Reactive Oxygen Species in Incense Smoke

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The objective of this study is to determine the concentration of reactive oxygen species (ROS) generated from burning incense in an experimental chamber with a volume of 288 liters and a continuous supply of filtered air at 15 l/min. A Micro-Environmental Monitor (MEM) collected PM_1 and $PM_{2.5}$ from the incense smoke on 37-mm polycarbonate membrane filters. The ROS in particles were extracted using dichlorofluorescin-horseradish peroxidase ($DCFH_2$-HRP) reagent. Additionally, two parallel sampling trains, each consisting of a filter cassette and 3 impingers connected in series, were employed to simultaneously collect ROS in particles and in gas phase. Each impinger contained 10 ml of the $DCFH_2$-HRP reagent for absorbing ROS in gas phase. The samples obtained by filter cassette were treated as the MEM samples. Subsequently, the extracts and impinger samples were incubated at 37 ℃ for 15 min and the fluorescence intensity of resulting dichlorofluorescein (DCF) was determined. Fluorescence intensity data were converted to equivalent H_2O_2 concentrations using calibration curves obtained from fluorescence measurement of standard DCF solutions. For samples collected 1 hr after one joss stick of black lignaloes was lit in the experimental chamber, the mean ROS concentrations in PM_1 and $PM_{2.5}$ were 15.60 ± 1.00 and 13.50 ± 1.30 nmol H_2O_2/mg of particles, respectively. In contrast, the mean ROS concentrations in PM_1 and $PM_{2.5}$ sampled from an apartment (free of incense smoke, cooking fumes, and cigarette smoke) were 6.54 ± 1.00 and 4.06 ± 2.04 nmol H_2O_2/mg, respectively. When one joss stick of black lignaloes was burned for 1 hr in the experimental chamber, the mean ROS concentration was 0.94 ± 0.06 nmol H_2O_2/l of air in gas phase and 1.06 ± 0.05 nmol H_2O_2/l in particles. The finding could have important health implications because particulate ROS in inhaled air could easily reach the alveolar region.

Keywords: Incense smoke, aerosol, reactive oxygen species

1. Introduction

Reactive oxygen species (ROS), a term used to collectively describe oxygen-containing species with strong oxidizing ability, can cause respiratory inflammation, lung cancer, and other adverse health effects. Both endogenous and exogenous sources of ROS exist. Biological systems produce ROS to defend against foreign

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organisms and other environmental challenges. Many studies of ROS have focused on their generation by biological systems (Kao et al., 1998; Vallyathan et al., 1995). On the other hand, Sagai et al. (1993) demonstrated that particles in diesel emissions can produce significant amounts of ROS, and do so in vivo without any biological activating systems. Photochemical reactions in polluted air also produce ROS. Earlier studies on atmospheric ROS have focused primarily on ROS in the gas phase and in rain and cloud droplets (Olszyna et al., 1988; Sakugawa and Kaplan, 1990; Hellpointner and Gäb, 1989). In a recent report on ROS in various particle fractions of Taipei aerosols, Hung and Wang (2001) showed that ROS concentrations in ambient particles correlate well with the intensity of photochemical reactions and that, for the same particle mass, smaller particles had higher ROS contents.

Combustion of organic materials such as cigarette and wood also can generate reactive oxygen species (Valavanidis et al., 1996; Leonard et al., 2000). A common source of combustion aerosols in Taiwan is incense burning, a religious practice followed by Taoists and Buddhists when they pray to gods and ancestors. Joss sticks are made of powdered incense wood coated on thin wooden sticks. ROS generated from burning joss sticks appear in both the gas and particulate phases. Particles in combustion aerosols are generally less than a few tenths of a micrometer in diameter and therefore have relatively high rates of deposition in the alveolar region of the respiratory tract. Consequently, ROS in combustion particles can reach the lower respiratory tract and therefore have greater health effects than the gas phase ROS, which are mostly absorbed by mucus in the upper respiratory tract.

Previous studies on aerosols generated from burning incense have focused mainly on particle concentrations, size distributions, and elemental and organic compositions. Cheng et al. (1995) reported the results of a study on particle sizes and concentrations in incense smoke using a 34 m^3 test room, while Kao and Lung (2000) measured concentrations of incense smoke at a residence as well as the concentration level to which worshipers were exposed at temples. Furthermore, Hu and Lung (2000) reported the rates at which particles were generated for various time intervals after the lighting of a joss stick. Other studies have also looked at aldehydes (Wang, 1992), elemental and organic compositions (Hung et al., 1993), and PAHs (Hsieh, 1996) in incense smoke. This study aims to determine the ROS concentrations in both the gas and particulate phases in incense smoke.

2. Materials and Methods

Rapid measurements of ROS can be taken using a fluorogenic probe. Dichlorofluorescin ($DCFH_2$), a non-fluorescent compound, forms highly fluorescent dichlorofluorescein (DCF) upon reaction with ROS. The dichlorofluorescin-horseradish peroxidase ($DCFH_2$-HRP) reagent solution used in this study was prepared using the procedure described by Black and Brandt (1974). Mixing 0.5 ml of 1 mM ethanol solution of 2',7'-dichlorofluorescin diacetate ($DCFH_2$-DA) with 2 ml of 0.01 N NaOH solution yielded an unstable $DCFH_2$ solution. This $DCFH_2$ solution was then incubated at room temperature in a darkened cabinet for 30 min and then mixed with 10 ml of sodium phosphate buffer solution to maintain a PH value of 7.2 in the resulting solution. The prepared $DCFH_2$ solution was refrigerated and stored in a darkened cabinet. To catalyze the reaction between $DCFH_2$ and ROS, horseradish peroxidase (HRP) was added to the $DCFH_2$ solution before it was used to extract ROS from the samples.

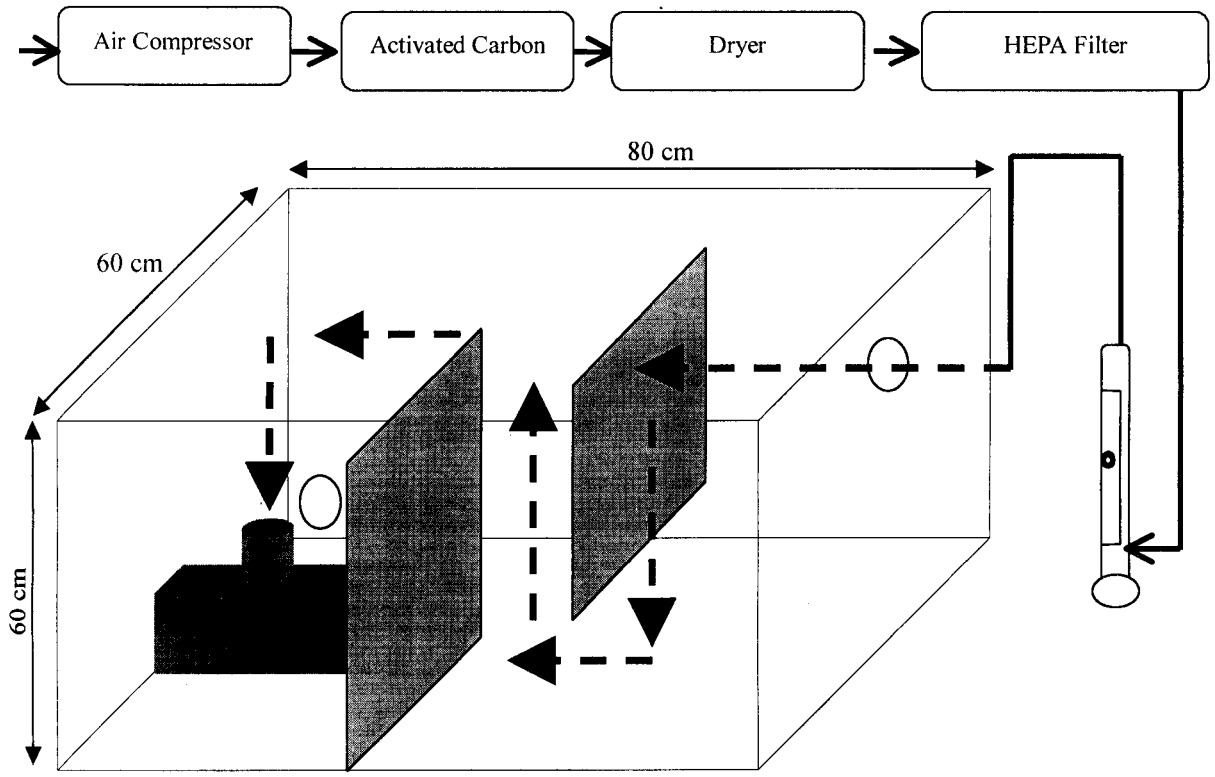

Figure 1 A schematic diagram of the experimental system

A Cytofluor 2300 microplate reader (Millipore, Bedford, MA, USA) was employed to determine the fluorescence intensity of the DCF formed from reactions between $DCFH_2$ and ROS in each sample. Measurements were made with an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. Data on the fluorescence intensity were converted to equivalent H_2O_2 concentrations using calibration curves, which were obtained from measuring the fluorescence of standard DCF solutions using the procedure described by Cathcart et al. (1983).

The incenses used in this study were red lignaloes, dark red lignaloes, and black lignaloes. The first two had identical ingredients, except that a dark dye was added to the second type, while the third type was made of a different kind of wood. The joss sticks were burned in an experimental chamber (Figure 1). Two plates were inserted in the chamber to direct air to flow around them, and the incense stick was placed between the two plates for each run. The chamber had a volume of 288 liters and a continuous supply of filtered air at 15 l/min. The average residence time of air in the chamber was 19.2 min, and therefore particle concentrations reached the steady state about one hour after an incense stick began to burn.

A Micro-Environmental Monitor (MEM, MSP Corporation, Minneapolis, MN, USA) was placed in the chamber to collect smoke particles on 37-mm polycarbonate membrane filters. An appropriate inlet allows the MEM to collect either PM_1 or $PM_{2.5}$ samples. The sampling flow rate was 10 l/min and the sampling period 1 min. For comparison, 8-hr particle samples were collected by an MEM from 8:00 to 16:00 in an apartment free of incense smoke, cooking fumes, and cigarette smoke.

All filters were weighed before and after sampling to determine the mass of particles collected. Following weighing, each filter sample

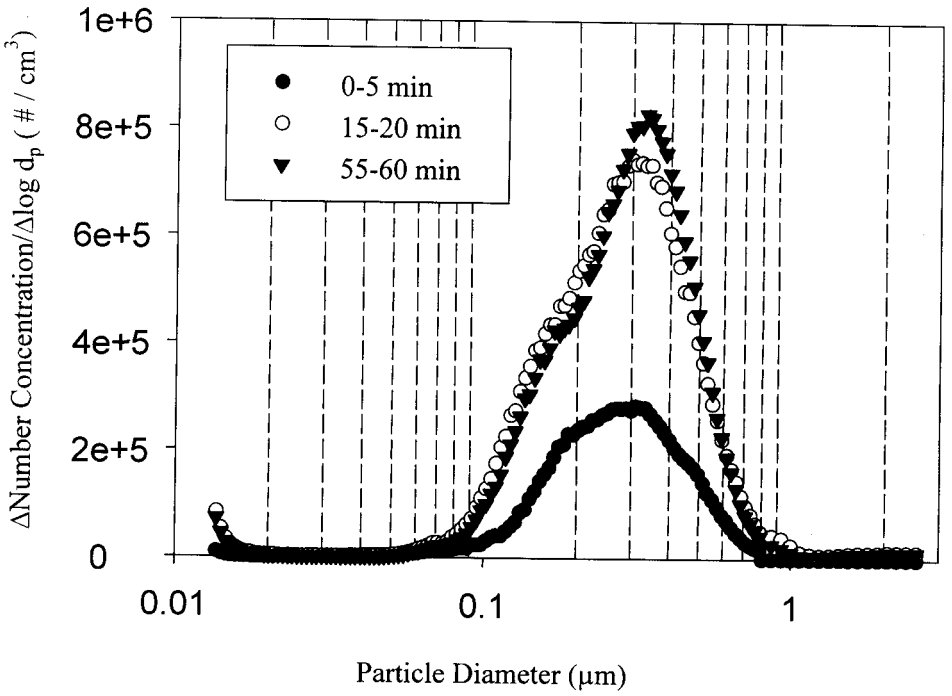

Figure 2 Size distributions of particles in black lignaloes smoke in the experimental chamber at various combustion times. Data in the range of 0.013- 0.80 μm were SMPS measurements and data in the range of 0.80-2.5 μm were APS measurements.

was mixed with 10 ml of 1 μM $DCFH_2$-HRP reagent and sonicated for 10 min to extract ROS from the particles.

Another sampling system, consisting of two parallel trains, each with a filter cassette followed by 3 impingers connected in series, was employed to collect ROS in particulate phase and gas phase simultaneously. Each impinger contained 10 ml of the $DCFH_2$-HRP reagent. The sampling flow rate was 1 l/min and the sampling period was 1 min. The particle samples obtained by the filter cassette were treated exactly the same as the MEM samples. The extracts and samples collected by impingers were incubated at 37 ℃ for 15 min and the fluorescence int of DCF resulting from reactions between D and ROS in each sample was determined.

A Scanning Mobility Particle Sizer (S Model 3934, TSI Inc., St. Paul, MN, USA an Aerodynamic Particle Sizer (APS, M 3310A, TSI Inc., St. Paul, MN, USA) employed to determine the size distributi incense smoke particles in the range 0.013-0.80 and 0.80-2.5 μm, respectively.

3. Results and Discussion

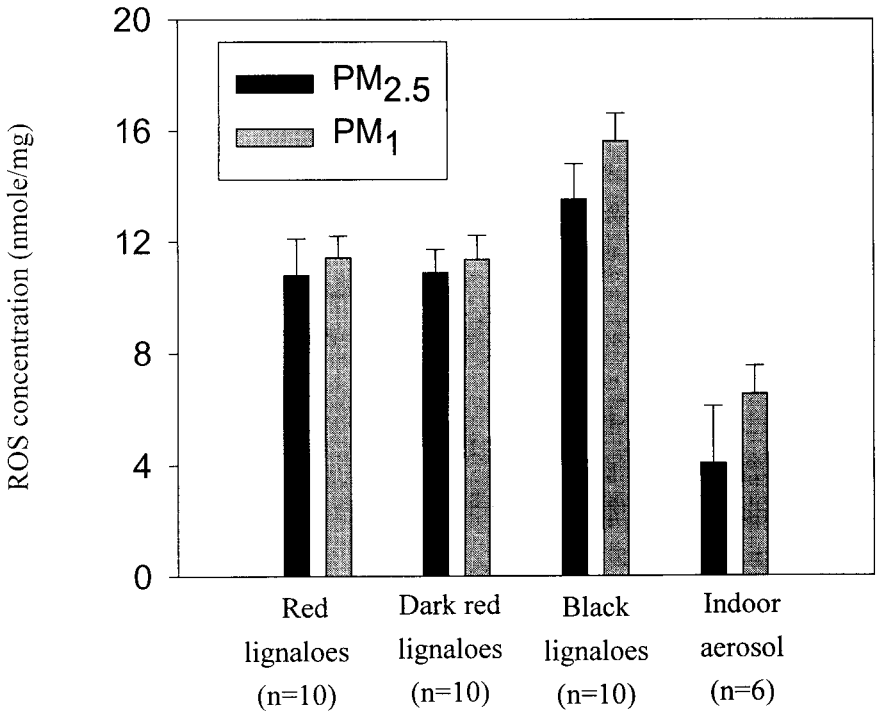

Figure 3 Comparison of ROS concentrations per unit mass of particles in PM_1 and $PM_{2.5}$ generated from burning of various incenses and in indoor aerosol. Each broad bar represents the mean of n samples and the error bar represents one standard deviation. The ROS concentration is expressed in terms of the equivalent H_2O_2 concentration.

Figure 2 shows some typical size distributions of smoke particles measured at various times after a joss stick of black lignaloes was lit in the experimental chamber. The curve for 0-5 min peaked at a particle size of about 0.3 μm. The number concentration of smoke particles increased with time because of particle accumulation in the chamber, while the peak shifted towards larger particles as a result of coagulation. Comparison of the curves for 15-20 min and 55-60 min indicates that the size distribution almost reached the steady state at 20 min. Particles generated from burning of red lignaloes and dark red lignaloes had size distributions similar to those shown in Figure 2, confirming that incense smoke particles were mainly smaller than 1 μm in aerodynamic diameter, as reported previously by Cheng et al. (1995).

For both PM_1 and $PM_{2.5}$, the mass concentration of particles generated from the burning of black lignaloes joss sticks was lower than for red lignaloes and dark red lignaloes joss sticks. The mean PM_1 concentration and the standard deviation determined 1 hr after a stick was lit in the chamber were 26,391±2,077 μg /m^3 for red lignaloes, 30,935±2,641 μg /m^3 for dark red lignaloes and 17,306±2,996 μg /m^3 for black lignaloes (all based on 10 samples). The rates at which PM_1 particles were generated from burning one joss stick in the experimental chamber, calculated from steady state mass concentration

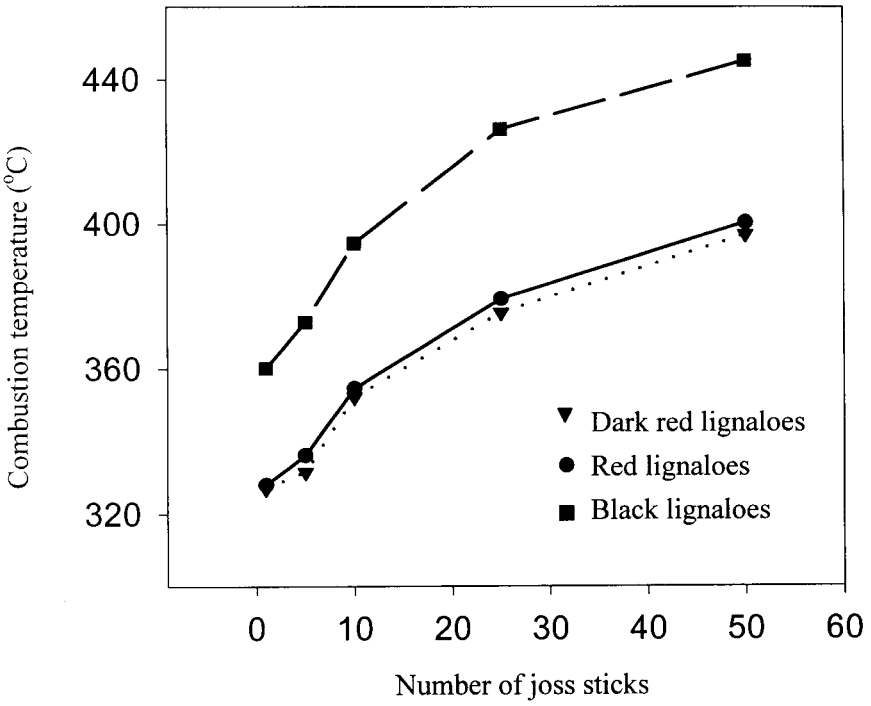

Figure 4 Combustion temperature as a function of the number of joss sticks for various incenses.

data, were 396, 464, and 260 μg/min for red lignaloes, dark red lignaloes, and black lignaloes, respectively. Among the three types of incense, black lignaloes had the lowest generation rate for both PM_1 and $PM_{2.5}$ particles. The rate at which particles were generated by the burning of one stick of black lignaloes obtained in this study was comparable to the rates of 230-280 μg/min reported by Cheng et al. (1995).

Figure 3 compares the ROS concentrations per unit mass of particles in PM_1 and $PM_{2.5}$ generated from burning various types of incense with the particles collected at the smoke free apartment. For samples collected after one joss stick had burned for 1 hr in the experimental chamber, the ROS concentration in PM_1 particles was 11.40 ± 0.80 nmol H_2O_2/mg of particles for red lignaloes, 11.60 ± 0.70 nmol H_2O_2/mg of particles for dark red lignaloes, and 13.50 ± 1.30 nmol H_2O_2/mg of particles for black lignaloes (all based on 10 samples). In contrast, unburned incense powder that was composed of very large particles had a mean ROS concentration of 2.54 ± 0.68 nmol H_2O_2/mg of particles (based on 3 samples).

The ROS concentration in particles from black lignaloes incense was appreciably higher than the ROS concentrations in particles from red and dark red lignaloes incenses. It is interesting to note that the burning temperature of black lignaloes incense was also considerably higher than that of the other two types of incense. Figure 4 shows the temperature at the burning tip of various incenses measured by a thermocouple. Temperature measurements were made for single sticks as well as for multiple sticks tied together in a bundle, and combustion temperature was found to increase with the number of joss sticks. For black lignaloes, the temperature increased from 360 ℃ at the tip of a burning stick to 450 ℃ at the tip of a bundle of 50 burning sticks. For a given number of sticks in a bundle, the combustion temperature of black lignaloes was at least 30 ℃ higher than those of the other two types of incense.

As expected, particles collected at the apartment had the lowest ROS concentration. The mean ROS concentrations in PM_1 and $PM_{2.5}$ particles collected 1 hr after a joss stick of black

lignaloes incense was lit in the chamber were 15.60 ± 1.00 and 13.50 ± 1.30 nmol H_2O_2/mg of particles, respectively. By contrast, the mean ROS concentrations in PM_1 and $PM_{2.5}$ particles sampled from the apartment were 6.54 ± 1.00 and 4.06 ± 2.04 nmol H_2O_2/mg of particles, respectively. It is, however, to be noted that the sampling duration for particles from burning joss stick was only 1 min, while that for particles in the apartment was 8 hr. As particle samples from the apartment were collected using a long sampling period and a high flow rate, the ROS concentrations could be underestimated. Particles generated from burning incense sticks and particle samples from the apartment both revealed more ROS in a unit mass of PM_1 particles than in a unit mass of $PM_{2.5}$ particles. Analyses of ash comprising large particles that had fallen from a burning incense stick gave a mean ROS concentration of 0.67 ± 0.37 nmol H_2O_2/mg of particles (based on 3 samples). These measured results indicate that smaller particles have a higher ROS content than larger particles with the same particle mass.

From the viewpoint of health effects, it is interesting to compare the ROS concentrations in the gas and particulate phases. Measurements were made 1 hr after a joss stick of black lignaloes was lit in the experimental chamber. For comparison, the ROS concentration is expressed in terms of nmol H_2O_2 per unit volume of air in the chamber. The mean ROS concentration was found to be 0.94 ± 0.06 nmol H_2O_2/l of air in the gas phase and 1.06 ± 0.05 nmol H_2O_2/l in the particulate phase (mean ± standard deviation of 5 samples). Expressed in terms of percentage of the total amount of ROS, the percentage of ROS in the particulate phase was 53 %, while that in the gas phase was 47 %.

ROS Measurements were also made for freshly generated particles, which were collected immediately after 15 joss sticks of black lignaloes began to burn in the experimental chamber. It was necessary to burn 15 joss sticks simultaneously in order to yield sufficiently high aerosol concentrations so that a 1-min sample would contain sufficient particles for analysis. To eliminate the effect of neighboring joss sticks on combustion temperature, the sticks were placed at least 3 cm apart. The ROS concentration in freshly generated particles was found to be 17.5 ± 1.3 nmol H_2O_2/mg of particles (mean ± standard deviation of 4 samples). By contrast, the ROS concentration in aged particles collected 1 hr after a joss stick was lit was 14.8 ± 0.9 nmol H_2O_2/mg of particles (mean ± standard deviation of 5 samples). ROS in particles decayed with time, probably owing to evaporation or reactions with reducing agents in the air. It is to be noted that particle samples collected 1 hr after a joss stick was lit actually consisted of particles with ages ranging from 0 to 60 min.

4. Conclusions

Burning of incense generated high concentrations of ROS in particles and in the gas phase. The ROS concentrations in both PM_1 and $PM_{2.5}$ generated from a burning incense stick were considerably higher than those in particles sampled from an apartment that was free of incense smoke, cooking fumes, and cigarette smoke. For both incense smoke particles generated in the experimental chamber and particles collected from the apartment, the amount of ROS in a unit mass of PM_1 particles exceeded that in a unit mass of $PM_{2.5}$ particles. When one joss stick of black lignaloes had burned for 1 hr in the experimental chamber, the percentage of ROS in particulate phase (53 %) was higher than the percentage of ROS in gas phase (47 %). As fine particles can reach the alveolar region of the respiratory tract, ROS in particles can cause greater adverse health effects

than ROS in the gas phase, which were mostly absorbed by mucus in upper lung airways. In view of the potential health effects of particulate ROS, further investigation is needed on the factors affecting concentrations of ROS in particles generated from incense burning.

Acknowledgments

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