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Characterization of Chemical Components and Bioreactivity of Fine Particulate Matter ($PM_{2.5}$) during Incense Burning

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Abstract

The chemical and bioreactivity properties of fine particulate matter ($PM_{2.5}$) emitted during controlled burning of different brands of incense were characterized. The mass of $PM_{2.5}$ emissions from supposedly environmentally friendly incenses were lower than from 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 ($\sum_7$PAHs/ΣOPAHs). 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.

Capsule:

Environmentally friendly incenses can produce higher emissions of carcinogenic PAH

compounds than traditional incenses, which are positively correlated with inflammatory responses.

Keywords:

Incense, Carbonyls, PAHs, OPAHs, Oxidative Stress

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 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 (Armstrong et al., 2004; Boffetta et al., 1997). 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 (•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 H., 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 μm: $PM_{2.5}$) 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 μg/ml (Matsumura et al., 2010). $PM_{2.5}$ 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 $PM_{2.5}$ (Lee et al., 2002; Lee and Wang, 2004).

This study evaluates the potential health effects of exposure to smoke from traditional and supposed environmentally friendly types of incense, deducing corresponding health outcomes. It identifies the chemical components of $PM_{2.5}$ and further characterizes the relationship between $PM_{2.5}$ 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* supposedly "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 m × 3.05 m × 2.05 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 $PM_{2.5}$ impactors (Airmetrics, OR, USA) were used to collect $PM_{2.5}$ 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. $PM_{2.5}$ samples were collected immediately after ignition on Teflon membrane (Φ = 47 mm, Pall Corporation, USA) and two quartz microfiber filters (Φ = 47 mm, Whatman, UK). The samples were collected for 1 hour after the incense was extinguished. A blank $PM_{2.5}$ sample (< 5 μg 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 °C) before chemical and biological analysis. The quartz filters were pre-heated at 900 °C for 3 hours to remove any organic vapors on the filters. All filters were pre-conditioned at 23±0.5 °C and 50±5% relative humidity (RH) for 48 hours before and after weighing the samples. Each filter was weighed on a microbalance (±1 μg precision, Sartorius AG MC5, Germany) before and after $PM_{2.5}$ sample collection.

The mass concentration of collected $PM_{2.5}$ 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 °C for 20 minutes. The extractant was transferred to a round-bottom flask and evaporated by rotary evaporator (RV10 Basic Rotary Evaporators, IKA Works, VWR, USA) at 30 °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 °C before analysis of carbonyls by reaction with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) followed by gas 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 hours. This solution was further extracted with 2 ml hexane, washed with 50 mg anhydrous Na_2SO_4, 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; Bandowe et al., 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 N_2 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 PACs using a gas chromatograph (Agilent 7890 N) coupled to a mass spectrometer (Agilent 5975 C). 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; Bandowe et al., 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., 2013). 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 onto surface-treated, 24-well Transwells at a density of 1×10^5 cells/ml and incubated for 24 h (3×10^4 cells/well; BD Biosciences, UK). Cells were cultured in RPMI containing 10% fetal bovine serum, penicillin, and streptomycin, and incubated in air at 37 °C, 95% humidity, and 5% CO_2. 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 OptEIATM 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-inflammation 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 Figure 1. The highest and lowest mass concentrations of $PM_{2.5}$ occurred in samples E (1265.2±59.2 $\mu g/m^3$) and D* (559.9±39.2 $\mu g/m^3$) respectively. The second-lowest mass concentration of $PM_{2.5}$ was in Incense C* (592.1±20.0 $\mu g/m^3$). The mass concentrations of $PM_{2.5}$ obtained from environmentally friendly Incense C* (592.1±20.0 $\mu g/m^3$) and D* (559.9±39.2 $\mu g/m^3$) 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 B. et al. (2006) found total $PM_{2.5}$ concentrations in traditional incense, aromatic incense, and church incense of 1391.0, 501.6, and 6024.8 $\mu g/m^3$, 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 D* in the present study.

3.2 Carbonyls

The average concentrations of high-molecular-weight (HMW) mono-carbonyl (C ≥ 6) and di-carbonyl compounds (glyoxal and methylglyoxal) in particulate phase are shown in Figure 2. Incense A (664.3±45.9 μg/g) showed highest total concentration of HMW mono-carbonyl and di-carbonyl compounds, whereas sample B recorded the lowest (188.3±27.7 μg/g). Glyoxal was the most abundant component in samples A (128.0±26.6 μg/g), B (31.2±4.2 μg/g), D* (61.1±10.1 μg/g), and E (88.5±3.3 μg/g), 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* (83.3±11.5 μg/g), accounting for ~18% of total carbonyl compounds. The remaining carbonyls in samples A–E contributed ~4–16% of overall composition. Samples C* and D* contained the lowest percentage compositions of hexanaldehyde (~7.4 and 10.5%) and nonanaldehyde (~6.7 and 6.3%) compared to the traditional incenses (≥ 14.4 and 8.4%). However, the percentage compositions of furfural (~15.0 and 12.9%), heptaldehyde (~6.6 and 10.5%), and methylglyoxal (~18.3 and 14.3%) in samples C* and D* were higher than in traditional incenses (≤ 10.2, 5.4 and 14.1% respectively). In total, incenses C* and D* showed the highest percentage compositions of a total of three carbonyl components and the

lowest composition of two “other components.” 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 (16.8±2.3–35.4±4.2 μg/g). Incense C* showed highest concentrations of benzo[a]anthracene (9.3±0.4 μg/g), chrysene and triphenylene (4.2±0.3 μg/g), benzo[b,j,k]fluoranthene (35.4±4.2 μg/g), indeno[1,2,3-cd]pyrene (15.7±3.5 μg/g), and benzo[a]pyrene (10.7±0.5 μg/g), whereas incense D* contained the highest concentration of benzo[ghi]perylene (4.3±0.5 μg/g). Of all seven Group B2 PAHs, the

highest concentrations were found in incenses C* (n=6) 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 variation, suggesting that all five types of incense shared a common PAH concentration profile. Both incense C* (65.6±13.0 μg/g) and D* (53.2±9.6 μg/g) demonstrated higher total concentrations of non-volatile PAHs (5- and 6 rings) compared to the traditional incenses (28.9±6.1-48.5±10.2 μg/g). Schauer et al. 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±20–110±30 μg/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, and 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 supposedly environmentally friendly incenses perform poorly for carcinogenic PAHs emissions.

3.4 Other PAHs, OPAHs, and AZAs

Figure 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 (12.0±4.1 and 9.6±1.4 μg/g). Incense C* also contained the highest concentrations of

5,12-naphthacenequinone (213.6±22.1 μg/g), 1-acenaphthenone (44.5±9.5 μg/g), benzo[a]fluorenone (26.5±3.8 μg/g), 4-H-cyclopenta(d,e,f)phenanthrene (10.5±0.6 μg/g), 1-methylphenanthrene (15.6±0.9 μg/g), and benzo[e]pyrene (14.2±1.7 μg/g). Based on the results, 4 out of 6 OPAHs in Figure 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 Figure 3 were most concentrated in environmentally friendly incenses, and Incense C* showed the highest concentrations for six components.

3.5 Bioreactivity

Figure 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 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. 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., 2013). 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 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 $PM_{2.5}$ 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, TNF-α, IL-6,

and IFN-γ; 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 TNF-α and any of the chemical species analyzed. Strong positive correlation was the only observation between IL-6 and total concentration of the OPAHs analyzed (R = 0.72). Strong positive correlations were also found between IL-6 and total concentration of Group B2 EPA, defined as probable human carcinogen PAHs (R = 0.79). Bioreactivity (determined by IL-6) was strongly correlated with benzo[a]anthracene (R = 0.70), chrysene and triphenylene (R = 0.71), benzo[b,j,k]fluoranthene (R = 0.76), and benzo[a]pyrene (R = 0.72). These four compounds are all potential human carcinogens and highly toxic in the environment. 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 ($\sum_7$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 Σ_7PAHs 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 IFN-γ responses. Strong positive correlation was observed between IL-6 and 5,12-naphthacenequinone (R=0.81); 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 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. 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 supposedly environmentally friendly incenses 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 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.

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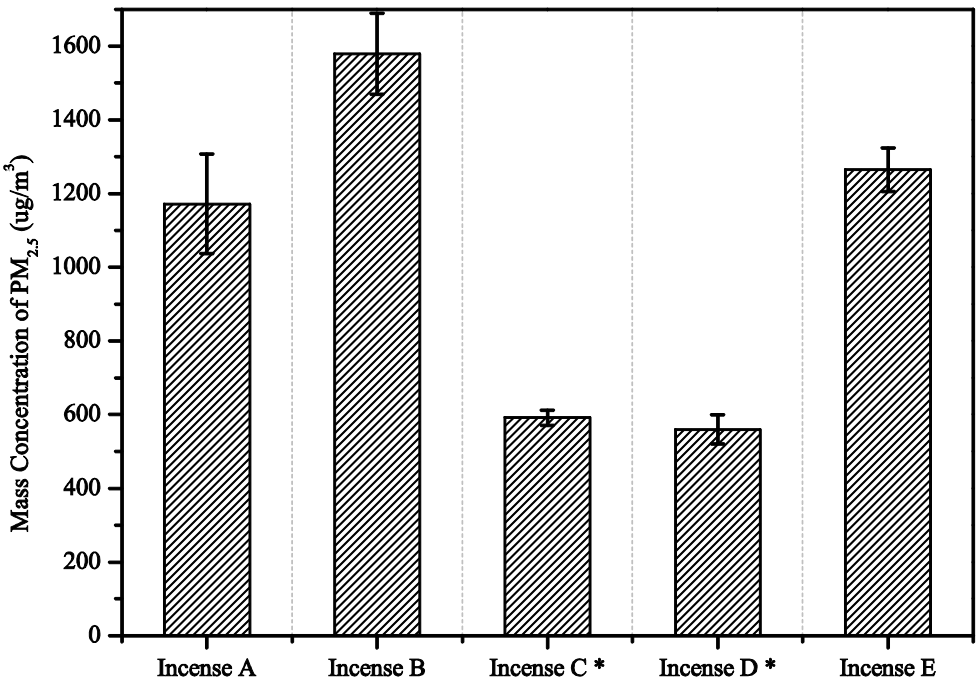
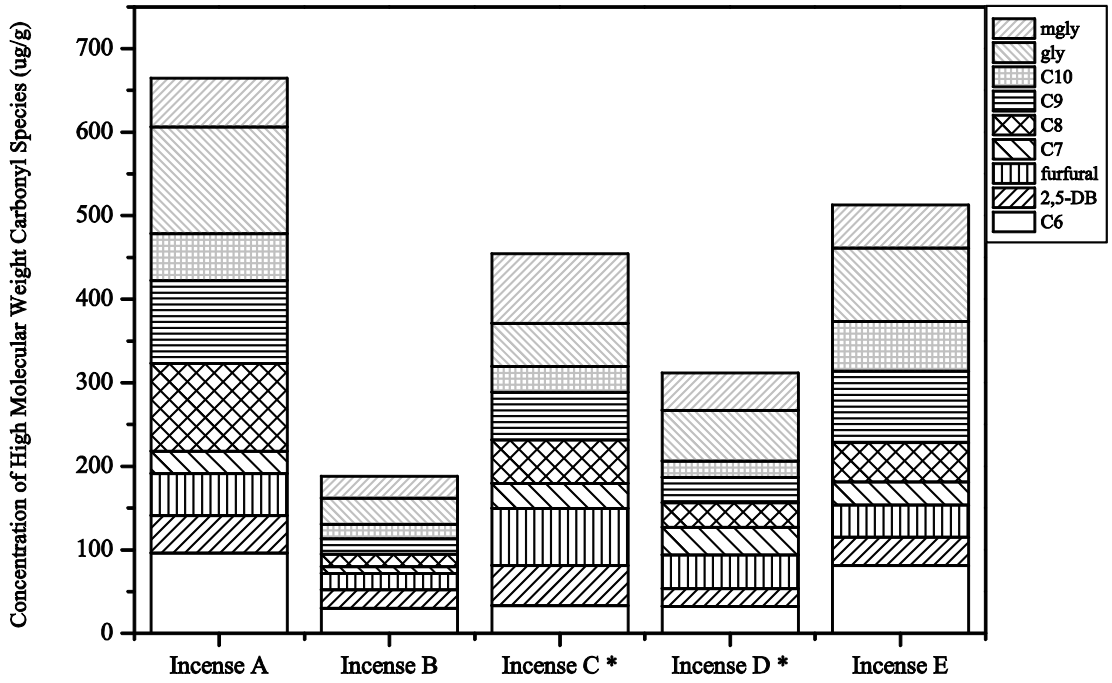
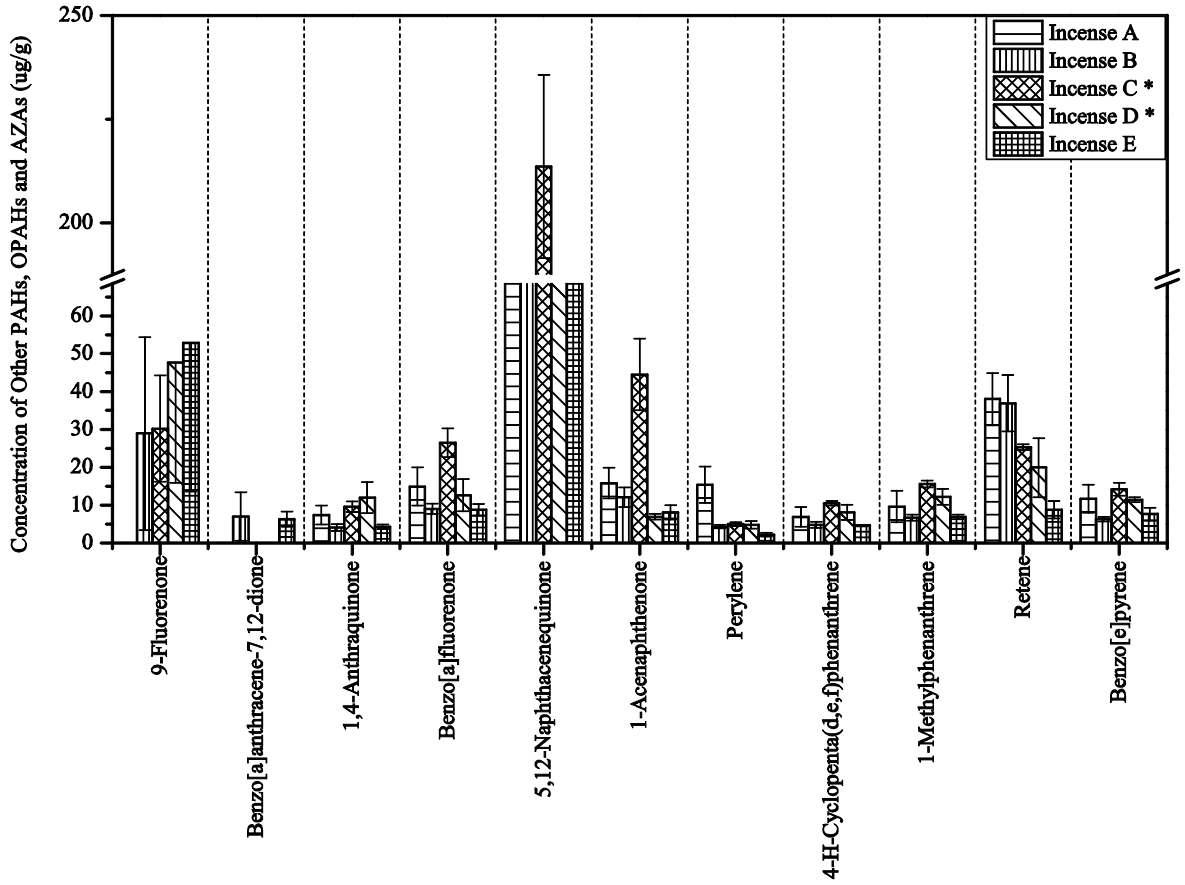


Figure 1. Mass Concentrations of $PM_{2.5}$ in Incenses



[a]Concentrations of particle-bound carbonyls species (μg/g) were defined as carbonyl entity mass (μg) of $PM_{2.5}$ and normalized by the unit mass (g) of incense burnt for incenses A to E.

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



[a]Particle-bound PAH/OPAH/AZA concentrations (μg/g) were defined as carbonyl/PAH/OPAH/AZA mass (μg) of $PM_{2.5}$ and normalized by the unit mass (g) of $PM_{2.5}$ for incenses A to E.

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

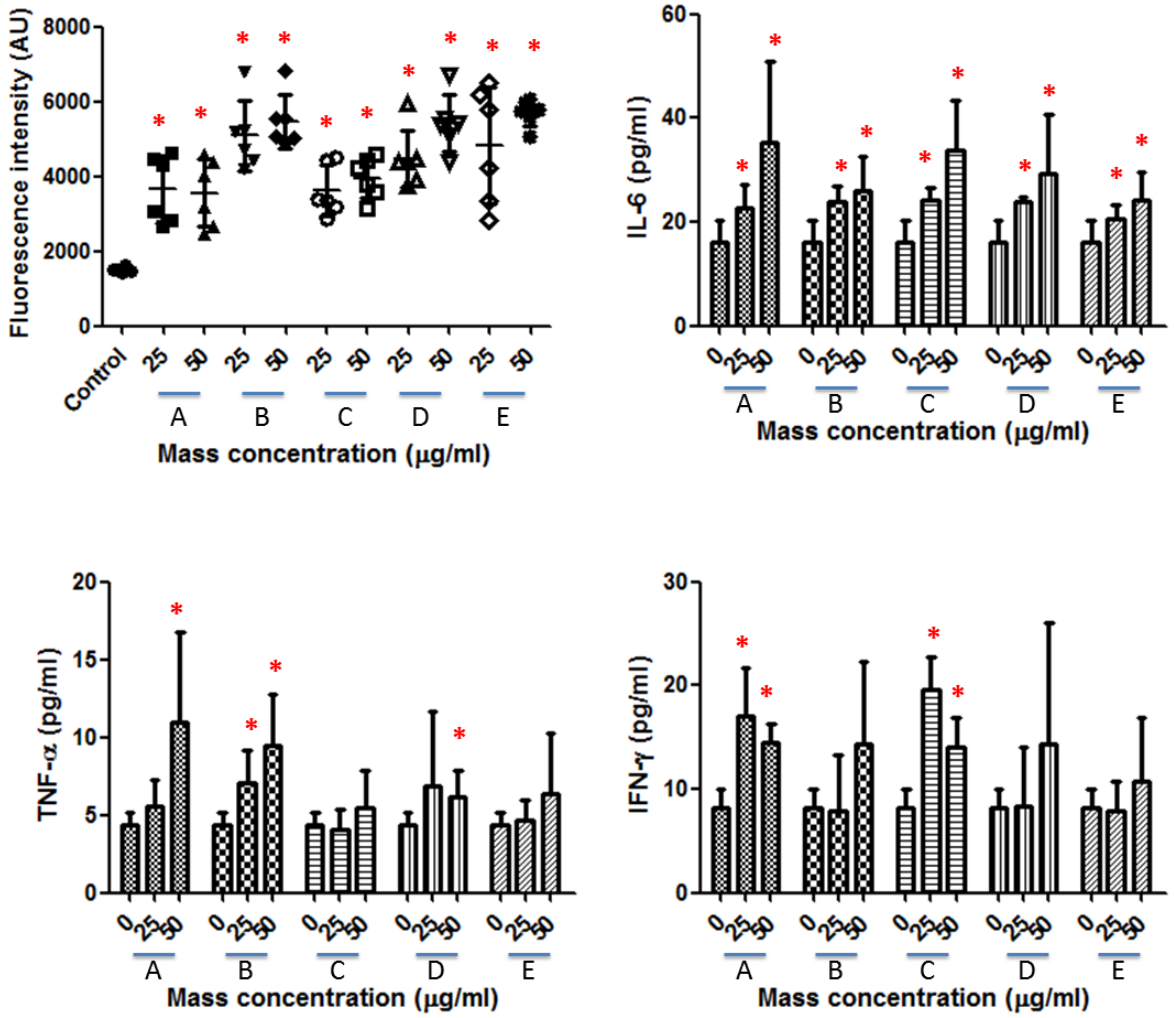


Figure 4. Oxidative Potentials, IL-6, TNF-α, and IFN-γ Production of Incenses (*p < 0.05 compared with control)

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 Incense 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*	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

Table 2. Descriptive Analysis and Relative Abundances of U.S. EPA Priority PAHs

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

[a#] Indicated by U.S. EPA as probable human carcinogen

[b]B.D. = below detection limit

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

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**	
Phenanthrene		0.69*[b]	
Anthracene		0.75 *	
Fluoranthene			
Pyrene		0.79**	
Benzo[a]anthracene#[c]		0.70*	
Chrysene and Triphenylene#		0.71*	
Benzo[b,j,k]fluoranthene#		0.76*	
Benzo[a]pyrene#		0.72*	
Dibenz(a,h)anthracene#			
Benzo[ghi]perylene#		0.66*	
Indeno[1,2,3-cd]pyrene#		0.65*	
Σ_7PAHs (Summation of total Group B2 PAHs)		0.79**	
Other PAHs, OPAHs, and AZAs			
9-Fluorenone	0.74*		
Benzo[a]anthracene-7,12-dione	0.77**		
1,4-Anthraquinone		0.65*	
Benzo[a]fluorenone		0.75*	
5,12-Naphthacenequinone		0.81**	
1-Acenaphthenone			0.66*
Perylene			0.65*
4-H-Cyclopenta(d,e,f)phenanthrene		0.83**	
1-Methylphenanthrene		0.75*	
Retene		0.75*	
Benzo[e]pyrene		0.76*	
ΣOPAHs (Summation of total OPAHs)		0.72*	

[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