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Abstract

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Hazard Assessment of UAE Incense Smoke

(Under the direction of Dr. Kenneth G. Sexton)

Burning UAE incense inside their homes is a common practice of Emeriti citizens and has been recognized as a source of indoor air pollution that can cause adverse effects to the individuals exposed to the compounds emitted. The goal of this study was to evaluate the hazards of burning incense indoors by first characterizing the particles and gases emitted from UAE incense when burned, then observing *in vitro* biomarkers of possible health effects in humans using cultured cells exposed to incense-derived compounds. To identify the emitted compounds, two types of UAE incense were burned in a specially designed indoor environmental chamber (22m³) to simulate the smoke concentration in a typical living room followed by analysis of the chamber air. Air analysis included determining particle (PM), carbon monoxide (CO), oxides of nitrogen (NOx) and formaldehyde (HCHO) concentrations as well as particle sizing, and carbonyl analysis. To examine potential health effects indicators, human lung cells were exposed to the incense emissions *in vitro* and the cell inflammatory responses were recorded. Analysis showed that the emissions from the incense burn included PM, CO, NOx and HCHO along with several other carbonyls. A significant inflammatory response was observed in response to the smoke components. Therefore, it is concluded that incense smoke contributes to indoor air pollution and can potentially be harmful to humans.

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Hazard Assessment of UAE Incense Smoke

1 Introduction

1.1 Background

The study serves as a hazard assessment of incense smoke exposure in the home.

Burning incense is a popular practice inside residential homes and in places of worship both in the UAE and around the world (Fang et al., 2002; Jetter et al., 2002; Yeatts et al., 2012).

Incense is burned in most Emirati residences to perfume both clothing and the home, as well as to remove the odors of fish or other food smells associated with indoor cooking.

Indoor air pollution and its sources have become of increasing concern due to the limited ventilation rate as compared to outdoors, and the fact that people spend 90% of their time indoors (Klepeis et al., 2001). To a small extent studies have examined indoor air quality resulting from specific indoor activities such as cooking or smoking cigarettes (Löfroth et al., 1991). Incense emissions in particular are a type of indoor air pollutant not well documented. In a low ventilation area such as a home, it is hypothesized that smoke emitted from incense would result in high concentrations of harmful chemical compounds.

One of the more common types of incense used in the UAE is Oudh, which is comprised of aromatic agarwood formed from the *Aquilaria agallocha* tree in response to a fungal infection (Alokail et al., 2011). Another popular type of incense is Bahkoor, which is derived from sandalwood tree resin and mixed with agarwood, essential oils, and other substances (Wahab and Mostafa, 2007). These types were chosen to represent opposite

ends of the spectrum; Oudh is homogenously made of agarwood, whereas Bahkoor is a pasty heterogeneous mixture of many ingredients molded into a small block.

1.2 Adverse Health Effects from Incense Smoke Inhalation

Incense undergoes a slow continuous burn with incomplete combustion emitting aromatic smoke with many compounds in the gas and particle phase. There are limited studies on the health effects of Arabian incense. A small case-control study of 200 Qatari children ages 2 to 12 explored the role of Arabian incense as a trigger of wheezing among children with asthma (Wahab and Mostafa, 2007). The researchers found that the 100 asthmatic children had significantly more exposures to Bahkoor and Oudh ($p=0.026$, $p=0.045$, respectively) than the control group. In a recent study in Oman, Al-Rawas et al. (2009) found conflicting results in a cross-sectional study of 2,441 ten-year old children. Although burning Bahkoor worsened wheeze in 38% of the asthmatic children, its increased frequency of use at home was not associated with the medical diagnosis of current asthma in the children. Studies have also shown that this smoke can cause an array of health effects from eye, nose, and throat irritation to contact dermatitis to respiratory symptoms & lung cell morphological changes (Al-Rawas et al., 2009; Böer, 1997; Lin et al., 2008).

Particle emissions are of concern as studies have observed PM emissions in the respirable particle size range which can exacerbate respiratory and cardiovascular disease (See et al., 2007). Small particles present in incense smoke are very effective at depositing in the lung periphery, eliciting adverse inflammatory responses (Bitterle et al., 2006). An inflammatory response was observed when human lung cells were exposed in vitro to Indian and Japanese incense particles re-suspended from filter collection at concentrations

of 10ug/mL (Mastsmura et al., 2010). The inflammatory response was measured by observing up regulation of many inflammatory genes including interleukin-8 (IL-8) and cyclooxygenase-2 (COX-2) (Mastsmura et al., 2010). Phagocytes release IL-8 upon exposure to inflammatory stimuli (Baggiolini and Clark-Lewis, 1992). This neutrophil-activating cytokine recruits neutrophils to the site of inflammation, in this case the lungs. COX-2 is expressed at low levels under normal conditions in most cells, but elevated levels are found during inflammation. The COX-2 enzyme has been found to catalyze the rate limiting step in the formation of inflammatory prostaglandins which control many strong physiological effects (Hla and Neilson, 1992). Incense smoke was also observed to be mutagenic in *Salmonella typhimurium* bacterium (Löfroth et al., 1991; Sato et al., 1980).

1.3 Compounds Emitted from Burning Incense

Compounds emitted from burning incense can be divided into 2 categories; particle and gas phase. They are emitted simultaneously, exposing recipients to a complex mixture. Due to variability in incense composition and study design, previous literature characterizing incense smoke is highly variable. However, recent published literature on incense emissions and household air pollution, which included emissions from incense, was used to guide us in our selection of target compounds. These compounds include particle matter (PM), carbon monoxide (CO), oxides of nitrogen (NO_x), formaldehyde (HCHO), and sulfur dioxide (SO₂) (Yeatts et al., 2012).

Table 1, summarizing incense emissions from incense around the world, contains the most applicable findings of peak emission concentrations and count median diameters (CMD). Incense PM_{2.5} concentrations ranged from 10.5-50mg/m³ and CO from 10-

65.5mg/m³ (Jetter et al., 2002; Lee and Wang, 2004; Mannix et al., 1996). In comparison cigarette smoke from one cigarette introduces 15mg of PM and 60mg of CO into the indoor air when smoked indoors (Croxford and Kynigou, 2005; Mannix et al., 1996). These data shows that concentrations of cigarette emissions are lower than incense emissions.

Table 1: Comparison of previous studies of incense and cigarette emissions. Peak emission concentrations are reported in parts per million (ppm) or milligrams per cubic meter (mg/m³) and refer to either PM_{2.5} concentrations, total particle matter concentrations (TPM) or overall CO or NOx concentrations. Count median diameter (CMD) is also recorded.

Burn Conditions	PM	CO	NOx
23 Asian & American types - 30m³ chamber⁽¹⁾	PM _{2.5} - 10.5mg/m ³ CMD = 0.03-0.649µm	8.1ppm (10mg/m ³)	0.020ppm (0.034mg/m ³)
3 Thailand, US, & Mexico types - 4m³ clean room⁽²⁾	TPM - 35mg/m ³ CMD = 0.37µm	53ppm (65.5mg/m ³)	1.5 ppm (2.8mg/m ³)
10 European & Asian types - 18m³ chamber⁽³⁾	PM _{2.5} - 38mg/m ³ CMD = N/A	37ppm (45.6mg/m ³)	20 ppm (38mg/m ³)
3 Chinese Joss sticks 34m³ chamber⁽⁴⁾	TPM - 0.533mg/m ³ CMD = 0.13-0.14µm	N/A	N/A
4 Asian sticks together 1.07m³ chamber⁽⁵⁾	PM _{2.5} - 50mg/m ³ CMD = 0.093-0.143µm	N/A	N/A
Cigarette 4m³ room⁽²⁾	TPM - 3.5mg/m ³ CMD = 0.24-0.4µm	27.4ppm (33.8mg/m ³)	1.14ppm (2.2mg/m ³)

1. Jetter, J. J., Z. Guo, J. A. McBrien & M. R. Flynn (2002) Characterization of emissions from burning incense. *Science of The Total Environment*, 295, 51-67.
2. Mannix, R. C., K. P. Nguyen, E. W. Tan, E. E. Ho & R. F. Phalen (1996) Physical characterization of incense aerosols. *Science of The Total Environment*, 193, 149-158.
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4. Cheng, Y. S., W. E. Bechtold, C. C. Yu & I. F. Hung (1995) Incense Smoke: Characterization and Dynamics in Indoor Environments. *Aerosol Science and Technology*, 23, 271-281.
5. See, S. W. & R. Balasubramanian (2011) Characterization of fine particle emissions from incense burning. *Building and Environment*, 46, 1074-1080.

Aside from PM, CO and NOx emissions, burning incense has also been shown to emit SO₂, HCHO, and other carbonyls. SO₂ emission rate has been observed to be 25.5mg/hr, this equates to 0.45mg/m³ from a cone of incense burned in an 30m³ environmental chamber (Jetter et al., 2002). Among the many carbonyls emitted from burning incense, previous research has shown formaldehyde is emitted at the highest rate. Formaldehyde has been

observed at peak concentrations ranging 40-2650ppb depending on size of chamber (ranging 1-82m³) and type of incense (Ho and Yu, 2002; Lee and Wang, 2004; Madany and Crump, 1994). Formaldehyde emission factors have been observed to range 1-25ppm per gram burned (Lin and Wang, 1994). Other carbonyls detected from burning incense include acrolein, methacrolein, acetaldehyde, valeraldehyde, acetone, glyoxal, methyl glyoxal, propanal and furfural (Chang et al., 2007; Ho and Yu, 2002; Lee and Wang, 2004; Lin and Wang, 1994; Madany and Crump, 1994; Tran and Marriott, 2007; Yang et al., 2007). These carbonyls have been observed at concentrations of 10-200ug/m³ in a 18.26m³ chamber (Lee and Wang, 2004).

1.4 Study Objectives

The purpose of this study is to assess the hazards associated with incense smoke exposure in the home. This study does not focus on one specific chemical emission component as most previous studies. Instead, this study takes advantage of several multi-disciplinary environmental monitoring devices and techniques to give a broad emission profile and also examine the potential health effect when inhaled.

Our goals were twofold: first to characterize the major particle and gaseous compounds emitted when burning two types of UAE incense in a large 22m³ indoor environmental chamber to simulate a room with realistic surface-to-volume characteristics and dilution rate, and second to observe the human inflammatory response to incense smoke *in vitro*. The incense chamber we developed was designed to simulate realistic burning conditions in a home with the large chamber size, ventilation rate, burn procedure, and cell exposure scenario. Particle and gas emission were analyzed by observing the peak concentration as

well as concentration time-series to observe decay of PM, CO, NO_x, HCHO, and SO₂ concentrations, their calculated emission rates, their mass emitted, and carbonyl concentrations. Particles alone were analyzed for particle size distribution and number and mass concentration over time. To observe potential health effect indicators, human epithelial cells were exposed *in vitro* to the chamber smoke and analyzed for levels of cell inflammatory markers with quantitative real-time polymerase chain reaction (qRT-PCR). Collective assessment of these data will significantly affect the evaluation of these incense products and their potential to invoke adverse health outcomes from comparisons to other studies of combustion sources and conditions, and allow for recommendations on how to reduce exposure.

2 Materials and Methods

2.1 Indoor Burn Simulation and Characterization System

All incense was burned in a 10x10x8ft (22m³) all-enclosed indoor environmental chamber, lined with non-reactive Teflon film, installed with a small mixing fan (Figure 1). Chamber ventilation rate was maintained at ½ chamber exchange per hour as observed in studies with similar chamber size (Lee and Wang, 2004), and monitored with a dilution tracer compound [carbon tetrachloride (CCl₄)] using a Varian CP-3800 Gas Chromatograph with an Electron Capture Detector (ECD-GC) (Figure 2). Initially the chamber was vented for an hour, and then background air was monitored for the next hour. The charcoal was then lit outside of the chamber and placed in an incense holder on top of a two foot tall stool

(Figure 1). A pre-weighed amount of incense was placed on top the smoldering incense and the door to the chamber was sealed shut.

Figure 1: Indoor chamber schematic. Setup of 10x10x8ft (22m^3) indoor environmental chamber. All instruments drew air from the chamber via Teflon tubing of specified length, aside from the real-time PM monitor which was placed inside the chamber.

Figure 2: Carbon tetrachloride dilution graph. Graph of the dilution rate in the chamber measured by the amount of carbon tetrachloride present every 30 minutes for 4.5 hours. The amount of carbon tetrachloride is at approximately $\frac{1}{2}$ of its original amount at 1 hour and closely follows an exponential decay over the 4.5 hours.

2.2 Experimental Plan and Test Protocol

Each type of incense was burned twice, totaling four experiments all taking place on different days. The two Bahkoor experiments burned 0.16-0.17g of Bahkoor Bait al Arab, while the two Oudh experiments burned 0.70-0.71g of Original Mahder Oudh (Table 2 & Figure 3). These amounts were chosen to standardize all runs by controlling the resulting similar peak particle concentrations ($\approx 1.25\text{mg/m}^3$), allowing direct comparison of compounds emitted for all burns. This target concentration corresponds to peak PM concentrations seen previously and allows ideal measurements of other compounds. Each type of incense was burned on two separate days denoted Bahkoor1/Oudh1 or Bahkoor2/Oudh2. Incense began burning as soon as it was placed on the smoldering charcoal, and continued to emit smoke for 8-10 minutes. Aside from the HCHO monitor, all burns utilized the real time PM monitor, particle sizer, CO, NO_x, and SO₂ monitors along with mister and filter samplers. To control for the smoke produced by the charcoal; smokeless, quick lighting charcoal, normally used to burn the incense, was burned alone in the chamber to access its emissions. Two trials were run; one with a full, and one with a half a briquette (Table 2). To further characterize incense emissions, the first of the two incense experiments utilized a full charcoal briquette, and the second of the two utilized a half a charcoal briquette. This allowed the study to additionally look at how the amount of charcoal effects emissions and health effects.

Table 2: Summary of burns. Documentation of what materials were used in each burn.

	Bahkoor1	Bahkoor2	Oudh1	Oudh2	Charcoal
Mass Burned (g)	0.16	0.17	0.70	0.71	Full – 6.50 Half – 3.75
Full/Half Charcoal	Full	Half	Full	Half	Both Individually
HCHO Monitor(y/n)	Yes	No	No	Yes	No
Cell Analysis (y/n)	Yes	Yes	Yes	Yes	No

Figure 3: Amount of incense burned. Both the amount of Oudh (middle) and Bahkoor (right) burned to produce 1.1-1.4mg/m³ of particles takes up less surface area than a quarter.

For each experiment, PM, CO, NO_x, HCHO and SO₂ concentrations were monitored in real-time before, during and after the incense was burning. Carbonyls samples were taken several times with a bubbler into liquid absorbing reagent, and concentrations were observed in background air, during smoke production, and after smoke was being visibly produced. Particle size distribution, number and mass concentration over time were also monitored for the particles emitted.

For all burns with incense, the smoke was collected in cell culture media using a bubbler and exposed to the A549 human epithelial cell line *in vitro*. Post-exposure, cells were analyzed for levels of inflammatory markers with qRT-PCR.

2.3 Emission Sampling Instruments

2.3.1 CO, NO_x, & SO₂ Monitors

CO was measured by an EPA approved method with a gas filter correlation CO analyzer manufactured by Thermo Environmental Instruments Inc. (Franklin, MA). Calibration tests were performed to determine which concentration of CO analyzer corresponded to the volt output read by the computer (Appendix A, Figure 16). CO concentrations over 50ppm were not recorded since 1volt read by the computer was the maximum value recorded. NO_x was measured by an EPA reference method with an Oxides of Nitrogen analyzer ML 9841 series manufactured by Teledyne Instruments Monitor labs (Englewood, CO). SO₂ was measured by a pulsed fluorescence SO₂ analyzer manufactured by Thermo Electron Instruments (Franklin, MA).

2.3.2 HCHO monitor

Real time HCHO concentrations were measured with a HCHO monitor (Dasgupta et al., 1988). Detection range was 0.1 – 800ppb with a lag time of 6.3 minutes between detection and recording (Dasgupta et al., 1988).

2.3.3 Carbonyl Trap

To trap carbonyls in the particle and gas phase, incense smoke was bubbled through a two compartment mister for 15 minutes at 15LPM (225L is ideal for detecting predicted

carbonyl concentrations) (Figure 4). Both compartments contained 10ml of high performance liquid chromatography (HPLC) grade water and O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) derivatizing agent at a 9:1 ratio (Figure 4). Mister liquid was derivatized for 24 hours then extracted with extraction solvent (4:1 mix of hexane to methylene chloride) and analyzed (Liu et al., 1999). PFBHA works by combining with carbonyls in the solution and gives it a mass fragment specifically identifying a carbonyl at a mass of 181 and ignores other compounds in the mixture (Yu et al., 1997). Other fragments recognized are unique to specific compounds and can be compared to standards to identify unknowns. HPLC measure of carbonyls from the mister was performed with a Varian Saturn II 2200 Gas Chromatograph and an ion trap Mass Spectrometer (GC/MS).

Figure 4: Mister and impinger setup. (A) Mister: Air is pulled from the chamber up through the bottom compartments and then through the top by the attached pump. (B) Impinger: Air gets pulled from the chamber on the left and gets bubbled through 4ml media at the bottom by a pump attached to the outlet on the right.

2.3.4 Filter

Chamber air particle concentration was measured by drawing air through pre-weighed 47mm Teflon filters (Pall Corporation, Port Washington, NY) at 20LPM (Figure 5). To obtain particle bound carbonyl data, the filter was sonicated for two minutes and also allowed to derivatize for 24 hours in HPLC grade water and PFBHA derivatizing agent at a 4:1 ratio in a filter jar.

Figure 5: Filters before and after loaded with incense smoke. Filter on the right had collected 1mg of smoke particles when 1360L of air from the chamber was pulled through at 20LPM.

2.3.5 Real-time PM Monitor

PM concentration was detected by a TSI Handheld DustTrak DRX Aerosol Monitor Model 8534 (Shoreview, MN) which pulled chamber air from inside the chamber at 3LPM. The concentrations measured by this device served as a quality control check that the incense was burning in accordance to the study protocol. The DustTrak measured size segregated mass fraction concentrations including $PM_{1\mu}$, $PM_{2.5\mu}$, respirable $PM_{4\mu}$, thoracic $PM_{10\mu}$, and total PM (TPM). The DustTrak can measure particles 0.1-15 μ m in diameter at concentrations in the range of 0.001-150mg/m³ in real time and assumes a particle density

of 2.65g/cm^3 (TSI, 2008). DustTrak PM concentrations had a correction factor applied of 0.4 to correct for the assumed particle density of 2.65g/cm^3 compared to 1.06g/cm^3 as seen previously (See and Balasubramanian, 2011). Reported concentrations were verified by the filter mass concentration measurement, which also assumed an aerosol density of 1.06g/cm^3 .

2.3.6 Scanning Mobility Particle Sizer

Scanning Mobility Particle Sizer (SMPS) (Shoreview, MN) drew chamber air at 2LPM to analyze the particle size distribution, particle number concentrations, and cumulative particle volume concentration simultaneously with the DustTrak. The SMPS system consisted of a TSI 3080 Electrostatic Classifier that separated particles by size and a TSI 3025A Ultrafine Condensation Particle Counter to measure particle number concentration (TSI, 2001). This system can detect particles from 17 to 980nm in diameter and assumes a particle density of 1g/cm^3 .

2.3.7 Impinger for Cell Exposure

To expose cells to incense smoke, smoke was bubbled into media in an impinger as outlined previously (Yu et al., 1997). The impinger used in this study holds 4mL of cell culture media containing F-12K, 0.015% bovine serum albumin (BSA), & 1% penicillin/streptomycin, and has air sampled at 4.8 LPM for an hour starting when the charcoal is lit (Figure 4). Volume of remaining media was measured and using the PM concentration observed by the DustTrak; then the concentration of particles trapped in the media was calculated and exposed to cells.

To ensure particles were effectively being trapped in the impinger liquid, an SMPS impinger smoke particle test was performed (Figure 6). Once the impinger began trapping particles the number concentration dropped approximately 70%, therefore particles in the smoke size range are assumed to be trapped with 70% efficiency.

Figure 6: SMPS impinger size distribution test. Graph shows particle size distribution in the room before the impinger pump was turned on at time 5 min and after it was tuned off at time 25 min.

2.4 Inflammatory Cell Response

2.4.1 Cell Culture and Exposure

Human A549 type II lung epithelial cells, derived from a human lung adenocarcinoma, were cultured according to standard protocol [ATCC (American Type Culture Collection), Manassas, VA]. These cells were cultured in growth media containing F-12K, 10% fetal bovine serum (FBS), & 1% penicillin/streptomycin and passaged when 90%

confluent as described previously (Rager et al., 2011). Cells were then plated on the bottom surface of a sterile 22mm 12-well plate. Cells were plated in 6 wells of each plate and after 24 hours of growth in 1ml of growth media a 90% confluent cell monolayer was established (~350,000cells/well). Once confluent, the 1ml of growth media was replaced with 1ml of starving media (media used in the impinger containing F-12K, 0.015% BSA, & 1% penicillin/streptomycin), lacking FBS needed for cell growth. The cells were incubated with starving media for 4 hours before they were exposed to media from the impinger to make the cells more susceptible to pollutants. Old starving media was removed from the incubated cells and the media from the impinger was aliquoted between 3 wells on the 12 well plate. Extra starving media, aside from media from the impinger, was added to the exposed cells to maintain 1mL of media in each well, while always maintaining a consistent mass of particles exposed to all wells. The other 3 wells acted as control and received fresh starving media with no incense smoke. Cells were again incubated for 24 hours to allow the particles to settle. Cells were recovered using 1ml of TRIzol® Reagent and frozen in labeled 1.5ml tubes for qRT-PCR analysis of target inflammatory genes IL-8 and COX-2 (Invitrogen).

2.4.2 qRT-PCR Analysis of Gene Expression Levels

Total RNA was isolated and first-strand cDNA was generated as described before (Jaspers et al., 1999). mRNA levels for two inflammatory response genes, IL-8 and COX-2, were quantified from exposed A549 cells by rtPCR according to (Blaschke et al., 2000) protocol. Numbers were compared to the expression of the two markers in the non-exposed A549 cells.

3 Results

3.1 PM, CO, NO_x, and HCHO Concentrations

Concentrations of PM, CO, NO_x, and HCHO were recorded for up to 180 minutes after the incense was lit (Figure 7&8). No SO₂ was observed to be emitted from burning Bahkoor or Oudh. Peak concentrations, the emission rates (mg/min), and emission factors (mg/g burned) were calculated for each compound and these ranges for the first and second burn are reported in Table 3. The amount of the dilution tracer CCl₄ was also observed in comparison to particle and gas concentrations detected in the chamber.

Incense was lit at time 0, burned out at time 8-10 minutes, and within five minutes of being lit concentrations of PM, CO, NO_x and HCHO increased (Figure 7&8). Particles decayed at the fastest rate due to wall loss experienced in the chamber (Figure 8). In contrast, HCHO concentrations were observed at steady high levels in the chamber for at least two hours post-burnout, experiencing slower dilution than the CCl₄ tracer.

We observed the charcoal emissions to be the main contributor to both the high CO and NO_x concentrations detected (Figure 7&8). As a result, the mass of charcoal was taken into account when calculating the emission factor of CO and NO_x (Table 3). Even though concentrations above 50ppm were not recorded by the program, a few peak CO concentration values for Bahkoor1 and Oudh1 were observed (Figure 7). CO concentrations peaked at 122ppm for Bahkoor1 and Oudh1 at time 60 minutes. When the amount of charcoal used in Bahkoor1 was cut in half in Bahkoor2, the peak CO detected went from 122ppm to 48.9ppm (Table 3). Also when a full charcoal briquette was burned alone in the

chamber, CO was observed at similar concentrations as when a full charcoal briquette was used to burn incense in Bahkoor1 and Oudh1. Decay rates of CO and NO_x were similar to the dilution rate (Figure 7&8).

To achieve comparable peak concentrations of all compounds, the mass of Oudh burned was 4.3 times the mass of Bahkoor. Based on the mass burned and the time it took to reach peak concentrations, Bahkoor has higher emission rates and emission factors than Oudh (Table 3).

Figure 7: Change in concentration of CO over time. Concentration recorded by the CO monitor for 180 minutes starting when the incense was lit. The program recording CO monitor readings could only record up to 50ppm, so only a few points above 50ppm in Oudh1/Bahkoor1 were recorded.

Figure 8: Change in concentration of PM, NOx, and HCHO over time. Concentration recorded by (A) DustTrak Real-time PM, (B) NOx and (C) HCHO monitor for 180 minutes starting when the incense was lit.

Table 3: PM, CO, NO_x, and HCHO emissions. Peak concentration, emission rate (mass emitted per minute), and emission factor (mass emitted per gram burned) for all compounds measured in real-time.

	Peak Concentration	Emission Rate	Emission Factor
Bahkoor			
PM	1.34-1.36mg/m ³	5.9mg/min	174.7-187.0mg/g
CO	48.9-122.1ppm	26.1-67.6mg/min	331.7-500.9mg/g
NO _x	0.2-0.3ppm	130.5-134.3ug/min	1.5-2.2mg/g
HCHO	72.0ppb	27.6ug/min	12.3mg/g
Oudh			
PM	1.12-1.42mg/m ³	2.9-3.9mg/min	35.3-44.6mg/g
CO	50.0-122.0ppm	23.1-52.2mg/min	339.2-509.3mg/g
NO _x	0.1-0.2ppm	93.6-111.3ug/min	0.7-1.3mg/g
HCHO	85.2ppb	17.8ug/min	3.6mg/g

3.2 Carbonyl Analysis

Chromatogram plots (Figure 9 & 10) display a complex mixture of carbonyls selected for by PFBHA. Chromatogram plots comparing mister samples of background, burn1 (during smoke production), burn2 (after smoke production), and filter from Bahkoor1&2 and Oudh1&2 in Appendix B, Figure 17-20. Also Appendix B, Figure 21 compares carbonyls detected in top and bottom mister compartments. Minimal carbonyls were detected when the charcoal was burned alone. Nine identifiable peaks were observed in both Bahkoor and Oudh burns, while peak labeled 7 appeared only in Oudh burns. Peaks 4 & 8 are identified as valeraldehyde and glyoxal (Table 4). Other carbonyls could not be absolutely identified due to similarities in compound structure and co-elution times in the GC-MS, however possible carbonyls identified include acetone or acrolein (peak 2) methyl vinyl ketone or crotonaldehyde (peak 3), isovaleraldehyde or butanal (peak 5), and diacetyl (peak 10). The area of each peak allowed direct comparison to previously run standards; formaldehyde, acrolein, methacrolein, acetone, propanol, acetaldehyde, glyoxal,

methylglyoxal, furfural, diacetyl and valeraldehyde at concentrations of $100\mu\text{g}/\text{m}^3$ (Lee, 2004). Again the emission factor of carbonyls emitted per gram of incense for Bahkoor is higher than Oudh and both emission factors and carbonyls concentrations for the first burns (Bahkoor1 & Oudh1) was higher than the second (Bahkoor2 & Oudh2) (Table 4). We hypothesize this is due to the amount of charcoal utilized.

The mister traps all carbonyls emitted in both the gas and particle phase, whereas the filter only traps carbonyls emitted in the particle phase. Filter carbonyl concentrations observed ranged from $0.53\text{--}5.12\mu\text{g}/\text{m}^3$ and mister carbonyl concentrations ranged from $86.82\text{--}299.61\mu\text{g}/\text{m}^3$ (Table 4). These values reveal that 98% of the carbonyls were emitted in the gas phase.

Figure 9: Chromatogram plots from Oudh1 filter, Bahkoor1 mister, Oudh1 mister, and charcoal. Carbonyls from Oudh1's filter, the bottom mister samples from Bahkoor1 and Oudh1 burns compared to burning the charcoal alone. Identifiable peaks are numbered 1-10.

Figure 10: Chromatogram plots from burn1 for Bahkoor1&2 and Oudh1&2. Bottom compartment mister samples taken from the beginning of all 4 burns. Identifiable peaks are numbered 1-10.

Table 4: Emitted carbonyl concentrations. Total and individual carbonyl concentrations emitted from both Bahkoor and Oudh burns. Carbonyls are reported in overall concentration ($\mu\text{g}/\text{m}^3$) and in mg emitted per gram of incense burned. Valeraldehyde and glyoxal concentrations are from mister samples.

	Bahkoor/Oudh1 Conc. ($\mu\text{g}/\text{m}^3$)	Emission Factor 1 (mg/g)	Bahkoor/Oudh2 Conc. ($\mu\text{g}/\text{m}^3$)	Emission Factor 2 (mg/g)
Bahkoor				
Total Mister	86.82	11.94	282.13	35.07
Total Filter	5.12	0.70	1.87	0.23
Valeraldehyde (4)	11.55	1.59	31.95	3.97
Glyoxal (8)	6.61	0.91	71.90	8.94
Oudh				
Total Mister	229.05	7.15	299.61	9.26
Total Filter	0.53	0.02	4.70	0.15
Valeraldehyde (4)	14.38	0.44	17.63	0.54
Glyoxal (8)	52.24	1.64	84.81	2.62

3.3 Particle Analysis

Particles emitted from Bahkoor had a count median diameter (CMD) of 65-85nm and ranged from 20-350nm, while particles emitted from Oudh had a CMD at 75-92nm and ranged from 20-300nm (Figures 11&12). Peak number concentrations were observed approximately 10min after the incense was lit, and in both types of incense the CMD increased by 20nm from time 0 to time 30 minutes after the incense was lit suggesting aggregation of the particles. Particles were eliminated quickly from the chamber, as seen with the DustTrak (Figure 8), with a half-life of only 30 minutes (Figure 13). Due to particle wall loss, this was much faster than the dilution tracer half-life of an hour.

Figure 14 shows the particle size range for particles emitted when the charcoal was burned alone. These particles were in the ultrafine particle size range (<100nm) and much smaller than particles produced by burning incense.

Figure 11: SMPS particle size of Bahkoor1 and Oudh1. (A)Bahkoor1 and (B)Oudh1 size distribution of particles in the chamber 0, 6, 12, 18 and 30 minutes after incense was lit, measured by number concentration.

Figure 12: SMPS particle size of Bahkoor2 and Oudh2. (A)Bahkoor2 and (B)Oudh2 size distribution of particles in the chamber 0, 6, 12, 18 and 30 minutes after incense was lit, measured by number concentration.

Figure 13: SMPS number and cumulative volume concentration throughout burn. Change in (A) number and (B) cumulative volume concentration of all 4 incense burns and full charcoal burn throughout their burn time.

Figure 14: SMPS full charcoal particle size distribution. Number concentration of particle emitted when a full charcoal was burned alone

Table 5 shows the overall particle mass collected on the Teflon filters compared to the time-weighted average (TWA) DustTrak particle concentration of the time smoke was being pulled through the filter.

Table 5: Particles trapped on filter. Total mass collected on the filter when smoke was pulled at 20 LPM compared to the TWA particle concentration observed by the DustTrak.

	Mass Collected (mg)	Time Pulled (min)	Liters Pulled (L)	Absolute Conc. (mg/m³)	DustTrak TWA Conc. (mg/m³)
Bahkoor1	0.592	60	1200	0.493	0.682
Bahkoor2	1.090	74	1480	0.736	0.597
Oudh1	1.410	68	1360	1.036	0.600
Oudh2	0.937	80	1600	0.585	0.512

Absolute filter concentration showed consistency with the DustTrak readings with the filter concentration being slightly higher than the DustTrak's. Discrepancies are from the assumed particle density applied to the DustTrak concentrations and error in particle

collection and analysis on the filter. Particle density was assumed to be 1.06g/cm^3 for both the filter and DustTrak, which was based off literature values for non-UAE incense particles (See and Balasubramanian, 2011). Also semi-volatile compounds can evaporate in the time 1200-1600L of chamber air gets pulled through the filter reducing its weight.

3.4 Cell Inflammatory Response

Using DustTrak data of airborne particles throughout the hour burn, the micrograms of suspended particles per milliliter of remaining media was calculated (Table 6). Assuming 70% particle retention efficiency from experiments we ran previously (Figure 6), the concentration exposed to all wells was 55ug/ml .

Table 6: Particle concentration in impinger media. Concentration of particles suspended in media after smoke is bubbled through 4ml of starving media for 1 hour.

	Final Media Volume (ul)	Particle Conc. in Media (ug/ml)	Volume Media Added to Wells (ul)	Mass Particles Added to Wells (ug)
Bahkoor1	400	404.21	133	55
Bahkoor2	800	202.10	267	55
Oudh1	1100	146.98	368	55
Oudh2	2000	80.84	667	55

To analyze the relative gene expression levels a $\Delta\Delta$ -Control Method was implemented to determine the average relative expression levels for both inflammatory markers IL-8 and COX-2, along with their standard deviation (Figure 15). The difference between target and control gene expression was calculated as Δ -Control and this difference was subtracted by the Δ -Control with the biggest Δ -Control from the previous step. The relative expression level was then calculated by using the equation $2^{(-\Delta\Delta\text{-Control})}$. Expression

levels from wells 1-3 for all 4 burns were then averaged along with incubator controls from Bahkoor and Oudh exposures.

Cells exposed to smoke from Oudh1 and Oudh2 burns had a relative IL-8 expression level of 34.5 ± 10.5 and 4.6 ± 0.75 compared to 1.7 ± 0.44 for control cells with no incense smoke exposure (Figure 15). These high relative expression levels of IL-8 compared to control cells indicate a significant inflammatory response to its emissions. Emissions from Bahkoor were highly variable and did not show a significant increase in IL-8 or COX-2 expression levels. However, relative expression levels of IL-8 in cells exposed to Bahkoor1 and Oudh1 were observed to be much higher than levels from Bahkoor2 and Oudh2.

Figure 15: Target gene relative expression bar graph. Relative expression levels for the (A) IL-8 and (B) COX-2 response from 24 hour exposure to particle and gas infused media after all 4 burns. Each well was exposed to 55ug of particles whereas the controls were exposed to 0ug.

4 Discussion and Conclusion

The main strength of this study is the realistic burn simulation experimental design and the use of the life-size burn simulator chamber and the simultaneous sampling for characterization and toxicological exposure system. Incense was burned in an identical manner to how it is burned within the home. The 22m³ chamber size and ventilation rate reflects a typical living room, the emissions were all measured in real-time while being exposed to cells *in vitro*. To our knowledge, this is the only study to observe UAE incense emissions and potential health effect indicators by both observing a broad emission profile, with several monitoring instruments, and exposing cells *in vitro* concurrently.

Both types of UAE incense when burned with charcoal emit PM, CO, NO_x, HCHO, and other carbonyls. These concentrations, emission rates, and emission factors were observed in a well-mixed room while simulating a room ventilation of 0.5exchange/hr and are not corrected for dilution. Previous literature values had a range of dilution rates and correction factors outlined (below Table 7, A-C) and the range represents the varying values reported from several incense types and burning conditions. No SO₂ was observed. Emission rates from this study were also compared to current outdoor regulation values, from EPA and WHO, and indoor regulation values, from California and Canadian health boards (Table 7).

Table 7: Emission rates, emission factors, and carbonyls detected in this study compared to other studies. CO, NO_x, PM, and HCHO are reported in emission rates (ER) and emission factors (EF) and glyoxal & valeraldehyde values are reported in overall concentration detected.

	This Study	Previous Literature	Current Regulations
CO	ER = 40-70ppm/hr EF = 331-509mg/g	ER = 0.39-14.4ppm/hr ^(A&B) EF = 106-228mg/g ^(A&B)	Outdoor = 35ppm/hr ⁽¹⁾ Indoor = 20-25ppm/hr ^(2&3)
NO_x	ER = 150-200ppb/h EF = 0.7-2.2mg/g	ER = 4-110 ppb/hr ^(A&B) EF = 0.4-3.3mg/g ^(A&B)	Outdoor = 100ppb/hr ⁽¹⁾ Indoor = 250ppb/hr ^(2&3)
PM_{2.5}	ER = 600-670ug/m ³ /hr EF = 35.32-187mg/g	ER = 480-6730 ug/m ³ /hr ^(A) EF = 13-205mg/g ^(B)	Outdoor = 35ug/m ³ /8hr ⁽¹⁾ Indoor = 100ug/m ³ /hr ⁽²⁾
HCHO	ER = 62-80ppb/30min EF = 3.55-12.32mg/g	EF = 0.45-1.70mg/g ^(B&C)	Outdoor = 80ppb/30min ⁽⁴⁾ Indoor = 100ppb/hr ⁽³⁾
Glyoxal	6.61-84.81ug/m ³	7.7ppb (19.7ug/m ³) ^(C)	400ug/m ³ ⁽⁵⁾
Valer-aldehyde	11.55-31.95ug/m ³	5-15ug/m ³ ^(B)	N/A

- A. **No ventilation due to direct sampling;** Jetter, J. J., Z. Guo, J. A. McBrien & M. R. Flynn (2002) Characterization of emissions from burning incense. *Science of The Total Environment*, 295, 51-67.
- B. **0.5/hr vent in 18.26m³ chamber, Dilution corrected;** Lee, S.-C. & B. Wang (2004) Characteristics of emissions of air pollutants from burning of incense in a large environmental chamber. *Atmospheric Environment*, 38, 941-951.
- C. **Natural 30m³ room vent, No Correction;** Ho, S. S. H. & J. Z. Yu (2002) Concentrations of formaldehyde and other carbonyls in environments affected by incense burning. *Journal of Environmental Monitoring*, 4, 728-733.
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One of the most notable emissions is CO and while CO concentrations accumulated as high as 122ppm inside the chamber, the main source was the charcoal used, not the incense itself. This CO emission rate for a full charcoal reached 70ppm/hr and half a charcoal CO emission rate reached 40ppm/hr, both exceeding regulation values and 0.39-14.4ppm/hr values observed previously in other studies (Table 7). NO_x levels as high as 0.225ppm were observed with an emission rate of 150-200ppb/hr depending on the

amount of charcoal used. Charcoal emissions were proven to be the main source of both CO and NO_x. HCHO emissions resulted in a peak concentration of 75-85ppb with averages of 62-80ppb/30min rate, comparable to the WHO outdoor standard of 81.8ppb per 30 minutes. Several other carbonyls were observed such as methyl glyoxal/glyoxal, biacetyl, valeraldehyde, and limonene. These were all observed at individual concentrations from 6-116ug/m³ and total carbonyl concentrations from 86-300ug/m³. Results explained previously from Table 4 indicate carbonyls are emitted 98% in the gas phase, experiencing slow decay rates similar to gases not particles.

HCHO was observed to decay with a half-life of over three hours and lingered in the chamber long after the incense produced visible smoke. CO and NO_x experienced decay rates similar to the room exchange rate of 0.5/hr. Conversely, particles were observed to decay quickly (half-life 30 minutes) while being emitted at 600-670ug/m³/hr in the respirable 20-350nm range. These findings suggest while particle concentrations peak quickly they become eliminated faster than the gaseous components due to wall loss. However, the chamber was designed with inert Teflon surfaces, making this wall loss minimal. This behavior has been seen previously where particles in similar sized Teflon lined chamber have experienced decay rate from wall loss much faster than the tracer gas (Kamans et al., 1994; Saucy et al., 1983). With the knowledge that different compounds emitted from incense peak and decay at different rates in the home, future studies using particle concentration data alone will not be sufficient in evaluating incense emissions.

When observing potential health effects indicators, emissions from Oudh appeared to induce an inflammatory response in A549 cells *in vitro* by causing an up-regulation of the

IL-8 cytokine. This response indicates a potential inflammatory response when incense smoke is inhaled into the airways, as observed in studies observing asthmatic response (Harving et al., 1991; Liu et al., 2009; Peters et al., 1997). All results together indicate a health risk when burning UAE incense indoors.

The number of burn replicates proved to be a weakness of this study due to of the amount of charcoal utilized each burn. While the charcoal alone did not emit carbonyls, the concentrations observed in burns with a full charcoal briquette, Oudh1 & Bahkoor1, was significantly lower than concentrations seen when half a charcoal briquette was used, Oudh2 & Bahkoor2 (Table 4). A possible explanation is that the amount of charcoal affected how complete the combustion process took place when incense burned, therefore affecting the emissions. More incomplete combustion of the incense occurred with less heat from less charcoal making the burn less 'clean' and emitted more carbonyls. A more complete combustion process of the incense occurred with more charcoal on the other hand, reducing the incense's carbonyl emissions. Also the inflammatory response observed from the cells *in vitro* to Bahkoor1/Oudh1 and Bahkoor2/Oudh2 was varied. IL-8 levels in cells exposed to Bahkoor1/Oudh1 were much higher than levels in their respective second experiments, Bahkoor2/Oudh2. This is the opposite of observed carbonyl concentrations where Bahkoor1/Oudh1 burns produced lower carbonyls concentrations and emission factors than Bahkoor2/Oudh2. This is consistent with the HCHO concentrations being higher and sustained longer in Oudh2 versus Bahkoor1, indicating that carbonyl emissions are not the dominating factor in cell inflammatory response; however the amount of charcoal could be. While the data presented here contributes to the goal of assessing UAE incense

emissions and their health effects, more replicates would add more strength to this hazard assessment.

To help reduce individual exposure indoors, proper ventilation must be implemented including but not limited to opening a door or window. Also using an alternative to charcoal to burn the incense would greatly reduce the CO and NO_x levels. Alternative ways to burn incense are on the market. Electric devices previously studied (Madany and Crump, 1994) were used to heat the incense eliminating the charcoal's deleterious emissions.

To give a complete profile of incense emissions, future studies should measure other hazardous compounds, observed previously, emitted from incense such as volatile organic compounds and polycyclic aromatic hydrocarbons (Lee and Wang, 2004; Lung et al., 2003; Madany and Crump, 1994; Navasumrit et al., 2008; Tran and Marriott, 2007; Yang et al., 2007). Also to evaluate individual exposure, personal monitoring equipment could be implemented. Lastly, a more in-depth analysis of inflammatory markers would help explore in more depth what type of inflammation is being experienced in the body.

5 Appendix A - CO Calibration

Figure 16: CO calibration. 10 ppm of pure CO was added to the chamber at time 11:23, 10 ppm more was added at 11:33 and lastly 10 ppm more totaling 30 ppm was added at 11:46. This graph shows that 0.2 volts corresponds to 10 ppm of CO.

6 Appendix B - Chromatogram Plots

Figure 17: Chromatogram plots from Bahkoor1. These graphs show carbonyls derivatized and extracted from the bottom of the mister during background, burn 1, burn2, and with filter analysis during Bahkoor1.

Figure 18: Chromatogram plots from Bahkoor2. Bottom mister samples from background, burn1, burn2, and filter from Bahkoor2.

Figure 19: Chromatogram plots from Oudh1. Bottom mister samples from background, burn1, burn2, and filter from Oudh1.

Figure 20: Chromatogram plot from Oudh2. Bottom mister samples from background, burn1, burn2, and filter from Oudh2.

Figure 21: Chromatogram plots comparing top and bottom compartment of mister. Top and bottom compartment carbonyl samples from Bahkoor1 and Oudh1. Data for this study came from carbonyls trapped in the bottom mister compartment since concentrations found in the top compartment are minimal.

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