell viability) were according to Wilson et al. (2002).

2.4.3. Determination of IL-6, TNF- α and IFN- γ

An enzyme-linked immunosorbent assay (ELISA) (BD OptEIA™ set, BD Biosciences, USA) was used to determine interleukin 6 (IL-6), tumor necrosis factor α (TNF- α) and interferon γ (IFN- γ) levels according to the manufacturers' instructions. Further explanation of IL-6, TNF- α and IFN- γ is available in the literature (Romagnani, 1997; Scheller et al., 2011).

2.5. Calculations and statistical analysis

Due to the small sample size and non-parametric and nature of the dataset, Spearman's rank was used to test for correlations between (1) oxidative stress and inflammation, and (2) all of the analyzed chemical compounds with oxidative-inflammatory cytokines. All the data were analyzed using SPSS (version 21.0, IBM, New York, NY) or GraphPad Prism (Version 5 for Windows) software. The individual chemical compounds and their calculated concentrations are shown in the Supplementary Material.

3. Results and discussion

3.1. Mass concentration of PM_{2.5}

Mass concentrations of PM_{2.5} obtained from incense samples A to E are shown in Fig. 1. The highest and lowest mass concentrations of PM_{2.5} occurred in samples B and D* respectively. The second-lowest mass concentration of PM_{2.5} was in Incense C*. The mass concentrations of PM_{2.5} obtained from environmentally friendly Incense C* and D* in the present study were within the ranges reported in previous study of environmentally friendly incenses (Lee and Wang, 2004).

A combustion chamber experiment by Wang et al. (2006) found total PM_{2.5} concentrations in traditional incense, aromatic incense,

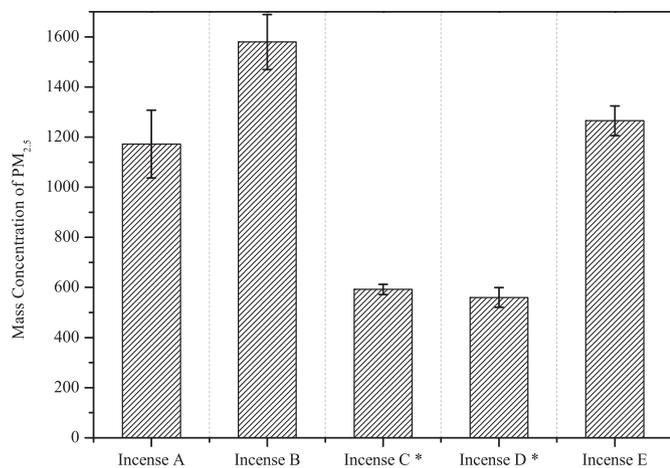


Fig. 1. Mass concentrations ($\mu\text{g}/\text{m}^3$) of $\text{PM}_{2.5}$ in incenses.

and church incense of 1391.0, 501.6, and 6024.8 $\mu\text{g}/\text{m}^3$, respectively. Those results for aromatic incense closely resembled the present findings for samples C* and D*, whereas the findings for traditional incense were similar to those for samples A, B, and E in the present study.

3.2. Carbonyls

The average concentrations of high-molecular-weight (HMW) mono-carbonyl ($C \geq 6$) and di-carbonyl compounds (glyoxal and methylglyoxal) in particulate phase are shown in Fig. 2. Incense A showed highest total concentration of HMW mono-carbonyl and di-carbonyl compounds, whereas sample B recorded the lowest. Glyoxal was the most abundant component in samples A, B, D*, and E, accounting for ~19%, 17%, 20%, and 17% of total carbonyl compounds (mono-carbonyls and di-carbonyls), respectively. Methylglyoxal was the most abundant component in sample C*, accounting for ~18% of total carbonyl compounds. The remaining carbonyls in samples A–E contributed ~4–17% of overall composition. Samples C* and D* contained the lowest percentage

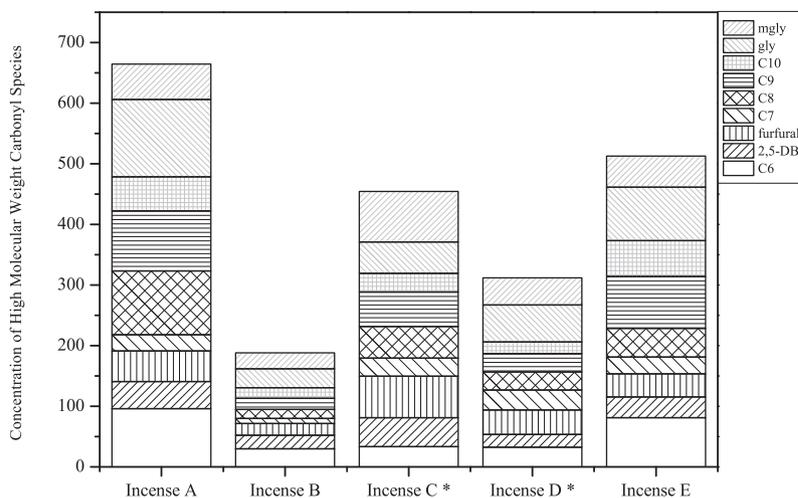
compositions of hexaldehyde (~7.4 and 10.5%) and decaldehyde (~6.8 and 6.2%) compared to the traditional incenses (≥ 14.4 and 8.4%). However, the percentage compositions of furfural (~15.0 and 12.9%), heptaldehyde (~6.5 and 10.5%), and methylglyoxal (~18.3 and 14.3%) in samples C* and D* were higher than in traditional incenses (≤ 10.3 , 5.4 and 14.0% respectively). In total, incenses C* and D* showed the highest percentage compositions of a total of three carbonyl components (furfural, heptaldehyde and methylglyoxal) and the lowest composition of two “other components” (hexaldehyde and decaldehyde).

Previous studies showed HMW mono-carbonyl compounds corresponded to anthropogenic (e.g., vehicular emission) and natural sources (e.g., biogenic emission) (Ho et al., 2006; Grosjean et al., 2002).

The results show that incense burning, together with other daily activities (e.g., meat cooking; Rogge et al., 1991), is a significant anthropogenic source of semi-volatile aldehydes. Overall, α -dicarbonyls were the most abundant components in the incenses. The detection of mono-carbonyls and di-carbonyls in particulate matter from incense emissions could provide further information about the particulate phase partitioning of semi-volatile compounds.

3.3. United States Environmental Protection Agency (U.S. EPA) priority PAHs

Table 2 shows concentration profiles of the U.S. EPA priority PAHs contained in samples A to E. Seven of these priority pollutant PAHs (#, termed Group B2) are considered probable human carcinogens. In all incenses, benzo[b,j,k]fluoranthene was the most abundant component in the Group B2 PAHs. Incense C* showed highest concentrations of benzo[a]anthracene, chrysene and triphenylene, benzo[b,j,k]fluoranthene, indeno[1,2,3-cd]pyrene, and benzo[a]pyrene, whereas incense D* contained the highest concentration of benzo[ghi]perylene. Of all seven Group B2 PAHs, the highest concentrations were found in incenses C* ($n = 5$) and D* ($n = 1$). The three most abundant components in incenses A–E were benzo[b,j,k]fluoranthene, indeno[1,2,3-cd]pyrene, and benzo[a]pyrene, which accounted for ~40–45%, ~13–21%, and ~11–16% of the total Group B2 PAHs composition, respectively. The individual PAHs showed minimal percentage



Concentrations of particle-bound carbonyls species ($\mu\text{g}/\text{g}$) were defined as carbonyl entity mass (μg) of $\text{PM}_{2.5}$ and normalized by the unit mass (g) of incense burnt for incenses A to E.

Fig. 2. Relative Concentration Contributions of Mono-carbonyl and Di-carbonyl (Glyoxal and Methylglyoxal) Compounds in Incenses.

Table 2
Descriptive analysis and relative abundances of U.S. EPA priority PAHs.

U.S. EPA priority PAHs ^c	Incense A ($\mu\text{g/g}$)	Incense B ($\mu\text{g/g}$)	Incense C* ($\mu\text{g/g}$)	Incense D* ($\mu\text{g/g}$)	Incense E ($\mu\text{g/g}$)
Naphthalene	20.9 \pm 4.9	22.4 \pm 6.1	108.8 \pm 15.2	191.7 \pm 176.9	50.5 \pm 4.1
Acenaphthylene	B.D. ^b	B.D.	B.D.	B.D.	B.D.
Acenaphthene	9.3 \pm 1.6	5.8 \pm 0.8	10.3 \pm 2.5	11.9 \pm 4.0	4.8 \pm 0.2
Fluorene	8.9 \pm 2.1	10.9 \pm 0.9	8.7 \pm 2.6	10.8 \pm 2.8	5.4 \pm 1.0
Phenanthrene	17.4 \pm 13.2	9.7 \pm 3.5	19.7 \pm 2.3	23.8 \pm 3.7	15.3 \pm 8.1
Anthracene	3.4 \pm 1.9	2.7 \pm 0.6	2.9 \pm 0.5	3.0 \pm 0.9	1.5 \pm 0.5
Fluoranthene	1.7 \pm 0.6	1.0 \pm 0.2	2.1 \pm 0.1	2.6 \pm 0.7	2.2 \pm 2.0
Pyrene	12.4 \pm 4.7	7.9 \pm 1.5	14.9 \pm 1.0	12.6 \pm 0.9	10.4 \pm 2.6
Benzo[a]anthracene ^a	8.6 \pm 2.5	7.0 \pm 1.2	9.3 \pm 0.4	7.8 \pm 0.6	7.7 \pm 1.9
Chrysene and Triphenylene ^a	4.0 \pm 1.4	3.5 \pm 0.6	4.2 \pm 0.3	3.4 \pm 0.5	3.5 \pm 0.7
Benzo[b,j,k]fluoranthene ^a	24.5 \pm 10.1	16.8 \pm 2.3	35.4 \pm 4.2	26.9 \pm 2.0	19.7 \pm 6.1
Benzo[a]pyrene ^a	9.5 \pm 4.7	5.0 \pm 0.5	10.7 \pm 0.5	8.8 \pm 1.7	4.9 \pm 1.0
Dibenz[a,h]anthracene ^a	B.D.	B.D.	B.D.	B.D.	0.5 \pm 0.82
Benzo[ghi]perylene ^a	2.4 \pm 0.2	1.8 \pm 0.5	3.8 \pm 0.1	4.3 \pm 0.5	2.0 \pm 0.3
Indeno[1,2,3-cd]pyrene ^a	12.0 \pm 6.5	5.3 \pm 0.5	15.7 \pm 3.5	13.3 \pm 1.5	7.9 \pm 3.0

^a Indicated by U.S. EPA as probable human carcinogen.

^b B.D. = below detection limit.

^c Particle-bound PAH concentrations ($\mu\text{g/g}$) were defined as PAH mass (μg) of $\text{PM}_{2.5}$ and normalized by the unit mass (g) of $\text{PM}_{2.5}$ for incenses A to E. The partition of PAHs is governed by temperature, relative humidity, pressure, and molecular weight of individual components.

variation, suggesting that all five types of incense shared a common PAH concentration profile. Both incense C* (65.6 \pm 13.0 $\mu\text{g/g}$) and D* (53.2 \pm 9.6 $\mu\text{g/g}$) demonstrated higher total concentrations of non-volatile PAHs (5- and 6 rings) compared to the traditional incenses (28.9 \pm 6.1–48.5 \pm 10.2 $\mu\text{g/g}$).

Schauer et al. (2003) analyzed PAHs in particulate matter from air sampled at urban traffic junctions and suburban residential areas in Munich, Germany, and found total concentrations of non-volatile PAHs (5- and 6 rings) within the range 30 \pm 20–110 \pm 30 $\mu\text{g/g}$. Burning incense (e.g., outside a temple) can contribute non-volatile particulate PAHs at levels comparable to that of automobile pollution at busy traffic junctions. A previous study showed PAHs attached to fine particles contained in environmental samples could enhance particle-induced inflammatory effects in lung tissues (Heinrich et al., 1994). The presence of particulate-phase PAHs in all incenses could cause the same condition; this requires further investigation.

Benzo[a]pyrene is listed as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2012), and was ranked 8th of 275 on the priority list of hazardous substances in 2011 by the Agency for Toxic Substances and Disease Registry (ATSDR, 2011). Benzo[a]pyrene is often used as a main indicator or marker of carcinogenic PAHs (Boström et al., 2002) and can be released by numerous burning processes (e.g., cigarette smoke) (ATSDR, 1995). The most pronounced carcinogenic effect in lungs due to sidestream smoke was attributed to PAH structures containing 4 or more rings (5 carcinomas of lungs in 35 rats) (Grimmer et al., 1988).

In a case-referent study by Tse et al. (2011), observed association between incense exposure and lung cancer was restricted primarily to smokers. Cigarette smoking coupled with high cumulative incense exposure at home produced a synergistic effect on lung cancer (i.e., compared with non-smokers who never used incense). In such circumstances, the presence of benzo[a]pyrene in samples A–E could enhance overall lung exposure to benzo[a]pyrene, potentially exacerbating the carcinogenic effect in the lungs. Incense C*, with the highest benzo[a]pyrene emissions, could have more noticeable carcinogenic effect, although further investigation is required. The findings for concentrations of Group B2 PAHs indicate that incenses marketed as being environmentally friendly perform poorly for carcinogenic PAHs

emissions.

3.4. Other PAHs, OPAHs, and AZAs

Fig. 3 shows concentrations of other PAH, OPAH, and AZA species in the five incenses. Incenses C* and D* showed the two highest concentrations of 1,4-anthraquinone. Incense C* also contained the highest concentrations of 5,12-naphthacenequinone, 1-acenaphthenone, benzo[a]fluorenone, 4-H-cyclopenta(d,e,f)phenanthrene, 1-methylphenanthrene, and benzo[e]pyrene. Based on the results, 4 out of 6 OPAHs in Fig. 3 were from environmentally friendly samples C* (3 components) and D* (1 component). Incense C* contained the highest concentrations of three out of five “other PAH” compounds. Overall, 7 out of 11 compounds in Fig. 3 were most concentrated in environmentally friendly incenses, and Incense C* showed the highest concentrations for six components.

3.5. Bioreactivity

Fig. 4 shows dose-dependent responses in oxidative potential, IL-6, TNF- α , and IFN- γ levels. Oxidative potentials of samples followed the sequence: E > B > D* > C* > A at 50 $\mu\text{g/ml}$. The levels of IL-6 followed the sequence: C* > A > D* > B > E. Samples A and B contained the highest levels TNF- α and IFN- γ , whereas the lowest levels were present in C* for TNF- α and E for IFN- γ .

Particulate-induced health effects are driven by the production of ROS in respiratory environments, causing inflammation and concomitant injury and disease (Poli and Parola, 1997). Incense burning is a common source of indoor air pollution, but there is limited data on combustion-derived products with respect to oxidative-inflammatory responses (Chuang et al., 2011a). Previous studies suggested that incense PM increased oxidative stress (Chuang et al., 2011b), inflammation (Lin et al., 2013), and cell cycle dysregulation (Chuang et al., 2013a).

In this study, the PM of incense samples shows differing degree of oxidative-inflammatory responses, which could be associated with the incense composition. Sandalwood-based incense A contained the highest increase of TNF- α and IFN- γ and the second-highest levels of IL-6. However, sandalwood-based incense E contained less IL-6, TNF- α and IFN- γ . The physicochemical

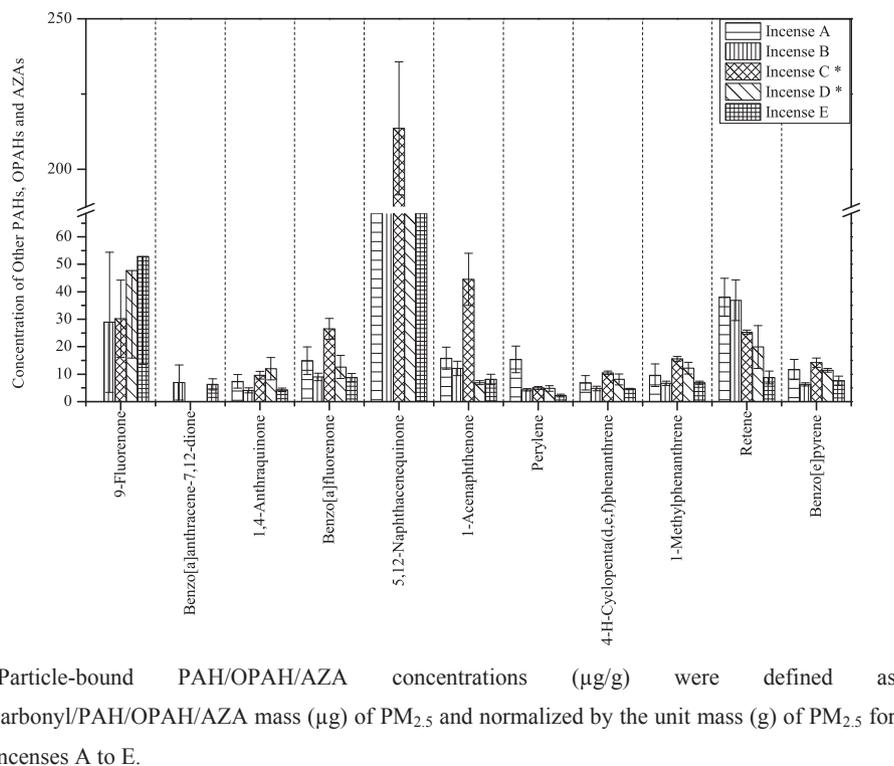


Fig. 3. Descriptive Analysis and Relative Abundances of other PAHs, OPAHs, and AZAs.

characteristics of PM are important for regulating particle toxicity. Therefore, potential health effects depend upon the chemical composition of PM and corresponding bioreactivity.

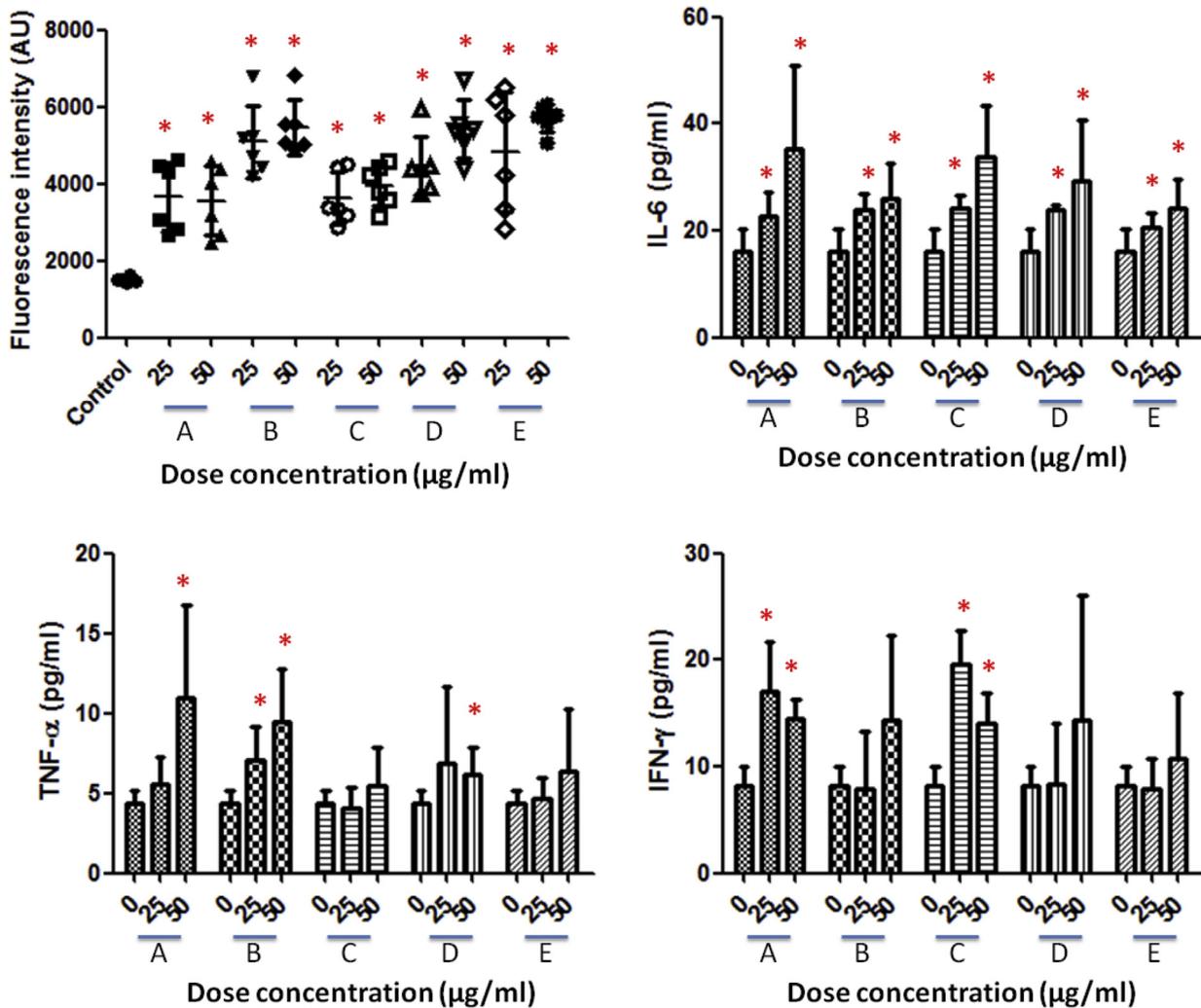
3.6. Correlation between chemical compounds and oxidative-inflammatory responses

To identify the concentrations of chemical compounds per unit mass (μg) of $\text{PM}_{2.5}$ and the association with bioreactivity during incense burning, Spearman correlation coefficients (R) were calculated between ROS-inflammatory activity and selected PM compounds (see Table 3). Only those chemical components that demonstrated moderate to strong correlations with ROS-inflammatory responses are presented. A total of 21 carbonyl and 4 azaarene compounds were analyzed for correlation between chemicals and oxidative stress and inflammatory cytokines. None of the carbonyl or azaarene compounds showed moderate to strong positive correlations with the oxidative-inflammatory response tests of samples A to E. A total of 42 PAH and OPAH compounds were analyzed: 22 (52%) demonstrated moderate to strong positive correlations with oxidative potential, $\text{TNF-}\alpha$, IL-6, and $\text{IFN-}\gamma$; 18 (~43%) showed moderate to strong positive correlation with IL-6; 11 of 16 U.S. EPA priority PAHs (69% of the total) showed moderate to strong positive correlations with IL-6 (Table 3); 6 of the 7 Group B2 PAHs showed moderate to strong positive correlations with IL-6. No correlations were found between $\text{TNF-}\alpha$ and any of the chemical species analyzed. Strong positive correlation was the only observation between IL-6 and total concentration of the OPAHs analyzed. Strong positive correlations were also found between IL-6 and total concentration of Group B2 EPA, defined as probable human carcinogen PAHs. Bioreactivity (determined by IL-6) was

strongly correlated with benzo[a]anthracene, chrysene and triphenylene, benzo[b,j,k]fluoranthene, and benzo[a]pyrene. These four compounds are all potential human carcinogens and highly toxic in the environment. When PAHs are inhaled into the lungs, they are converted to their hydroxyl derivatives, such as quinones, by cytochrome P450, epoxide hydrolase and dihydrodiol dehydrogenase, leading to oxidative-inflammatory responses (Xia et al., 2004). For example, previous reports showed that benzo[a]pyrene induced oxidative stress imbalance and inflammatory reaction *in vivo* (Shahid et al., 2016) and *in vitro* (Shi et al., 2015).

As shown in Table 2, despite being marketed as environmentally friendly, incense C* emitted the highest concentrations of potentially carcinogenic PAH compounds that show strong positive correlation with inflammatory responses. The summation of Group B2 PAHs ($\Sigma_7\text{PAHs}$) further demonstrated strong positive correlation with IL-6. Consequently, although individual dibenz(a,h)anthracene compound did not show moderate to strong positive correlation with IL-6, the overall $\Sigma_7\text{PAHs}$ result suggests that the correlation between dibenz(a,h)anthracene and inflammatory response should not be underestimated and possibly plays a role in the overall inflammatory response.

Six of 15 OPAH compounds (40%) revealed moderate to strong positive correlations with oxidative potential, IL-6, and $\text{IFN-}\gamma$ responses. Strong positive correlation was observed between IL-6 and 5,12-naphthacenequinone; furthermore, emissions from environmentally friendly incense C* were ~3.1 times greater than those from the lowest traditional incense E. Only 3 out of 15 OPAH compounds showed moderate to strong positive correlations with IL-6; however, moderate to strong positive correlation was demonstrated between IL-6 and total concentration of OPAHs analyzed. Individually, the other 12 OPAH compounds did



Oxidative potentials of PM_{2.5} followed the sequence: E > B > D* > C* > A at 50 μg/ml. The levels of IL-6 followed the sequence: C* > A > D* > B > E. Samples A and B contained the highest levels TNF-α and IFN-γ, whereas the lowest levels were present in E and C* respectively.

Fig. 4. Oxidative Potentials, IL-6, TNF-α and IFN-γ Production of Incenses. (*p < 0.05 compared with control).

not show significant correlation; nonetheless, the strong positive correlation between inflammatory response and total OPAH concentration suggests that these 12 OPAH compounds in combination, along with the three previously identified OPAH compounds, are implicated in the production of IL-6. Therefore, the concentration impact of the 12 individual OPAH compounds on inflammatory responses should not be overlooked. Knecht and colleagues examined 38 different OPAHs and the resultant developmental outcomes in Zebrafish embryos (Knecht et al., 2013). They suggested that the OPAHs contained adjacent diones on 6-carbon moieties or terminal, para-diones on multi-ring structures are the most toxic, which can increase oxidative-inflammatory response.

The Spearman correlation coefficients demonstrated that a substantial number of PAH and OPAH compounds showed moderate to strong positive correlations with oxidative potential, IL-6, TNF-α, and IFN-γ. This finding warrants further investigation from appropriate regulatory bodies, and possibly the

introduction of new regulations and guidelines for the content and use of incense.

4. Conclusions

The characteristics were investigated of fine particulate matter (PM_{2.5}) emitted by burning incense. The incenses marketed as being environmentally friendly generated less PM_{2.5}; however, mixed results were obtained for carbonyl, PAH, OPAH, and AZA emissions. Taken together, incenses C* and D* emitted the highest percentage compositions of three carbonyl components (furfural, heptaldehyde, and methylglyoxal). The environmentally friendly incenses emitted higher total concentrations of non-volatile PAHs than did traditional incenses; and the highest concentrations for 4 out of 6 listed OPAHs. More than half of the measured PAH and OPAH compounds showed moderate to strong positive correlations with oxidative-inflammatory responses. The summation of Group B2 PAHs and total concentration of OPAHs

Table 3

Spearman correlation coefficients (R) of reactive oxygen species (ROS) and inflammatory activity in various chemical compounds (R > 0.60; 0.05 significance level).

U.S. EPA priority PAHs	Oxidative potential (AU)	IL-6 (pg/ml)	IFN- γ (pg/ml)
Naphthalene			
Acenaphthylene			
Acenaphthene		0.77 ^a	
Fluorene		0.89 ^a	
Phenanthrene		0.69 ^b	
Anthracene		0.75 ^b	
Fluoranthene			
Pyrene		0.79 ^a	
Benzo[a]anthracene ^c		0.70 ^b	
Chrysene and Triphenylene ^c		0.71 ^b	
Benzo[b,j,k]fluoranthene ^c		0.76 ^b	
Benzo[a]pyrene ^c		0.72 ^b	
Dibenz[a,h]anthracene ^c			
Benzo[ghi]perylene ^c		0.66 ^b	
Indeno[1,2,3-cd]pyrene ^c		0.65 ^b	
Σ_7 PAHs (Summation of total Group B2 PAHs)		0.79 ^a	
Other PAHs, OPAHs, and AZAs			
9-Fluorenone	0.74 ^b		
Benzo[a]anthracene-7,12-dione	0.77 ^a		
1,4-Anthraquinone		0.65 ^b	
Benzo[a]fluorenone		0.75 ^b	
5,12-Naphthacenequinone		0.81 ^a	
1-Acenaphthenone			0.66 ^b
Perylene			0.65 ^b
4-H-Cyclopenta(d,e,f)phenanthrene		0.83 ^a	
1-Methylphenanthrene		0.75 ^b	
Retene		0.75 ^b	
Benzo[e]pyrene		0.76 ^b	
Σ OPAHs (Summation of total OPAHs)		0.72 ^b	

^a Correlation is significant at the 0.01 level (2-tailed).^b Correlation is significant at the 0.05 level (2-tailed).^c Indicated by U.S. EPA as probable human carcinogen.

showed strong positive correlation with inflammatory response.

The findings support claims that incense burning is associated with health problems. The results suggest the need to revise current regulations on the content and usage of incense products.

Acknowledgments

This study was supported under the Research Grants Council of the Hong Kong Special Administrative Region China (Project No. CUHK 412612). The authors thank Chi-Sing Chan and Michael Wendler for laboratory assistance, and Xiao-Cui Chen for valuable comments on the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2016.02.053>.

References

- Agency for Toxic Substances and Disease Registry (ATSDR), 2011. The priority list of hazardous substances that will be the subject of toxicological profiles. <http://www.atsdr.cdc.gov/SPL/index.html>.
- Ballester, F., Medina, S., Boldo, E., Goodman, P., Neuberger, M., Iniguez, C., Künzli, N., 2008. Reducing ambient levels of fine particulates could substantially improve health: a mortality impact assessment for 26 European cities. *J. Epidemiol. Community Health* 62, 98–105.
- Bandowe, B.A.M., Lueso, M.G., Wilcke, W., 2014. Oxygenated polycyclic aromatic hydrocarbons and azaarenes in urban soils: a comparison of a tropical city (Bangkok) with two temperate cities (Bratislava and Gothenburg). *Chemosphere* 107, 407–414.
- Bandowe, B.A.M., Shukurov, N., Kersten, M., Wilcke, W., 2010. Polycyclic aromatic hydrocarbons (PAHs) and their oxygen-containing derivatives (OPAHs) in soils from the Angren industrial area. *Uzb. Environ. Pollut.* 158, 2888–2899.
- Bandowe, B.A.M., Sobocka, J., Wilcke, W., 2011. Oxygen-containing polycyclic aromatic hydrocarbons (OPAHs) in urban soils of Bratislava, Slovakia: patterns, relation to PAHs and vertical distribution. *Environ. Pollut.* 159, 539–549.
- Bandowe, B.A.M., Wilcke, W., 2010. Analysis of polycyclic aromatic hydrocarbons and their oxygen-containing derivatives and metabolites in soils. *J. Environ. Qual.* 39, 1349–1358.
- Bitterle, E., Karg, E., Schroepel, A., Kreyling, W., Tippe, A., Ferron, G., Schmid, O., Heyder, J., Maier, K., Hofer, T., 2006. Dose-controlled exposure of A549 epithelial cells at the air–liquid interface to airborne ultrafine carbonaceous particles. *Chemosphere* 65, 1784–1790.
- Boldo, E., Medina, S., Le Tertre, A., Hurley, F., Mücke, H.-G., Ballester, F., Aguilera, I., 2006. Apeis: health impact assessment of long-term exposure to PM_{2.5} in 23 European cities. *Eur. J. Epidemiol.* 21, 449–458.
- Boström, C.-E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T., Rannug, A., Törnqvist, M., Victorin, K., Westerholm, R., 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ. Health Perspect.* 110, 451.
- Charrier, J.G., McFall, A.S., Richards-Henderson, N.K., Anastasio, C., 2014. Hydrogen peroxide formation in a surrogate lung fluid by transition metals and quinones present in particulate matter. *Environ. Sci. Technol.* 48, 7010–7017.
- Chen, C.-C., Lee, H., 1996. Genotoxicity and DNA adduct formation of incense smoke condensates: comparison with environmental tobacco smoke condensates. *Mutat. Res. Genet. Toxicol.* 367, 105–114.
- Chuang, H.C., Jones, T.P., Bérubé, K.A., 2011a. Combustion particles emitted during church services: implications for human respiratory health. *Environ. Int.* 40, 137–142.
- Chuang, H.C., Jones, T., Chen, T.T., Bérubé, K., 2013a. Cytotoxic effects of incense particles in relation to oxidative stress, the cell cycle and F-actin assembly. *Toxicol. Lett.* 220, 229–237.
- Chuang, H.C., Jones, T.P., Lung, S.C., Bérubé, K.A., 2011b. Soot-driven reactive oxygen species formation from incense burning. *Sci. Total Environ.* 409, 4781–4787.
- Chuang, H.C., Cheng, Y.L., Lei, Y.C., Chang, H.H., Cheng, T.J., 2013b. Protective effects of pulmonary epithelial lining fluid on oxidative stress and DNA single-strand breaks caused by ultrafine carbon black, ferrous sulphate and organic extract of diesel exhaust particles. *Toxicol. Appl. Pharmacol.* 266, 329–334.
- Clancy, L., Goodman, P., Sinclair, H., Dockery, D.W., 2002. Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study. *Lancet* 360, 1210–1214.
- Cohen, R., Sexton, K.G., Yeatts, K.B., 2013. Hazard assessment of United Arab Emirates (UAE) incense smoke. *Sci. Total Environ.* 458, 176–186.
- Dai, W.-T., Ho, S.S.H., Ho, K.-F., Cao, J.-J., 2012. Characterization of particulate-phase high molecular weight mono-carbonyls (C#> 5) and dicarbonyls in urban atmosphere of Xi'an, China. *Aerosol Air Qual. Res.* 12, 892–901.
- Grimmer, G., Brune, H., Dettbarn, G., Naujack, K.-W., Mohr, U., Wenzel-Hartung, R., 1988. Contribution of polycyclic aromatic compounds to the carcinogenicity of sidestream smoke of cigarettes evaluated by implantation into the lungs of rats.

- Cancer Lett. 43, 173–177.
- Grosjean, D., Grosjean, E., Moreira, L.F., 2002. Speciated ambient carbonyls in Rio de Janeiro, Brazil. *Environ. Sci. Technol.* 36, 1389–1395.
- Heinrich, U., Roller, M., Pott, F., 1994. Estimation of a lifetime unit lung cancer risk for benzo (a) pyrene based on tumour rates in rats exposed to coal tar/pitch condensation aerosol. *Toxicol. Lett.* 72, 155–161.
- Ho, K., Lee, S., Cao, J., Kawamura, K., Watanabe, T., Cheng, Y., Chow, J.C., 2006. Dicarboxylic acids, ketocarboxylic acids and dicarbonyls in the urban roadside area of Hong Kong. *Atmos. Environ.* 40, 3030–3040.
- World Health Organization International Agency for Research on Cancer (IARC), 2012. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. http://monographs.iarc.fr/ENG/Classification/vol1_113.php.
- Jetter, J.J., Guo, Z., McBrien, J.A., Flynn, M.R., 2002. Characterization of emissions from burning incense. *Sci. Total Environ.* 295, 51–67.
- Knecht, A.L., Goodale, B.C., Truong, L., Simonich, M.T., Swanson, A.J., Matzke, M.M., Anderson, K.A., Waters, K.M., Tanguay, R.L., 2013. Comparative developmental toxicity of environmentally relevant oxygenated PAHs. *Toxicol. Appl. Pharmacol.* 271, 266–275.
- Lee, Y., Calori, G., Hills, P., Carmichael, G., 2002. Ozone episodes in urban Hong Kong 1994–1999. *Atmos. Environ.* 36, 1957–1968.
- Lee, S.-C., Wang, B., 2004. Characteristics of emissions of air pollutants from burning of incense in a large environmental chamber. *Atmos. Environ.* 38, 941–951.
- Lee, K.-Y., Wong, C.K.-C., Chuang, K.-J., Bien, M.-Y., Cao, J.-J., Han, Y.-M., Tian, L., Chang, C.-C., Feng, P.-H., Ho, K.-F., Chuang, H.-C., 2014. Methionine oxidation in albumin by haze fine particulate matter: an in vitro and in vivo study. *J. Hazard. Mater.* 274, 384–391.
- Lin, L.-Y., Liu, I.J., Chuang, H.-C., Lin, H.-Y., Chuang, K.-J., 2013. Size and composition effects of household particles on inflammation and endothelial dysfunction of human coronary artery endothelial cells. *Atmos. Environ.* 77, 490–495.
- Lundstedt, S., Bandowe, B., Wilcke, W., Boll, E., Christensen, J., Vila, J., Grifoll, M., Faure, P., Biache, C., Lorgeoux, C., 2014. First intercomparison study on the analysis of oxygenated polycyclic aromatic hydrocarbons (oxy-PAHs) and nitrogen heterocyclic polycyclic aromatic compounds (N-PACs) in contaminated soil. *TrAC Trends Anal. Chem.* 57, 83–92.
- Mannix, R.C., Nguyen, K.P., Tan, E.W., Ho, E.E., Phalen, R.F., 1996. Physical characterization of incense aerosols. *Sci. Total Environ.* 193, 149–158.
- Masih, J., Singhvi, R., Kumar, K., Jain, V., Taneja, A., 2012. Seasonal variation and sources of polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air in a semi arid tract of northern India. *Aerosol Air Qual. Res.* 12, 515–525.
- Matsumura, F., Vogel, C.F., Kobayashi, R., Liu, X., Wong, P., Wu, D., Kaur, R., Chiang, T., Kado, N., 2010. Assessment of health impacts of particulate matter from indoor air sources phase I: development of in vitro methodology. *Contract* 5, 302.
- Medina, S., Plasencia, A., Ballester, F., Mücke, H., Schwartz, J., 2004. Apehis: public health impact of PM10 in 19 European cities. *J. Epidemiol. Community Health* 58, 831–836.
- Poli, G., Parola, M., 1997. Oxidative damage and fibrogenesis. *Free Radic. Biol. Med.* 22, 287–305.
- Rogge, W.F., Hildemann, L.M., Mazurek, M.A., Cass, G.R., Simoneit, B.R., 1991. Sources of fine organic aerosol. 1. charbroilers and meat cooking operations. *Environ. Sci. Technol.* 25, 1112–1125.
- Romagnani, S., 1997. The th1/th2 paradigm. *Immunol. Today* 18, 263–266.
- Shahid, A., Ali, R., Ali, N., Kazim Hasan, S., Rashid, S., Majed, F., Sultana, S., 2016. Attenuation of genotoxicity, oxidative stress, apoptosis and inflammation by rutin in benzo(a)pyrene exposed lungs of mice: plausible role of NF-kappaB, TNF-alpha and Bcl-2. *J. Complementary Integr. Med.* 13 (1), 17–29.
- Schauer, C., Niessner, R., Pöschl, U., 2003. Polycyclic aromatic hydrocarbons in urban air particulate matter: decadal and seasonal trends, chemical degradation, and sampling artifacts. *Environ. Sci. Technol.* 37, 2861–2868.
- Scheller, J., Chalaris, A., Schmidt-Arras, D., Rose-John, S., 2011. The pro-and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica Biophysica Acta (BBA)-Mol. Cell Res.* 1813, 878–888.
- Shi, Q., Haenen, G.R., Maas, L., Arlt, V.M., Spina, D., Vasquez, Y.R., Moonen, E., Veith, C., Van Schooten, F.J., Godschalk, R.W., 2015. Inflammation-associated extracellular beta-glucuronidase alters cellular responses to the chemical carcinogen benzo[a]pyrene. *Archives Toxicol.* <http://dx.doi.org/10.1007/s00204-015-1593-7>.
- Tse, L.A., Yu, I.T.-s., Qiu, H., Au, J.S., Wang, X-r., 2011. A case-referent study of lung cancer and incense smoke, smoking, and residential radon in Chinese men. *Environ. Health Perspect.* 119, 1641.
- U.S. Centers for Disease Control Agency for Toxic Substances and Disease Registry (ATSDR), 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). <http://www.atsdr.cdc.gov/toxprofiles/tp69.pdf>.
- Wang, B., Lee, S., Ho, K., 2006. Chemical composition of fine particles from incense burning in a large environmental chamber. *Atmos. Environ.* 40, 7858–7868.
- Wang, H., Joseph, J.A., 1999. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic. Biol. Med.* 27, 612–616.
- Wilson, M.R., Lightbody, J.H., Donaldson, K., Sales, J., Stone, V., 2002. Interactions between ultrafine particles and transition metals in vivo and in vitro. *Toxicol. Appl. Pharmacol.* 184, 172–179.
- Xia, T., Korge, P., Weiss, J.N., Li, N., Venkatesen, M.I., Sioutas, C., Nel, A., 2004. Quinones and aromatic chemical compounds in particulate matter induce mitochondrial dysfunction: implications for ultrafine particle toxicity. *Environ. Health Perspect.* 112, 1347–1358.
- Yu, J., Jeffries, H.E., Le Lacheur, R.M., 1995. Identifying airborne carbonyl compounds in isoprene atmospheric photooxidation products by their PFBHA oximes using gas chromatography/ion trap mass spectrometry. *Environ. Sci. Technol.* 29, 1923–1932.