



## Surface Characterization of Secondary Organic Aerosols from Ozonolysis of Monoterpene and the Effects of Acute Lung Injury in Mice

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### ABSTRACT

Monoterpene is a biogenic volatile organic compound commonly found in cleaning products and air fresheners. It can react rapidly with indoor oxidants, such as ozone (O<sub>3</sub>), to produce secondary organic aerosols (SOAs) in indoor environments, and the reactions are potentially influenced by ammonia (NH<sub>3</sub>). This study simulated the reactions of O<sub>3</sub> and monoterpene with and without the presence of NH<sub>3</sub> in an environmental chamber and investigated the surface characterization (elemental components and carbon states) of the PM<sub>2.5</sub> generated by these reactions. We found that the generated particles possessed a higher content of nitrogen-containing organic compounds when NH<sub>3</sub> was present. Unsubstituted aromatic carbon and aliphatic carbon were the main carbon structures, exhibited by over 60% of the carbon-containing compounds. Additionally, in the presence of NH<sub>3</sub>, more amide carbon and carboxylic carbon formed during the reactions. We also examined acute lung injury in mice caused by new particle formation under different reaction conditions. Oxidative stress was observed in the bronchoalveolar lavage fluid of the mice, as evidenced by a decrease in antioxidant enzymes (superoxide dismutase) and antioxidants (glutathione) as well as an increase in malondialdehyde. Moreover, the SOAs generated in the presence of NH<sub>3</sub> lowered glutathione levels, indicating a rise in oxidative stress. Hence, fine particles formed by indoor oxidative reactions may trigger acute lung injury in humans, potentially causing further respiratory disease.

**Keywords:** Indoor air pollution; SOA; Oxidative stress; Acute lung injury.

### INTRODUCTION

Secondary organic aerosols (SOAs), mainly generated from the oxidative reactions of unstable chemical compounds (i.e., volatile organic compounds [VOCs]) in the atmosphere, account for a considerable proportion of organic aerosols in the urban atmosphere (Hallquist *et al.*, 2009). The chemical characteristics of SOAs generated in indoor environments are different from those generated outdoors as a result of divergent emission sources and formation processes (Colbeck *et al.*, 2010; Perrino *et al.*, 2016). The organic compounds generated indoors can substantially contribute to personal PM<sub>2.5</sub> (particulate matter in diameter < 2.5 μm) exposure owing to the long exposure time in indoor environments (Morawska *et al.*, 2013). Epidemiological and toxicological

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studies have confirmed the association between PM<sub>2.5</sub> and damage to human pulmonary and cardiovascular systems (Kappos *et al.*, 2004; Kim *et al.*, 2015). Although SOAs contain many toxic compounds (i.e., metals, polycyclic aromatic hydrocarbons [PAHs] and carbonyls), evidence for their adverse effects on the human respiratory system is still limited (McDonald *et al.*, 2012).

Ozone (O<sub>3</sub>) is a common initiator of indoor gas-phase oxidation. It was estimated that average daily indoor intake of ozone oxidation products was one-third to two times compared to that from outdoor ozone inhalation (Weschler, 2006). Biogenic volatile organic compounds (BVOCs) are also common indoor pollutants. O<sub>3</sub> and BVOCs can react to and initiate most indoor chemical reactions (Matsumoto, 2014). The presence of unsaturated carbon double bonds within the molecular structures of BVOCs makes these compounds highly reactive with indoor oxidants, eventually leading to the formation of various secondary pollutants (Yuan *et al.*, 2013). Hydroxyl radicals (OH) and nitrate radicals

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(NO<sub>3</sub>) can also result in the formation of SOAs (Wang *et al.*, 2016). BVOCs are present at high concentrations in indoor environments due to the use of cleaning products and air fresheners (Huang *et al.*, 2011). The most common indoor terpenoids are monoterpenes; d-limonene, for example, can react rapidly with ozone and has high particle formation potential (Nørgaard *et al.*, 2014). The formation of ultrafine particles produced by ozone and limonene reactions was observed in indoor air under low and closed ventilation conditions, and it was revealed that excess O<sub>3</sub> led to higher particle concentrations (Langer *et al.*, 2008). Indoor gas-phase reactions can be affected by various factors in real indoor environments, including temperature, relative humidity and concurrent gaseous pollutants. NH<sub>3</sub> is one of the important indoor gaseous pollutants, mainly emitted from household cleaners, refrigeration units and addition urea-based antifreeze admixtures (Pei *et al.*, 2016). NH<sub>3</sub> can interact with gas-phase organic acids, which are the major products of indoor BVOC reactions, to form condensable salts, thereby enhancing SOA formation (Na *et al.*, 2007).

Particulate matter suspended in the atmosphere can be effectively deposited in the extrathoracic airways, whereas PM<sub>2.5</sub> or PM of a smaller size can easily pass through the respiratory tract to the alveoli. These particles penetrate deeper into the airways and alveoli and undergo a slow clearance process, with particles usually remaining for weeks or months (Kim and Hu, 1998). PM-mediated oxidative stress through the generation of reactive oxygen species (ROS) is considered a crucial molecular mechanism of PM-induced toxicity (Shen and Anastasio, 2011). Oxidative stress refers to a critical imbalance between ROS production during oxidative phosphorylation and antioxidant defense, and ROS has been identified as signaling molecules in regulating pathways (Deng *et al.*, 2013). Riva *et al.* (2011) revealed that acute exposure to low doses of PM<sub>2.5</sub> could induce lung inflammation and oxidative stress in a dose-dependent manner in mice. Generally, the adverse effect of PM<sub>2.5</sub> is determined by its surface characterization and chemical composition (Daher *et al.*, 2014; Niu *et al.*, 2017). Studies have reported that PM<sub>2.5</sub> chemical compounds including aliphatic and chlorinated hydrocarbons, PAHs and alkyl derivatives, ketones, and quinones are responsible for the formation of oxidative stress and inflammation through the generation of free radical species (Oh *et al.*, 2011; Yi *et al.*, 2014; Ho *et al.*, 2016b).

The chemical states of carbon and nitrogen and their atomic concentrations in PM critically determines their impact on human health due to their specific surface areas (Cheng *et al.*, 2013). SOAs are important PM<sub>2.5</sub> components contributing to numerous health effects including increased respiratory symptoms, exacerbation of chronic respiratory and cardiovascular diseases, and decreased lung function (Kim *et al.*, 2015). Therefore, it is crucial to study the characteristics of SOAs' functional groups and the related adverse health effects from O<sub>3</sub> and monoterpene reactions. Most previous studies on PM<sub>2.5</sub> health effects were focused on ambient PM<sub>2.5</sub> or specific air pollution sources (Dieme *et al.*, 2012; Chuang *et al.*, 2013; Dilger *et al.*, 2016; Ho *et al.*, 2016a). An environmental chamber can be used to simulate

the formation of SOAs in the atmosphere, and the surface characterization of SOAs collected can be determined and used for toxicity experiments. The objective of this study is to (1) identify the surface characterization (main elemental composition and carbon states) of SOAs formed in the reactions of O<sub>3</sub> and monoterpene, (2) investigate the variations of functional groups of SOAs in the reactions with and without NH<sub>3</sub>, and (3) evaluate the oxidative potential in the respiratory system caused by SOAs generated from O<sub>3</sub> and monoterpene reactions.

## METHODS

### Chamber Study

An environmental chamber with an effective volume of 18.26 m<sup>3</sup> (3.2 m × 3.2 m × 2.5 m) were used for the simulation experiments of SOA generation. Details of the chamber can be found in Huang *et al.* (2012). Prior to use, the interior surfaces of the chamber were thoroughly cleaned and conditioned for 4 hours at the desired physical conditions with the air exchange rate (ACH) of 0.36 h<sup>-1</sup>, relative humidity (RH) of 75%, and temperature of 23°C. The background level of total volatile organic compounds (TVOCs) and individual VOCs were below 10 µg m<sup>-3</sup> and 2 µg m<sup>-3</sup>, respectively, in each experiment. d-Limonene, as a common monoterpene, was chosen for this study. To reach an initial BVOC concentration of approximately 200 ppb in the chamber, 60 µL of d-limonene was introduced to the chamber using a 40-L air bag (Tedlar; SKC Inc., Eighty Four, PA, USA). The air bag with zero air generated from a Zero Air Supply (Model 111; Thermo Environmental Instruments) was injected with d-limonene using a 100-µL syringe (Hamilton, Reno, NV, USA). After complete vaporization, the gas-phase d-limonene was introduced into the chamber by an AirChek sampler (Model 224-44XR; SKC Inc.) at a flow rate of 1 L min<sup>-1</sup>. Before the d-limonene introduction, ozone was introduced into the chamber using an ozone generator (Model 2001; Jelight Company Inc., Irvine, CA, USA) at a constant flow rate of 63 mL min<sup>-1</sup> to approximately 70 ppb. After 30 min, the gaseous d-limonene prepared in the sampling bag was injected. The O<sub>3</sub> supply was terminated 120 min after the injection of d-limonene. In the NH<sub>3</sub> experiments, NH<sub>3</sub> was introduced to the chamber 30 min before the introduction of O<sub>3</sub>. The NH<sub>3</sub> was supplied from a compressed gas cylinder at a concentration of 1000 ppm (N<sub>2</sub> balance, 99.999%; BOC Gas, UK). NH<sub>3</sub> was measured by a thermal oxidizer (Thermal Converter Model 501; Teledyne API) and detecting the oxidized ammonia as NO (Chemiluminescence NH<sub>3</sub> Analyzer Model 201A; Teledyne API). Each experiment was repeated three times to demonstrate reproducibility.

### SOA Sampling

Two mini-volume samplers (Airmetrics, Springfield, OR, USA) with a flow rate of 5 L min<sup>-1</sup> were used for PM<sub>2.5</sub> collection in the environmental chamber during O<sub>3</sub> and d-limonene reactions. The samplers were turned off at the beginning of the experiments. The sampling inlets were placed at ~1.2 m above ground level. The PM<sub>2.5</sub> samples were collected on 47-mm quartz filters (Whatman, Germany).

The filters were preheated at 900°C for 3 h to reduce the amount of organic vapors. All filters were equilibrated at a condition of ~50% RH and ~23°C for over 48 h prior to weighing. Before and after sampling, each filter was weighed at least twice on a microbalance (M5; Sartorius, Göttingen, Germany). The precision was < 15 µg per filter before sampling and < 20 µg after sampling. The exposed filters were stored in a refrigerator at ~4°C until the chemical analysis was conducted to prevent the evaporation of volatile compounds. Operation blank samples were processed in two groups of background experiments.

### **X-ray Photoelectron Spectroscopy Analysis**

The particles on the filter surface, consisting of the elements and their bonding states, from O<sub>3</sub> and d-limonene reactions were analyzed using X-ray photoelectron spectroscopy (XPS) in the spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a monochromatic Al K $\alpha$  X-ray source (1486.6 eV). XPS has been used as a usual method for surface analysis, which can provide information on elemental contributions. Chemical states of elements can also be determined by peak synthesis. Samples can be directly examined by XPS without any sample preparation. The PM<sub>2.5</sub> samples were punched from the 47-mm filters with an area of 0.526 cm<sup>2</sup>. High-resolution spectra were collected at a resolution of 0.2 eV for binding energy, 100 ms per step and 20.0 eV pass energy. All peaks were referenced to the C1s binding energy for adventitious carbon at 284.8 eV. Elements oxygen (O), silicon (Si), carbon (C), sodium (Na), and nitrogen (N) were identified from the spectra.

### **Animal Exposure to PM<sub>2.5</sub>**

Male Kunming mice (20–25 g) were purchased from the Experimental Animal Center, Xi'an Jiaotong University (Xi'an, China). They were maintained under standard conditions with ~50% RH, ~23°C, and a 12-h light/dark cycle. Animals were allowed free access to standard laboratory food and water. All experimental handling and safety procedures were in accordance with National Institute of Health guidelines.

All filter samples were extracted with distilled deionized water through ultrasonication to a final concentration of 50 µg mL<sup>-1</sup>. A total of 48 mice were randomly divided into four groups with 12 mice in each group, and then treated using intranasal instillation with 100 µL of different solutions: (i) experimental control (*control*) treated with autoclaved phosphate-buffered saline (PBS) solution, (ii) field blank (*blank*) treated with suspension liquid from the blank samples, (iii) reaction in the absence of NH<sub>3</sub> (*without NH<sub>3</sub>*) treated with PM<sub>2.5</sub> extractions collected without NH<sub>3</sub> reaction, and (iv) reaction with the presence of NH<sub>3</sub> (*with NH<sub>3</sub>*) treated with PM<sub>2.5</sub> extractions collected through an NH<sub>3</sub> reaction. The mice were immobilized in a 60°-inclined supine position and the extractions were delivered in a dropwise manner to the nares using an automatic pipette.

### **Collection and Analysis of Bronchoalveolar Lavage Fluid**

After intranasal stimulation for 6 and 12 h, the bronchoalveolar lavage fluid (BALF) was collected

repeatedly three times using a tracheal cannula with autoclaved PBS. Each sample was instilled up to a total volume of 1.3 mL. The recovery rate of BALF was approximately 90%. The collected BALF was immediately centrifuged at 1500 rev min<sup>-1</sup> for 10 min at 4°C and the supernatants were collected for further analysis.

The superoxide dismutase (SOD) activity in the supernatant sample was determined using commercial SOD kits following the manufacturer's instructions. The supernatant was measured by a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at an absorbance of 450 nm. The BALF malondialdehyde (MDA) level of mice was determined by the thiobarbituric acid (TBA) method using a TBARS Assay Kit. The absorbance of the supernatant was measured at 532 nm, and the standard curve was obtained using 1,1,3,3-tetramethoxypropane. The glutathione (GSH) level was determined using the standard method explained by Moron *et al.* (1979). The absorbance of the supernatant was measured at 412 nm and compared with the GSH standard. The kits for SOD, MDA and GSH were purchased from Jiancheng Bioengineering Institute (Nanjing, China).

### **Statistical Analysis**

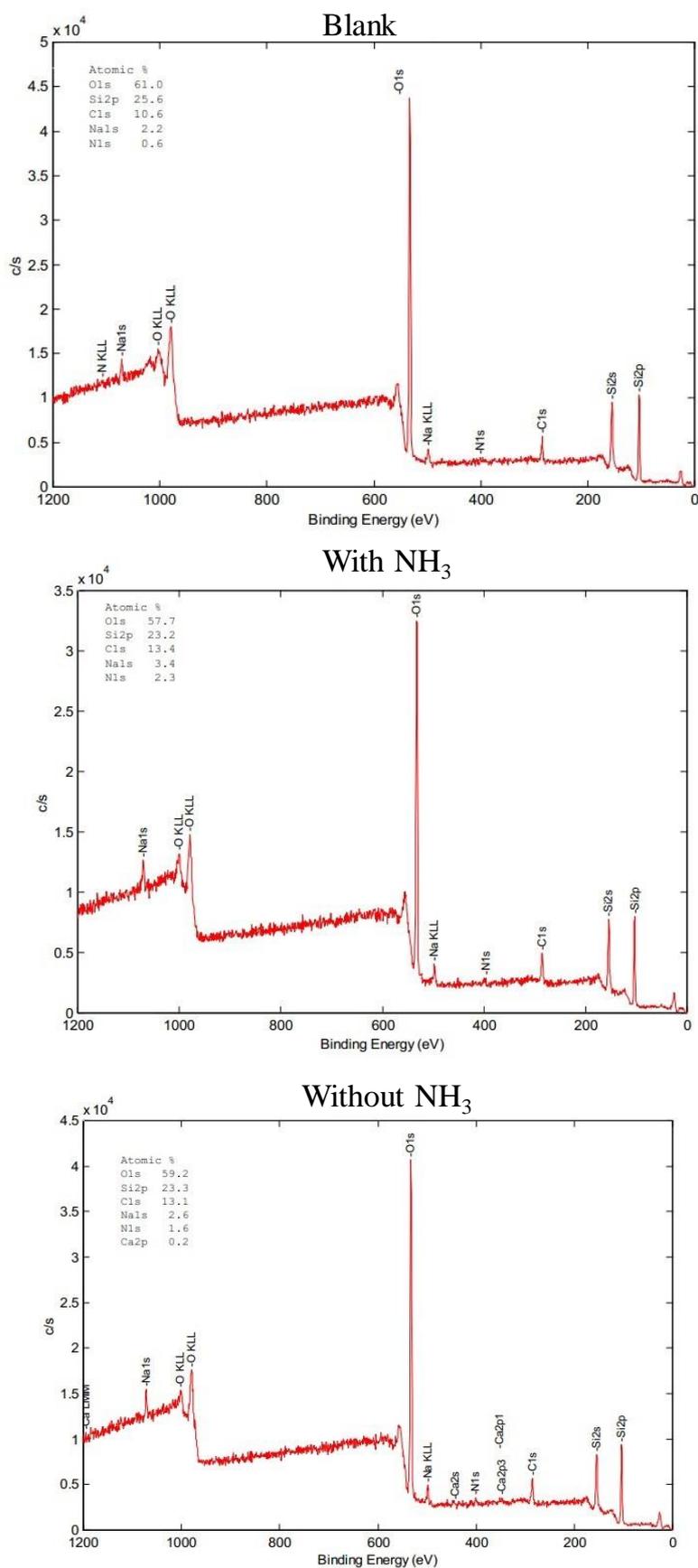
All statistical analyses were performed using SPSS software (version 21.0; IBM, New York, NY, USA). One-way analysis of variance (ANOVA) was conducted to evaluate statistical significance between groups. A p value of < 0.05 was considered to represent statistical significance.

## **RESULTS AND DISCUSSION**

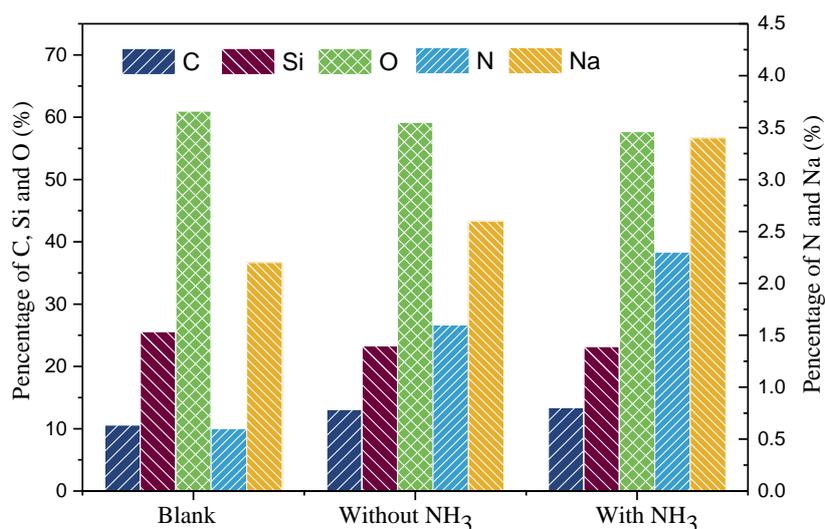
### **Surface Characterization of SOAs**

The average PM<sub>2.5</sub> mass concentrations from the reaction of O<sub>3</sub> and monoterpene were 257.9 ± 19.9 µg m<sup>-3</sup> and 172.9 ± 3.8 µg m<sup>-3</sup> with and without the presence of NH<sub>3</sub>, respectively. A substantial increase was observed in SOA generation in the reaction with NH<sub>3</sub>, which indicated the positive effect of NH<sub>3</sub> on O<sub>3</sub> and monoterpene reactions. These results agreed with the study of Waring and Siegel (2013), which investigated the contribution to gas-phase SOA formation of ozone reactions with surface-sorbed d-limonene and demonstrated that surface reactions promoted nucleation more than gas-phase reactions.

XPS survey scan was used to identify the elements on the surface of SOAs generated from O<sub>3</sub> and monoterpene reactions. The XPS survey spectra were collected at an intensity of 1100 eV for three particle samples (Fig. 1), including a blank filter. Fig. 2 presents the distinct elemental surface composition distributions of SOAs derived from the different conditions of O<sub>3</sub> and monoterpene reactions. Atomic compositions for each sample were calculated using narrow-scan peak areas and the appropriate sensitivity factors for each element (Atzei and Rossi, 2004). Carbon was the third most prominent element on the filter surface (approximately 10.6–13.4%), which is representative of the normal composition of organic matter existing in filters, and new organic aerosols also formed during the reactions of ozone and monoterpene. Only 0.6–2.3% of N was observed on the surface of the filter; a large proportion of N was generated from the organic matter



**Fig. 1.** XPS survey spectra for SOAs on the blank sample; sample with and without NH<sub>3</sub> present in O<sub>3</sub> and monoterpene reactions.



**Fig. 2.** Elemental distribution of SOAs generated from different conditions of O<sub>3</sub> and monoterpene reactions.

that formed during the reaction of ozone and monoterpene. The increase of N indicated the transformation of gas-phase N (i.e., NO, NO<sub>2</sub>, N<sub>2</sub>O<sub>5</sub>, HNO<sub>3</sub>, HONO, alkyl nitrates, and PAN) to solid phase with new formation of organic particles in the reactions.

Secondary pollutants were generated in the process of reaction, with the main element being C (Coleman *et al.*, 2008). In addition, O<sub>3</sub> and monoterpene reactions produced hydroxyl radical, which triggered further reactions with the organics generated; therefore, more carbon-constituted aerosols were generated (Chen and Hopke, 2010). When NH<sub>3</sub> was involved in the reaction, the atomic carbon composition of carbon was slightly (2.3%) but not significantly higher than in the reaction without the presence of NH<sub>3</sub>. NH<sub>3</sub> can considerably enhance the generation of SOAs in O<sub>3</sub> and monoterpene reactions (Huang *et al.*, 2012), and more organic matter with carbon was generated, which enhanced the carbon distribution of SOAs. The contribution of N increased by as much as 167% in the reactions of O<sub>3</sub> and monoterpene without NH<sub>3</sub> compared with the blank sample. The substantial growth of N indicated the transformation of gas-phase N into solid phase with new formation of organic particles. With the presence of NH<sub>3</sub>, the contribution of N element was 43.8% higher than in the reaction without NH<sub>3</sub>. The increase could be attributed to the positive effect of NH<sub>3</sub> on the O<sub>3</sub>/monoterpene reactions that enhanced the formation of SOAs, including nitrogen organic matter such as cyanide and organic matrix. A small quantity of NH<sub>3</sub> adhered to the surface of the quartz filter in the reaction, which may have also contributed to the N increase.

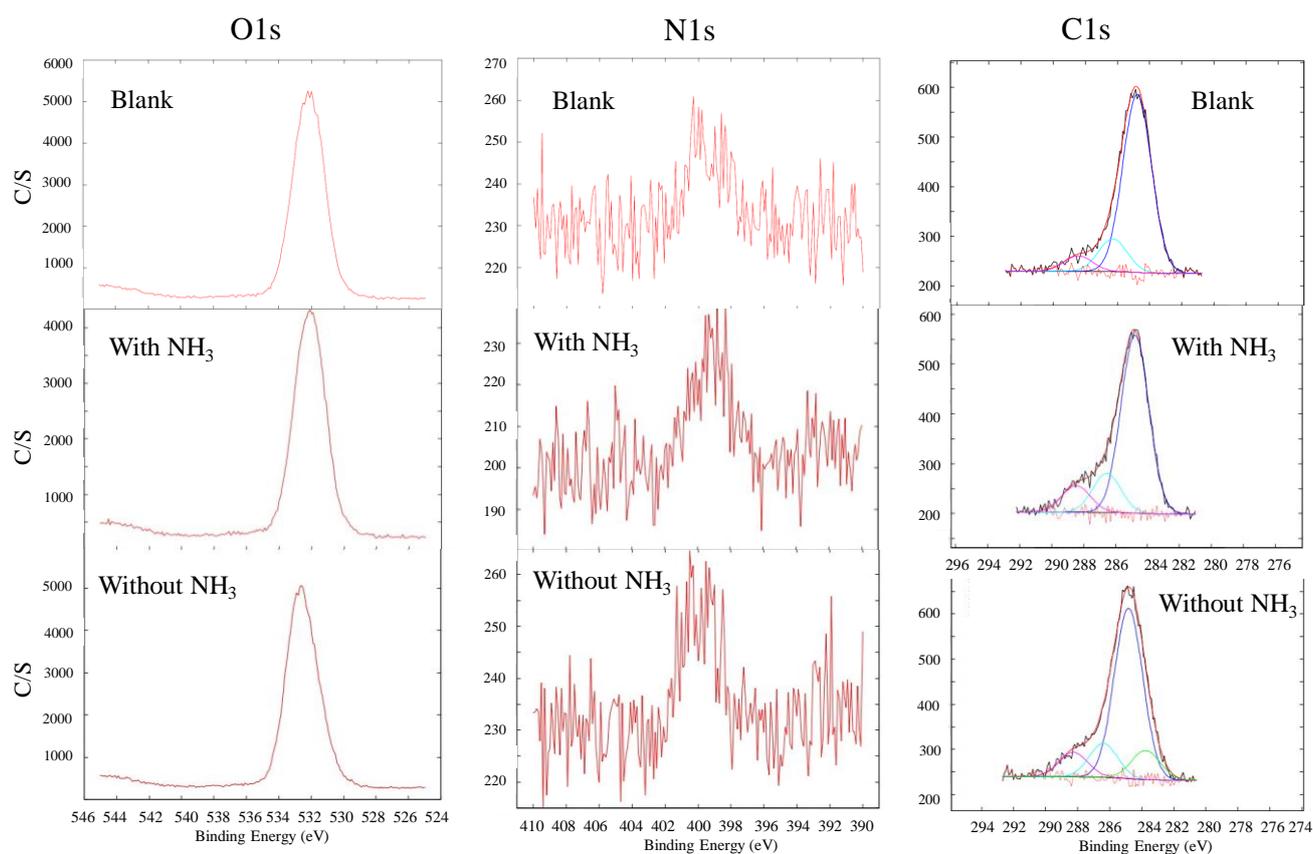
The atomic ratio of elements has been widely used to analyze the chemistry of environmental matrices (Song and Huang, 2005; Shchukarev and Sjöberg, 2005; Qi *et al.*, 2006). The O/C value of the blank sample (5.75) was substantially higher than that of O<sub>3</sub> and monoterpene reactions with NH<sub>3</sub> (4.31) and without NH<sub>3</sub> (4.52). Large amounts of new organic matter were generated in the reaction, and the high ratio of carbon in the organic particles could have resulted in a decrease in the O/C ratio. The declining trend of O/C

with the presence of NH<sub>3</sub> may be because the continuously generated new organic particles enhanced the organic compound yields. The N/C value of the group without NH<sub>3</sub> (0.122) increased compared with the blank sample (0.057), which was attributed to the compounds in the gas phase (including NO, NO<sub>2</sub>, N<sub>2</sub>O<sub>5</sub>, HNO<sub>3</sub>, HONO, *et al.*) reacting with the BVOCs and generating large amounts of nitrogen-containing organic compounds in the solid phase. With the presence of NH<sub>3</sub>, the N/C ratio could be as high as 0.172. The atoms were more active due to the effect of NH<sub>3</sub> in the reaction of O<sub>3</sub> and monoterpene and enhanced the generation of nitrogen-containing organic compounds.

#### Chemical States of Elements

The high-resolution O1s, N1s, and C1s spectrum of the filter samples from the O<sub>3</sub> and monoterpene reactions with and without the presence of NH<sub>3</sub> are shown in Fig. 3. The binding energy of N1s in samples with and without NH<sub>3</sub> in the reactions of O<sub>3</sub> and monoterpene were 399.8 and 399.6 eV, respectively. The nitrogen-containing compounds could be NH<sub>3</sub>, cyanide, or an organic matrix; however, the exact percentage of each possible component is still unknown. As a result of the contribution from pyrrolic and amide nitrogen forms, some of the nitrogen in aerosol particles is assignable to amides, which was also shown by the C1s peak of C(O)N (Song and Peng, 2009). With the oxidation effect of O<sub>3</sub>, the nitrogen-containing gas-phase compounds (e.g., N<sub>2</sub> and NH<sub>3</sub>) reacted with monoterpene, and the C-C and C-H resolved and formed new bonds such as C-N and C(O)N.

More than one chemical embedment was identified in the carbon spectrum. To quantify the high-energy tail of the C1s signal in detail, all particle sample carbon concentrations was classified into four types: (1) unsubstituted aromatic carbon (C-C/C-H, 283.8 eV), (2) aliphatic carbon (C-C/C-H, 284.8 eV), (3) amide carbon [C(O)N, 286.3 eV], and (4) carboxylic carbon [C(O)O, 288.5 eV] according to the C1s spectra (Monteil-Rivera *et al.*, 2000). The chemical assignments and experimental area percentages for each component of the C1s spectra of the three samples are shown in Table 1. More



**Fig. 3.** High-resolution X-ray photoelectron spectrum of O1s, N1s, and C1s for SOAs.

**Table 1.** Percentage area (%) for chemical peaks under the C1s envelopes.

Possible compound	Aromatic C-C/C-H	Aliphatic C-C/C-H	C-O/C-N	COO
Atomic %				
Blank	–	78.72	14.45	6.83
Without NH <sub>3</sub>	11.33	66.10	13.08	9.49
With NH <sub>3</sub>	–	72.92	16.01	11.07

than 70% of the carbon was in CH state, and these C-H functional groups included aromatic C-H and aliphatic C-H. In the reaction without NH<sub>3</sub>, an aromatic hydrocarbon of 11.3% was observed in all the carbon-containing organic compounds. The original carbon structure of monoterpene, including aromatic C-H, was not completely separated by the reaction with O<sub>3</sub>. With the presence of NH<sub>3</sub>, no aromatic C-H was observed in the SOAs, indicating that all monoterpenes in the reactions had transformed into aliphatic C-H and other carbon-containing organic compounds. Studies have revealed that reactions of O<sub>3</sub> with constituents containing unsaturated C-C bonds are highly reactive and always serve as a larger source of secondary pollutants (Westbrook *et al.*, 2013). The O<sub>3</sub> and monoterpene in the chamber reacted rapidly and generated secondary pollutants by decomposing unsaturated C-C bonds. This also explained the decreasing trend of C-C and C-H contribution after reaction. BVOCs are labile and can be oxidized in the atmosphere, thereby resulting in many oxygen-containing polar compounds aggregating on the surface of aerosol particles (Kroll and Seinfeld, 2008).

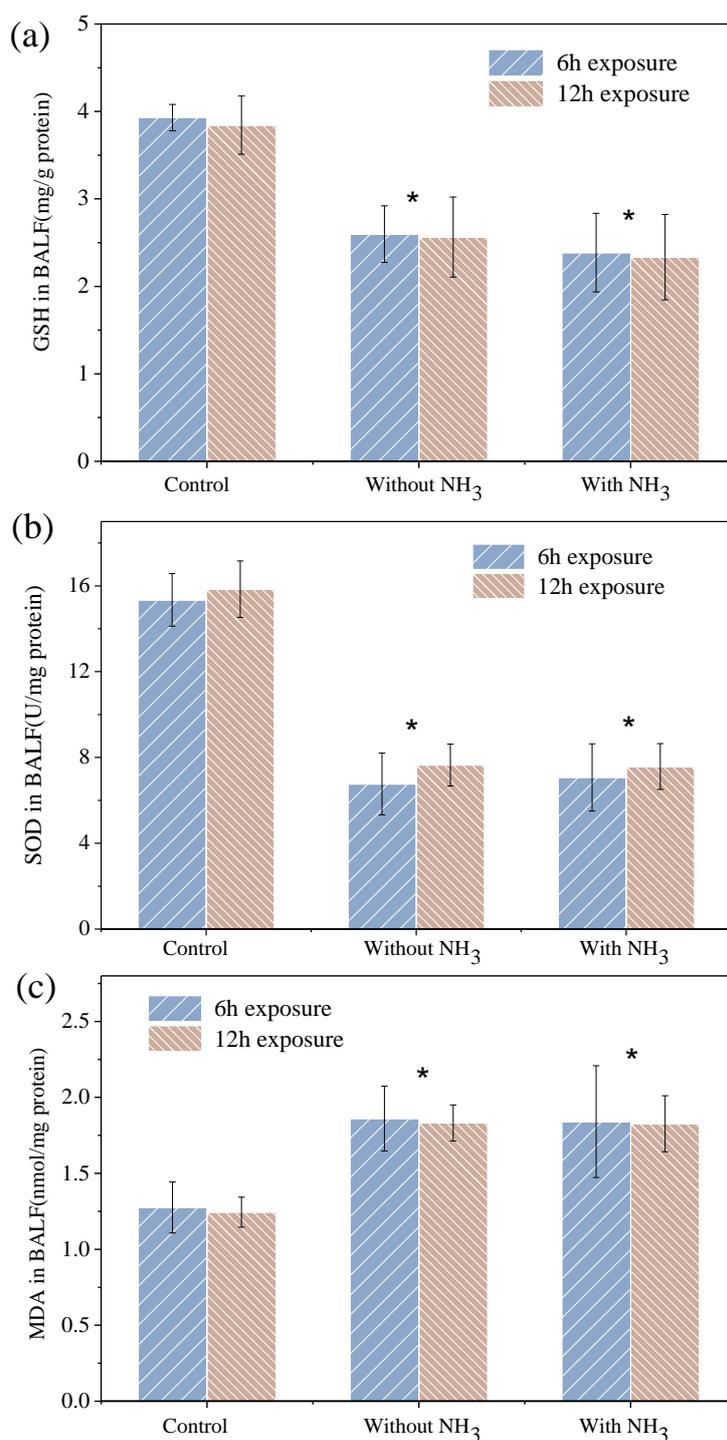
CO/CN and COO structures were detected in the surface particles. The primary oxygen functional groups (i.e., carboxylic and phenolic moieties) can react with O<sub>3</sub> and form secondary organic particles containing CO/CN and COO functional groups. With the presence of NH<sub>3</sub>, more organic compounds in CO/CN and COO structures were generated compared with the reactions without NH<sub>3</sub>, this may be attributed to the active atoms leading to the formation of new bonds in the reactions.

#### **Oxidative Stress Induced by SOAs**

Antioxidants (e.g., GSH) and antioxidant enzymes (e.g., SOD, glutathione peroxidase, and catalase) prevent the generation of ROS and subsequent oxidative damage to various cell constituents and can provide antioxidant defenses to the respiratory system (Blokhina *et al.*, 2003). When an imbalance between excessive oxidant and antioxidant defenses exists, oxidative stress is triggered (Leeuwenburgh and Heinecke, 2001). The oxidative stress and antioxidative responses in mice BALF after exposure to PM<sub>2.5</sub> from O<sub>3</sub>

and monoterpene reactions are presented in Fig. 4. The production of SOD, MDA, and GSH after exposure to samples generated with and without the presence of  $\text{NH}_3$  differed significantly compared with control groups ( $p < 0.05$ ). The concentration of SOD declined significantly (55.9% for the group without  $\text{NH}_3$  and 54.0% for the group with  $\text{NH}_3$ ) after 6 h of exposure to  $\text{PM}_{2.5}$  compared with the control group. No significant difference was observed

between the group with and without the presence of  $\text{NH}_3$ . GSH also exhibited a decreasing trend compared with the control group in the biomarker of antioxidation. After 6 h of exposure to  $\text{PM}_{2.5}$ , the GSH concentrations of the groups with and without  $\text{NH}_3$  declined to 2.4 and 2.6  $\text{mg g}^{-1}$  protein, respectively. The generation of MDA increased significantly compared with the control group, indicating that oxidative stress was triggered by  $\text{PM}_{2.5}$  in mice BALF. No significant



**Fig. 4.** Activity levels of (a) SOD, (b) MDA, and (c) GSH in BALF after mouse exposure to  $\text{PM}_{2.5}$  from  $\text{O}_3$  and monoterpene reactions. \*  $p < 0.05$  compared with the control group.

difference was observed between the groups with and without  $\text{NH}_3$ . It is inferred that the additional chemical compounds generated in  $\text{O}_3$  and monoterpene reactions with  $\text{NH}_3$  were not the main contributors to  $\text{PM}_{2.5}$ -triggered oxidative reactions.

Oxidative stress has been confirmed as a common pathway for oxidative damage caused by  $\text{PM}_{2.5}$  (Bonetta *et al.*, 2009). The generation of free radicals and oxidative stress would further indicate that the occurrence of inflammatory responses in the human respiratory systems is associated with  $\text{PM}_{2.5}$  exposure (Dilger *et al.*, 2016). In our study, the SOAs generated from the reactions of  $\text{O}_3$  and monoterpene caused oxidative stress in mice lungs with short-term exposure. The differences in oxidative stress levels in different reaction groups are associated with the chemical compositions of  $\text{PM}_{2.5}$ , especially organic components (Gualtieri *et al.*, 2009; Ho *et al.*, 2016a). The oxygen-containing polar compounds in SOAs generated from  $\text{O}_3$  and monoterpene reactions were important contributors to the oxidative responses. The oxidative stress in mice BALF could be triggered by different sources, including (1) the direct generation of ROS on the surface of the particle, (2) Fenton reactions with transition metals and organic compounds, (3) the activation of inflammatory cells able to generate ROS and reactive nitrogen species, and (4) alteration in mitochondrial function or NADPH oxidase (Risom *et al.*, 2005; Lakey *et al.*, 2016). The compounds generated in  $\text{O}_3$  and monoterpene reactions that collected on the filter including various chemical compounds would cause oxidative stress via different pathways. Riva *et al.* (2011) revealed that low doses of PM could induce lung oxidative stress, inflammation, and worsened lung impedance and histology in mice. Rohr *et al.* (2002) suggested that oxidation products of ozone and unsaturated hydrocarbons may have medium-term adverse effects on the upper airways and pulmonary regions. A related study confirmed upper airway irritation in mice and a reduction of respiratory rate after exposure to a mixture of terpenes and  $\text{O}_3$  (Rohr *et al.*, 2002).

Our results indicated that short-term exposure to  $\text{PM}_{2.5}$  from  $\text{O}_3$  and monoterpene reactions induced the inhibition of antioxidant enzymatic activities of SOD and antioxidant generation of GSH. Longer exposure time induced more considerable reductions of GSH, whereas time-related responses for SOD and GSH were not significant due to the relatively short exposure time in this study. The group with  $\text{NH}_3$  exhibited lower GSH levels compared with the group without  $\text{NH}_3$ , indicating the relatively higher oxidative ability of SOAs from reactions with the presence of  $\text{NH}_3$ . More organic compounds with C-C/C-H, C-O/C-N, and COO function groups were generated with the addition of  $\text{NH}_3$  reaction, which could be responsible for the elevated oxidative stress. SOD could catalyze the dismutation of  $\text{O}_2^-$  to  $\text{H}_2\text{O}_2$ , which protects cells from oxidation (Go and Kim, 2001). The ROS induced by  $\text{PM}_{2.5}$  in mice lungs can interact directly with antioxidant enzymes, which would further cause a reduction in enzymatic activities (Pamplona and Costantini, 2011). The excessive generation of ROS in radical form causes severe damage to DNA, RNA, and proteins in the respiratory system (Vattanasit *et al.*, 2014). These results are in agreement with the study indicating that  $\text{PM}_{2.5}$  can

elicit oxidative stress by inducing ROS generation and causing a loss of antioxidant enzymatic activity (Deng *et al.*, 2013). The decline in GSH levels after exposure to  $\text{PM}_{2.5}$  from  $\text{O}_3$  and monoterpene reactions is probably caused by a GSH peroxidase reaction, GSH transferase reaction, or GSH efflux (Riva *et al.*, 2011). Our results were in agreement with the study in which pulmonary inflammation was caused by PM exposure in the airways of neonate rats, which also reported a diminished GSH related to the inflammatory process (Ding *et al.*, 2010). Weldy *et al.* (2011) confirmed that GSH synthesis can mediate diesel exhaust particulate-induced lung inflammation through mice exposure experiments.

With the inhibition of SOD as antioxidative enzymes in oxidative stress conditions in mice BALF, the excessive ROS easily attack the cell membrane and form MDA (Gehling *et al.*, 2014). MDA is a product of ROS-mediated lipid peroxidation and has been used as the biomarker for cell membrane damage (Bo *et al.*, 2016). In our study, MDA concentrations in different reaction groups increased significantly after exposure to  $\text{PM}_{2.5}$  extractions. The SOAs generated from  $\text{O}_3$  and monoterpene reactions could cause oxidative stress in the pulmonary system of mice and damage cell membranes. Exposure to the SOAs led to an imbalance of pro-oxidants and antioxidants in mice BALF and resulted in the occurrence of oxidative stress (Dianat *et al.*, 2016). With the reactions of  $\text{O}_3$  and monoterpene, the generation of organic compounds in SOAs including C-O/C-N and COO functional groups enhanced the damage to cell membranes. The effect of SOAs from reactions with and without the presence of  $\text{NH}_3$  did not reveal significant differences. This may be due to the slight differences in the  $\text{PM}_{2.5}$  chemical compositions from reactions with and without the presence of  $\text{NH}_3$ . This indicates that  $\text{NH}_3$  did not strongly affect the acute respiratory oxidative damage caused by  $\text{O}_3$  and monoterpene reactions. The release of MDA caused by  $\text{PM}_{2.5}$  exposure did not show significant time-dependent responses. Li *et al.* (2015) revealed that the changes of mitochondrial fission induced by  $\text{PM}_{2.5}$  exposure were accompanied by an increase in MDA. A related study on the toxicity of  $\text{PM}_{2.5}$  revealed oxidative damage to human alveolar epithelia cells through the induction of lipid peroxidation and the alteration of MDA activity (Kouassi *et al.*, 2010).

## CONCLUSION

This study investigated the ozone-initiated formation of indoor SOAs containing monoterpene in a large environmental chamber and their corresponding respiratory health effects. The presence of  $\text{NH}_3$  potentially enhanced the formation of SOAs from reactions between  $\text{O}_3$  and monoterpene and also altered the surface characteristics of these SOAs. More organic compounds with C-N and COO functional groups were also generated when  $\text{NH}_3$  was present during the reactions. Furthermore, we examined oxidative stress in the BALF of mice following exposure to these SOAs. The antioxidant and oxidative biomarkers displayed no significant differences between the groups with and without  $\text{NH}_3$ , indicating that  $\text{NH}_3$  exerts no distinct influence through the generated SOAs on acute lung injury in mice.

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