https://dx.doi.org/10.1016/j.scitotenv.2016.11.018

Indoor secondary organic aerosols formation from ozonolysis of monoterpene: An example of d-limonene with ammonia and potential impacts on pulmonary inflammations

Xinyi Niu [a,b], Steven Sai Hang Ho [b,c], Kin Fai Ho [e], Yu Huang [b,d*], Junji Cao [b,d,f*], Zhenxing Shen [g], Jian Sun [g], Xiumei Wang [h], Yu Wang [h], Shuncheng Lee [i], Rujin Huang [b,d]

[a]*School of Human Settlements and Civil Engineering, Xi'an Jiaotong University, Xi'an, China*
[b]*Key Lab of Aerosol Chemistry & Physics, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, China*
[c]*Division of Atmosphere Sciences, Desert Research Institute, Reno, NV89512, United States*
[d] *State Key Lab of Loess and Quaternary Geology (SKLLQG), Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 710061, China*
[e]*The Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong, China*
[f]*Institute of Global Environmental Change, Xi'an Jiaotong University, Xi'an, China*
[g]*Department of Environmental Sciences and Engineering, Xi'an Jiaotong University, Xi'an, China*
[h]*School of Pharmacy, Xi'an Jiaotong University, Xi'an, China*
[i]*Department of Civil and Structural Engineering, Research Center for Environmental Technology and Management, The Hong Kong Polytechnic University*

** Corresponding author: Key Lab of Aerosol Chemistry & Physics, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an, 710061, China.*
E-mail addresses: huangyu@ieecas.cn (Y. Huang), cao@loess.llqg.ac.cn (J.J. Cao).
Telephone number: +86-029-62336261; +86-029-62336205
Tax number: 86-029-62336234

Abstracts:

Monoterpene is one class of biogenic volatile organic compounds (BVOCs) which widely presents in household cleaning products and air fresheners. It plays reactive role in secondary organic aerosols (SOAs) formation with ozone (O_3) in indoor environments. Such ozonolysis can be influenced by the presence of gaseous pollutants such as ammonia (NH_3). This study focuses on investigations of ozone-initiated formation of indoor SOAs with d-limonene, one of the most abundant indoor monoterpenes, in a large environmental chamber. The maximum total particle number concentration from the ozonolysis in the presence of NH_3 was 60% higher than that in the absence of NH_3. Both of the nuclei coagulation and condensation involve in the SOAs growth. The potential risks of pulmonary injury for the exposure to the secondary particles formed were presented with the indexes of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) expression levels in bronchoalveolar lavage fluid (BALF) upon intratracheal instillation in mice lung for 6 and 12 hours. The results indicated that there was 22-39% stronger pulmonary inflammatory effect on the particles generated with NH_3.This is a pilot study which demonstrates the toxicities of the indoor SOAs formed from the ozonolysis of a monoterpene.

Key words: indoor air quality; secondary organic aerosols; monoterpene; ammonia effect; pulmonary inflammation

1. Introduction

Indoor air quality (IAQ) has been drawn more public and governmental authorities' concern in recent years. Hundreds of illness outbreaks, directly or indirectly related to IAQ, occurred in our societies, where include offices, schools, and open accessible buildings (Cao et al., 2012; Che et al., 2015; Godish, 1989; Jiang and Bell, 2008). Higher levels of many pollutants were often seen indoor than outdoor (He et al., 2005; Jones et al., 2000; Parker et al., 2008; Zhou et al., 2016). The United States Environmental Protection Agency (U.S. EPA) highlighted that people spend approximately 90% of their lifetime in indoor environments. Indoor exposure is thus particularly important. Many epidemiological studies proved that either short- or long-term pulmonary exposures to toxic respirable particulate matter (PM) are highly associated with increases of morbidity and mortality (Huang et al., 2012a; Lin et al., 2005; Madureira et al., 2015; Ostachuk et al., 2008).

Ozone (O_3) and ammonia (NH_3) are important indoor gas pollutants. The O_3 is often generated from electric devices such as office equipment (e.g., photocopiers and laser printers) and ozone/ion generator, and its indoor levels (with a typical range of 10-100 ppbv) can be greatly influenced by outdoor-to-indoor conversion subjected to the efficiency of air exchange (Britigan et al., 2006; Waring and Siegel, 2011a, 2013). Indoor NH_3 is mainly emitted from household cleaners, refrigeration units, tobacco smoke and addition urea-based antifreeze admixtures (Bai et al., 2006; Koistinen et al., 2008; Pei et al., 2016; Sarigiannis et al., 2011).

Monoterpenes, a well-known class of biogenic volatile organic compounds

84 (BVOCs) with a basic chemical formula of $C_{10}H_{16}$, are widely used as an active
85 ingredient or a fragrance in cleaning products and air fresheners (Singer et al., 2006a;
86 Singer et al., 2006b). d-limonene and α-pinene are the two most abundant
87 monoterpenes present in indoor environment (Brown et al., 1994). Leungsakul et al.
88 (2005) outlined secondary organic aerosols (SOAs) formation mechanism from the
89 chain reactions of d-limonene in presence of oxides of nitrogen and natural sunlight in
90 an environmental chamber. The important role of O_3 in the gas-to-particle reactions
91 with surface-absorbed d-limonene onto the SOAs was demonstrated by Waring and
92 Siegel (2013) as well. The generations of SOAs between indoor O_3 and a single
93 terpenoids were investigated in many studies, in which few focused on a mixture of
94 BVOCs emitted from the household products (Huang et al., 2011a; Lamorena and Lee,
95 2008; Nazaroff and Weschler, 2004; Waring et al., 2011b).
96 Exposures to indoor O_3 and oxidized products have connections with
97 morbidity/mortality (Breysse et al., 2013; Tamas et al., 2006; Weschler, 2006). The
98 impacts on the irritants formation from limonene and isoprene in functions of reaction
99 time, relative humidity (RH) and initial concentrations of the reactants were
100 investigated (Wilkins et al., 2003). Rohr et al. (2002) suggested that the terpene/ozone
101 reaction products may have moderate-lasting adverse effects on both the upper
102 airways and pulmonary regions to human. In addition, more evidences showed that
103 PM inhalation can perpetually damage pulmonary and cardiovascular systems (Astort
104 et al., 2014; Bates et al., 2015; de Brito et al., 2014; Magnani et al., 2011); A wide
105 variety of toxicities caused were also reported *in vivo* and *in vitro* studies, by means of

inflammatory cytokines induction as exposure response to different PM components, (Ho et al., 2016; Leung et al., 2014; Michael et al., 2013; Seagrave et al., 2006). However, those studies seldom discussed on the pulmonary injury related to the ozonolysis of BVOCs.

There is little work on the investigation of SOAs formation between O_3 and BVOCs in the presence of NH_3; and, more particularly, their potential health impact on human respiratory system is still a lack. In this study, d-limonene was used to be an example to demonstrate the indoor SOAs formation from the ozonolysis of monoterpene and the effects of presence of NH_3 on the basis of chains of chamber experiments. With the collections of PM onto filter matrix, the degrees of pulmonary injury of the exposures to the secondary particles formed were presented by the indexes of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) expression levels in bronchoalveolar lavage fluid (BALF). The theme of this research is critical and advances our knowledge on their potential health risks for those abundant indoor pollutants.

2. Methods

2.1 Environmental chamber

The experiments were conducted in a stainless-steel environmental chamber (3.2 m × 3.2 m × 2.5 m). The effective volume is 18.26 m^3 with a surface area to volume ratio of 2.87 (m^2/m^{-3}). The schematic diagram of the chamber is shown in Fig. 1 and details of the operating principle were presented in our previous publication (Huang et

al., 2011a). Prior to each experiment, the chamber interior surfaces were fully cleaned by a sponge mop, and then adjusted and maintained to desired physical conditions [air exchange rate (ACH) of 0.36 /h, relative humidity (RH) of 75% and temperature (T) of 23° C for 4 hours, simulating to a real indoor environment. The background level for total volatile organic compounds (TVOCs) and individual VOC were below 10 μgm^{-3} and $2\mu gm^{-3}$, respectively (Huang et al., 2011b; USEPA, 1999).

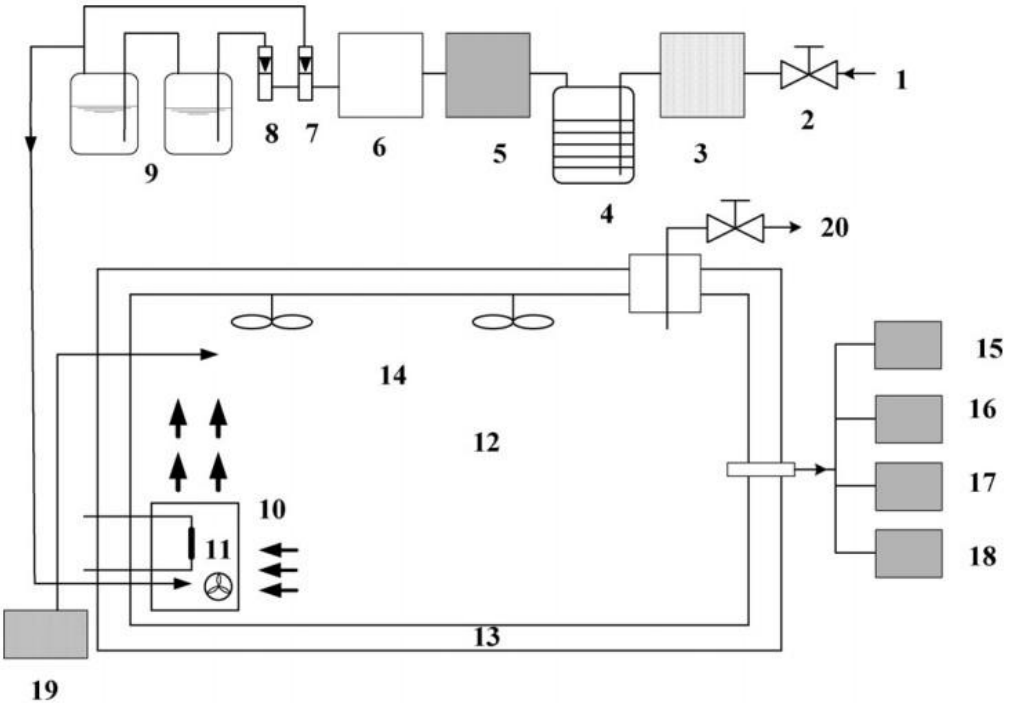

Fig. 1 Schematic diagram of the chamber study set-up: (1) air inlet; (2) valve; (3) blower; (4) active charcoal filters; (5) HEPA filters; (6) mass flow controllers; (7) flow controller dry air; (8) flow controller wet air; (9) humidifier; (10) rotating cylinder; (11) heating unit; (12) large environmental test chamber; (13) insulation layer; (14) mixing fan; (15) SMPS/CPC; (16) ozone monitor; (17) ammonia monitor; (18) H_2O_2 analyzer; (19) ozone generator; and (20) air outlet.

2.2 Gas introductions and experiment process

d-limonene was chosen as a representative of monoterpene in this study. The initial concentration of d-limonene inside the chamber was set as 200 ppbv, which was prepared by injection of standard gaseous by sampling bag method. A 40-L Tedlar air

bag (SKC Inc., Eighty Four, PA, USA) was fully cleaned and filled with air generated by a zero air supply (Model 111, Thermo Environmental Instruments, Frankin, MA, USA). Sixty microliter of d-limonene (Technical Grade, Chem Service, West Chester, PA, USA) was injected into the air bag with a 100 µl syringe (Hamilton, Reno, Nevada, USA). The liquid was completely vaporized in an oven at 70°C. The airs in the bag was then introduced into the centre of the chamber through a plastic tube by an aircheck sampler (Model 224-44XR, SKC Inc.) at a flow rate of 1 L/min.

O_3 was produced by an ozone-generator (Model 2001, Jelight Company Inc., Irvine, CA, USA), which was fed with purified air at a pressure of 20 psi. Initially, the O_3 was introduced into the chamber with a constant flow rate of 63 ml/min. After 30 minutes, the gaseous d-limonene prepared in the sampling bag was then injected. The O_3 supply was terminated 120 minutes after the injection of d-limonene. In the NH_3 effect tests, NH_3 was introduced into the chamber 30 minutes before the d-limonene injection. The NH_3 gas stream was directly supplied from a compressed gas cylinder (N_2 balance, 99.999%, BOC Gas, UK). Each experiment was repeated 3 times to demonstrate the reproducibility.

2.3 Sampling and analytical methods

The variations of O_3, NH_3 and TVOC concentrations and particle number size distribution were measured in the chamber. The NH_3 level was monitored by connecting the air from the chamber to a thermal oxidizer (Model 501, API, San Diego, CA, USA), which oxidized ammonia to nitrogen monoxide (NO) at 825° C, and finally measured by a Chemiluminescent NO_x Analyzer (Model 201A, API). The

thermal oxidizer was properly calibrated using a certified cylinder of NH_3. The O_3 concentration was monitored by a photometric ozone analyzer (Model 400E, Teledyne Instruments, San Diego, CA, USA). A ppbRAE monitor (Model PGM 7240, RAE Systems, Sunyvale, CA, USA) was used to measure the TVOCs concentrations. Particle size distribution of PM with the diameters ranging from 14.1 to 737 nm was measured by a scanning mobility particle sizer (SMPS) system with a differential mobility analyzer (Model 3080, TSI Inc., MN, USA) coupled to a condensation particle counter (Model 3022, TSI Inc.). All of the real-time instruments were well-calibrated before uses. The concentrations of O_3, NH_3 and TVOCs were recorded at 1-min interval, while particle number size distribution was monitored at 4-min interval continuously.

$PM_{2.5}$ formed in the reaction between O_3 and d-limonene was collected with a mini-volume sampler (Airmetrics, Springfield, OR, USA) in the environmental chamber. The sampling inlet was set at 1.2 m above ground level and the actual volumetric flow rate was 5 L/min. The $PM_{2.5}$ was collected on 47 mm quartz microfiber filters (Whatman, Germany), which were preheated at 900 ℃ for 3 h. The sample contained filter was unloaded from the filter holder immediately after the sampling and stored in a refrigerator at 4 ℃ to avoid evaporation of volatile compounds. Before gravimetric measurement, all filters were maintained in a condition chamber with a 50% RH at 25 ℃ for over 24 h. The mass of $PM_{2.5}$ were obtained by weighing the filters prior and after the sample collection, respectively, on a microbalance (M5, Sartorius, Göttingen, Germany) with an accuracy of 1 μg. Each

filter weighed at least twice to ensure the reproducibility with a d-value less than 0.015 mg. Operation filed blanks were collected to investigate organics passively absorbed onto the filter during sampling. The amounts of analytes on the sample filters were deducted from those on the blanks to reduce the error due to positive absorption artifact.

2.4 Animal exposure to PM and model of acute lung injury (ALI)

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 of RH of 55±5%, a 12 h light/dark cycle, and at 23±2℃ with standard laboratory chow and water. All experimental handing and safety procedures were in accordance with guidelines recommended by the National Institute of Health (Institute of Laboratory Animal Resources et al., 1985).

All PM and filed blank samples were extracted with 10ml distilled–deionized water (18.2MΩ resistivity) using ultra-sonication for 60 min, and then shaken with a mechanical shaker for 60 min. A total of 48 mice were randomly divided into four groups for the tests, including (i) experimental control, (ii) field blank, (iii) ozonolysis in the presence of NH_3, and (iv) ozonolysis in the absence of NH_3. The mice were treated by intranasal instillation with 100 μL of the autoclaved phosphate buffered saline (PBS) solution (for the control group), the suspension liquid from the blank samples (for the blank group), and the suspension of $PM_{2.5}$ samples collected in absence and absence of NH_3. They were then immobilized in a 60° inclined supine position while the same dose of 50μg/ml suspension was delivered dropwisely to the

nares by an automatic pipette. The acute pulmonary injuries to mice by $PM_{2.5}$ formed in the chambers were euthanized after exposures for 6 h and 12 h respectively.

2.5 Collections of bronchoalveolar lavage fluid (BALF) and BALF analysis

After the intranasal stimulation for 6 and 12 h respectively, the collections of bronchoalveolar lavage fluid (BALF) were repeatedly performed three times through 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 > 90%. The collected BALF was immediately centrifuged at 1500 rpm at 4 ℃ for 10 min and the supernatants were collected for inflammatory evaluation.

The enzyme-linked immunosorbent assay (ELISA) kits for mice of TNF-α, IL-6, and IL-10 tests were obtained from R&D Systems (Minneapolis, MN, USA). The levels of TNF-α, IL-6, and IL-10 in BALF were determined with the corresponding ELISA kits according to the manufacturer's instructions. In brief, 100 μL of the samples were loaded and added up with biotin conjugated secondary antibodies. The streptavidin-HRP and substrate solution were used as indicators for the reactions. The products were measures at an absorbance of 450 nm with an ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

2.6 Statistical analysis

All statistical analyses were using SPSS software (version 12.0; SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was applied for statistical significance of different groups. The level of significance for all statistical analyses was set as $p < 0.05$.

3. Results and discussion

3.1 Ozone and ammonia variation

Fig. 2 presents the variations of O_3 and NH_3 concentrations in the environmental chamber. In the test without NH_3, O_3 was introduced into the conditioned chamber 30 minutes before the introduction of d-limonene (Phase I). The O_3 level steadily increased to above 60 ppbv. In Phase II, the injection of d-limonene began, and the concentration of O_3 slightly increased to ~70 ppbv and then dropped to 60 ppbv in 20 minutes. This can be explained by the oxidation off d-limonene, which had a consumption of O_3. The injection of d-limonene to the chamber stopped in the beginning of Phase III. The O_3 level dropped a little to 50 ppbv but then rose to 100 ppbv in 100 minutes due to continuous complement of O_3. The introduction of O_3 was terminated in Phase IV. There was thus a continuous and shape decrease in O_3 level while the concentration of TVOCs was still being reduced (TVOCs <100 ppbv). Rather than uptake by the oxidation, O_3 can be self-degraded in the chamber or absorbed by inter-walls of the chamber. The decreasing rate of O_3 became steady in the last 60 minutes. It could be ascribed to almost no reaction with d-limonene.

In the demonstration tests for NH_3 effect, NH_3 was continuously supplied into the chamber with O_3 since the start in Phase I. While d-limonene was injected in Phase II, there was a more rapidly decline in O_3 level (to 55ppbv) compared to the case without NH_3. The result demonstrated that more O_3 uptake in the oxidation of d-limonene in the presence of NH_3, suggesting the strong catalytic effects of NH_3. This function can be further confirmed by the phenomenon in Phase IV, while the introduction of both O_3 and NH_3 were terminated. In the coexistence of NH_3, more remained O_3 was

consumed for the reaction of d-limonene, resulting in a faster decline on the O_3 concentration compared with the case without NH_3.

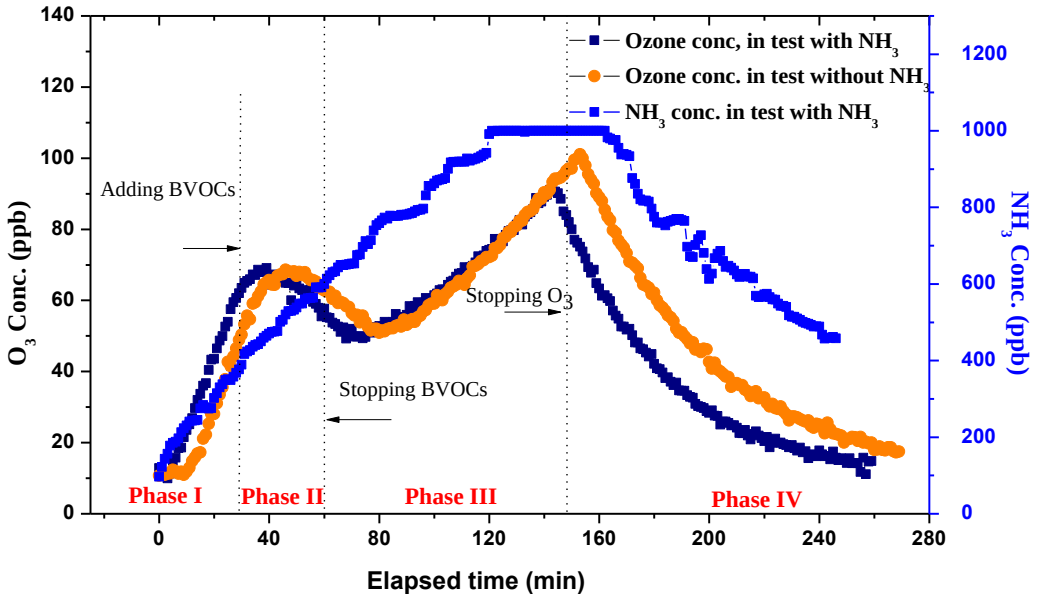

Fig. 2 Ozone and ammonia concentration variations during experiment with the presence of NH_3 and without the presence of NH_3

3.2 Effects of NH_3 on SOAs formation

Fig. 3 shows the particle size distributions with a diameter of 14.1-737 nm from the ozonolysis of d-limonene with and without the presence of NH_3 measured by SMPS. While the introducing of d-limonene into the O_3 atmosphere, particles formations were seen at once. More evident "burst" growth of SOAs was observed in the presence of NH_3, especially with particle diameter ranging from 50 to 200 nm, of which these fine particles were dominant to the total particle number concentrations in the chamber atmosphere. Previous studies demonstrated that gasphase SOA formation commonly occurred from the reactions between surface-adsorbed d-limonene and O_3 in indoor environment (Waring et al., 2011b), and the subsequent

intermediate and by-products could also advance with the growth rate (Sarwar and Corsi, 2007). This finding is also consistent with the previous reports on the formation of SOAs that the particles could be generated from household product emissions (Chen and Hopke, 2010; Destaillats et al., 2006; Rossignol et al., 2013).

In Phase I, the total particle number concentration kept at a low value (below 10^3#cm^{-3}), indicating that there were no any particle formations. When d-limonene was injected into the chamber in Phase II, an obvious growth of particles could be seen, especially for the particles with diameter ranging from 10 to 300 nm. Proceeding of the reactions with NH_3, the particle number concentration (in diameter ranging from 100 nm to 200 nm) increased rapidly to the highest level of 5.94×10^5 #cm^{-3} (Fig. 3a), which was 55% higher than the maximum value of that in the absence of NH_3 (Fig. 3b).

In Phase III, the growth of SOAs continued but then reduced slightly. The highest particle number concentrations were as high as 5.83×10^5 #cm^{-3}, which was also much greater than the maximum without NH_3. In comparison, the particles ranging from 100 to 200 nm in diameter had relatively higher abundances than others in the formation. Due to the degradation and consumption of O_3, NH_3 and d-limonene and ventilation in the chamber environment, there was a huge decline in particle number concentration to 1.0×10^4 #cm^{-3} to 1.0×10^5 #cm^{-3} with a maximum, with size ranging from 180 to 210 nm.

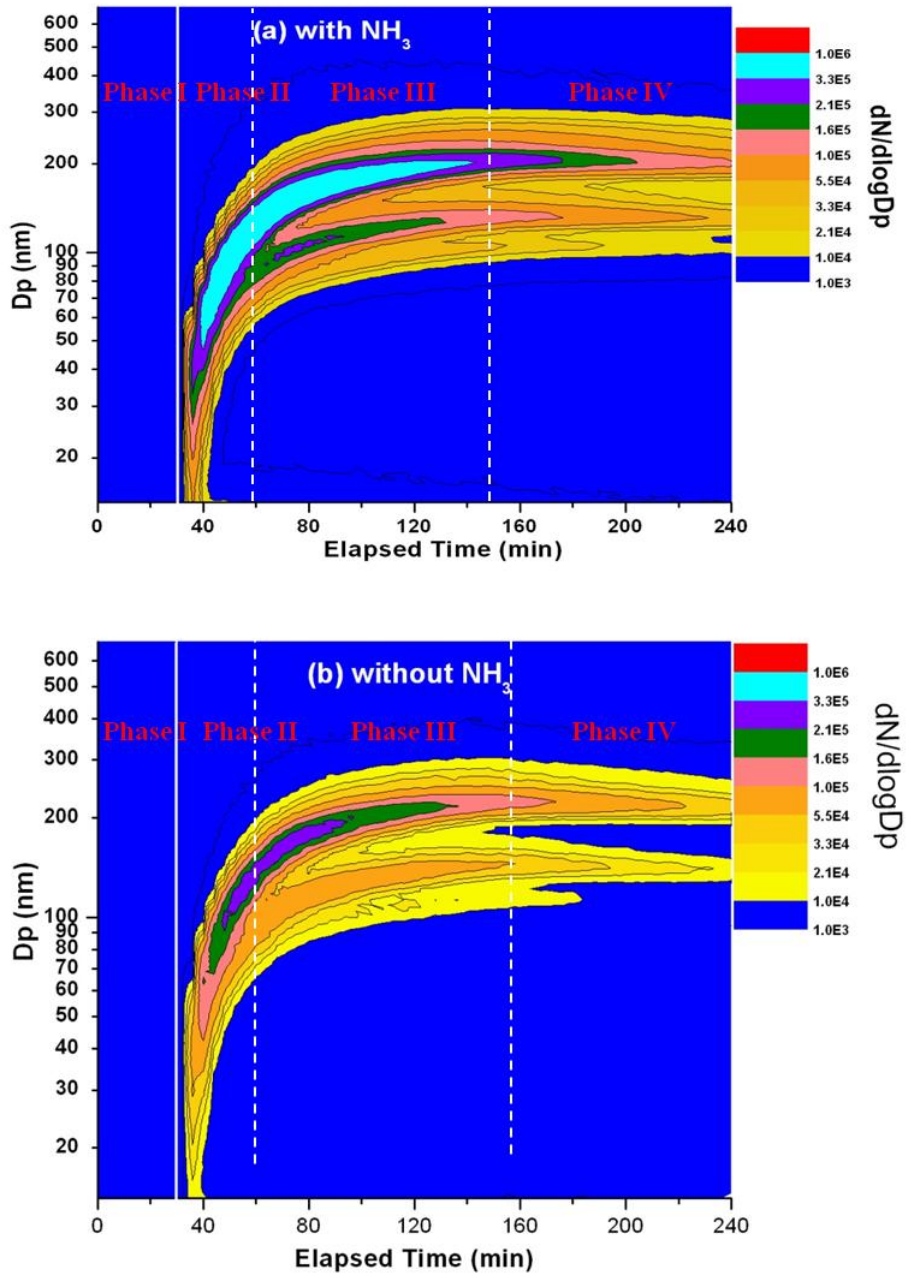

Fig. 3 Temporal evolutions of particle size distributions with a diameter of 14.1-737 nm measured with the SMPS equipment with (a) and without (b) NH_3 presence in the initial chamber atmosphere

The presence of NH_3 in the reaction systems could enhance the oxidation of d-limonene, and thereby generated much more SOAs. Huang et al. (2012b) demonstrated that the extent of NH_3 effect on SOAs formation from the ozonolysis of BVOCs was component-dependent. The results from this study proved the significant of ultrafine particles formations in the coexistence of NH_3, leading to a

302 better understanding of indoor ozone chemistry.

303 Na et al (2006) suggested a detailed explanation to the impact of SOAs formations
304 with the presence of NH_3. NH_3 can interact with gas-phase organic acids, products
305 from oxidation of organics, to form condensable salts (e.g., ammonium salts) and
306 thereby enhance the SOAs formation. As shown in Na et al (2007), the condensable
307 salts could be formed from the α-pinene ozonolysis system while NH_3 was present. It
308 has been generally found that low volatility organic acids such as pinic acid and
309 pinonic acid could be generated during the oxidations of biogenic hydrocarbons
310 (Kavouras et al., 1999; Yu et al., 1999). In our chamber reaction system, the organic
311 acids were also expected to be the major by-products from the ozonolysis of
312 d-limonene which could react with NH_3 to form the condensable salts of low volatility,
313 contributing to the increases of the particle number concentrations.

314 ***3.3 Effect of NH_3 on particles number concentrations and geometric mean***
315 ***diameters***

316 The variations of total particle number concentrations with and without NH_3
317 presence are shown in Fig. 4 (a). The O_3 concentration is plotted as a reference. The
318 highest total particle number concentration was 1.4×10^5#cm^{-3} in the presence of
319 NH_3, which was 60% higher than that without NH_3. The higher concentration
320 suggests that NH_3 can enhance gas-to-particle conversion and promote new
321 formations of particles. NH_3 may contribute to the condensation of vapors onto the
322 existing particles and hence lead the coagulation of particles in the nuclei mode. The
323 highest total particle numbers were seen few minutes after d-limonene was

introduced into the chamber at the initial stage of Phase II. This suggests that the SOAs formation underwent in a very short time at the defined conditions. In the presence of NH_3, the reactions were more energetic and rapid in production of the particles. Decline trends in the total particle number concentrations were also seen from the maximum values either presence or absence of NH_3. Less formation of SOAs occurred in the later phases since more and more d-limonene had been consumed or ventilated, additional with the termination of introduction of O_3 and NH_3 in Phase IV.

Fig. 4 (b) shows the geometric mean diameters of the particles formed in the chamber experiment. The mean diameters of particles increased during the ozonolysis taken place. In the presence of NH_3, a slightly larger mean diameter was observed, which is consistent with the finding from our previous study (Huang et al., 2012). In Phase I, only trace amount of particles, identified as the background, were presented in the chamber. The geometric mean diameters of these particles were between 120-140 nm in the presence of NH_3, which were slightly higher than those without NH_3 (in a range of 100-120nm). However, these should not be related to any SOAs formation as the organic levels were very low in the clean environment. Once d-limonene was introduced, the geometric mean diameters of the particles reduced to 32.5 nm. Along the elapsed times in Phase II, the particle sizes continuously enlarged which had diameters close to 150 nm until the end of this phase. This can be ascribed with the nuclei coagulation and condensation reactions, leading to absorption onto the preexisting particles. More particularly, the particle diameters were slightly lower

in the presence of NH_3 than those formed without NH_3. NH_3 can activate the ozonolysis that has a higher tendency to form new and small particles in the chamber, instead of the condensation of newly-generated particles onto the pre-existed particles. Such particle growth was continuous but had a slower rate even though the introduction of d-limonene terminated at the beginning of Phase III. The activity of ozonolysis obviously reduced the geometric mean particle diameters raised to 180 nm. In the last stage of Phase IV, the reaction almost complete and thus, the geometric mean particle diameters only kept at a constant level.

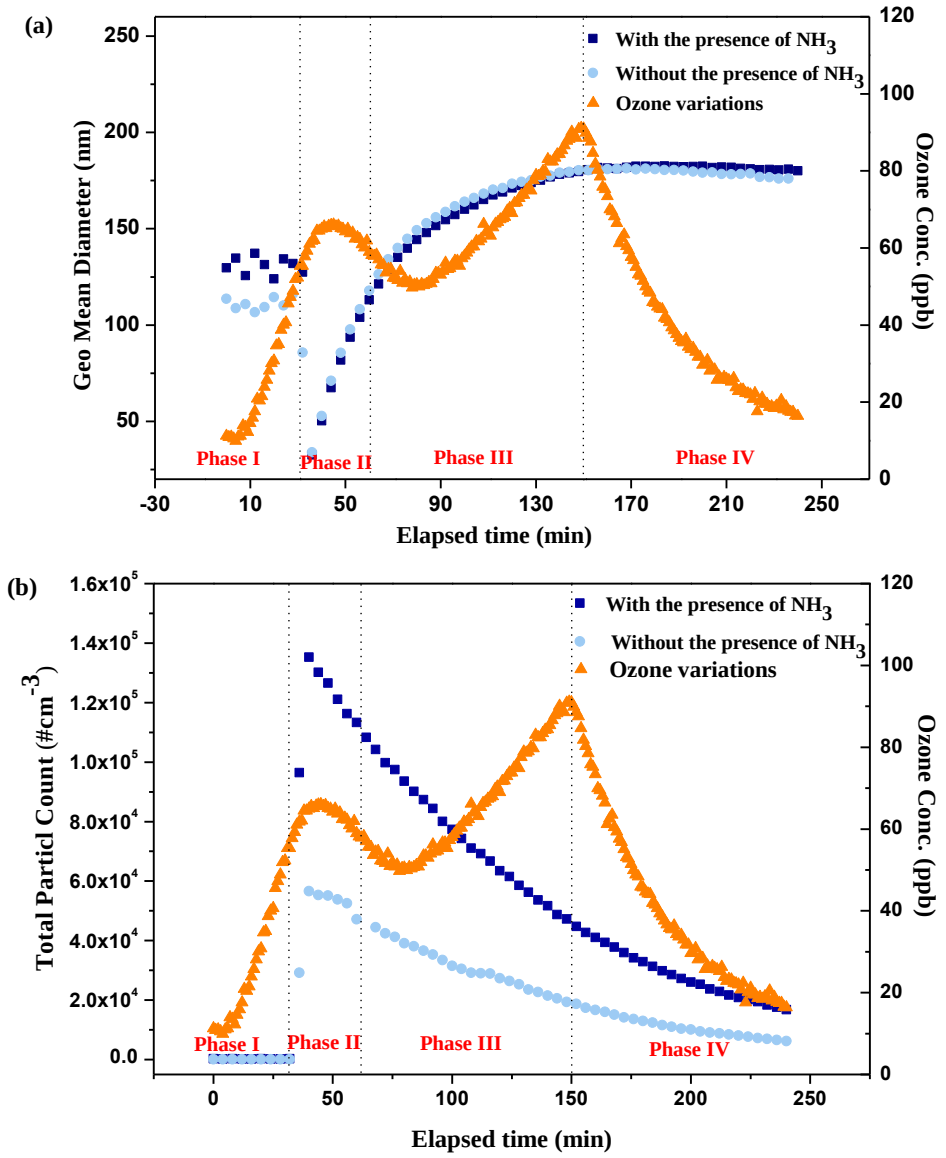

Fig. 4 Ozone concentration and (a) total particle number concentration of particles; (b) geometric mean diameters of particles with and without NH_3 presence in the initial chamber atmosphere

3.4 Effect of NH_3 on toxicity of the particle formed

Fig.5 gives a schematic overview of the biological responses in mice BALF upon treatment with the different PM suspensions. The production of TNF-α, IL-6 and IL-10 in the PBS blank control group has no significant difference with the blank group, while the blank samples still have slightly effect on inflammation, which may caused by quartz fibers and initial elements in the filter. However, the levels of

368 TNF-α, IL-6, and IL-10 in BALF were markedly elevated after PM samples
369 instillation both with and without the presence of NH_3 in 6h and 12h, which were
370 significantly different from control group ($p<0.05$ or $p<0.01$). In addition,
371 inflammatory cytokines levels of TNF-α, IL-6 and IL-10 reached a maximum at 6 h
372 and then gradually declined at 12h. Comparing to PM samples without the presence
373 of NH_3, the levels of TNF-α and IL-6 were 22% ~ 39% higher than the levels in the
374 samples with NH_3, while anti-inflammatory cytokine of IL-10 reduced when exposed
375 to NH_3.
376
377

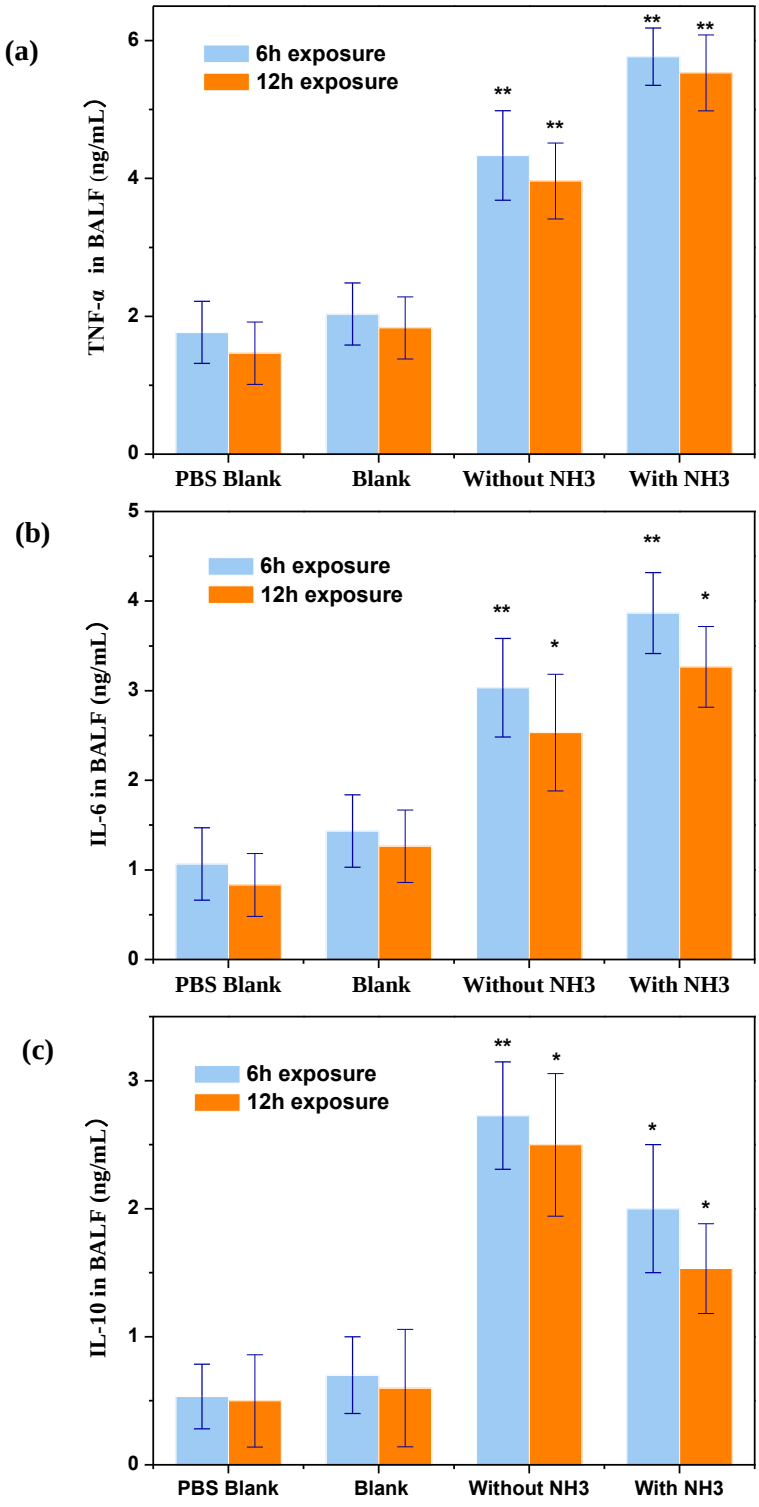

Fig. 5 Effects of NH_3 on (a) TNF-α; (b) IL-6; (c) IL-10 levels in ozone and monoterpene reactions. *p<0.05, **p<0.01

TNF-α is a representative and pleiotropic proinflammatory cytokine in the

inflammation response. It stimulates the expression of COX-2 and iNOS and triggers inflammation, injury and carcinogenesis in various tissues (Goldring and Goldring, 2004). Another significant proinflammatory cytokine is IL-6, which is generated by different cells and possesses pleiotropic effects on different tissues. IL-6 regulates genes expression involved in cell cycle progression and suppression of apoptosis (Lin and Karin, 2007). In present study, it was found that the levels of TNF-α and IL-6 in BALF was significantly increased after PM samples instillation with and without NH_3 presence compared with blank groups, the results are consistent with previous observations where particles from different sources trigger pulmonary inflammation (Dick et al., 2003; Perez et al., 2007). In the reactions of ozone and monoterpene with the presence of NH_3, the proinflammatory cytokine levels was increased comparing to reactions without NH_3 when induced the same dose of PM suspensions, which indicated that the effect of NH_3 not only has enhanced the generations of SOAs, but also increased the probabilities of cell injury or cell death, and pulmonary inflammation, injury or carcinogenesis would be triggered more easily, which may caused by the new generated chemical species with enhanced toxicity.

IL-10, a significant anti-inflammatory cytokine, is characterized by anti-inflammatory and immunosuppressive activities. IL-10 acts in a pleiotropic way by inhibiting antigen presentation, decreasing cell-surface expression of cytokine receptors and inducing expression of endogenous cytokine antagonists (Rennick and Fort, 2000). In this study, the production of IL-10 in BALF was found to be notably

reduced in ozone and monoterpene reactions with the presence of NH_3 comparing to the reactions without NH_3, which indicated that the effect of NH_3 in the reactions would weaken the anti-inflammatory abilities of cells and tissues, with weak defense, inflammation, injury and carcinogenesis on mice lung would be happened in greater chances.

Generally, particles undergo a variety of physicochemical transformations during their transport from sources to receptors, resulting in the formation of secondary inorganic and organic PM species. In our study, large amount of secondary organic aerosols were generated from the chemical and physical reactions between O_3 and monoterpenes, and NH_3 drives gas-phase organic acids into particle-phase organics which enhanced SOAs generations. Recently more and more researches have focus on the toxicity of secondary particles, they found that the most significant responses always occurred in more complex oxidized scenarios, which indicated that photochemically aged particles are more toxic than primary particles (Diaz et al., 2011; Godleski et al., 2011; Lemos et al., 2011; Verma et al., 2009; Wellenius et al., 2011). The responses included increases in *in vivo* chemiluminescence of the heart and the lung, change in breathing patterns, increases in total cell count and macrophage number on bronchoalveolar lavage, and the increases of pro-inflammation cytokines and reactive oxygen species (ROS). In addition, Delfino et al. (2010) concluded that organic components were more strongly and significantly associated with systemic inflammation, whereas organic components related to secondary photochemical aging of particles were more strongly and

significantly associated with airway inflammation.

In conclusion, above in vivo results suggest that inflammatory cytokines of TNF-α, IL-6, and IL-10 may be responsible for the effects observed with different PM suspensions. SOAs from reactions of ozone and monoterpene with the presence of NH_3 from the simulated indoor environment induced the potent inflammatory reaction upon intratracheal instillation in mice lung, meanwhile particles from reactions without the presence of NH_3 could also induce pulmonary inflammatory with weaker effects. However, it remains to be investigated whether the in vivo effects as observed with PM samples simulated from different conditions from this study also hold true for samples from other reactions and conditions. In addition, considering the complexity of particle composition related to multiple kinds of reactions with varies SOAs, it is still not easy to separate which components are responsible for activating the molecular mechanisms able to induce TNF-a , IL-6 and IL-10 secretion, which need a combination and initial correlation studies on characteristics of different chemical composition and size distribution-specific activities with inflammation effects.

4. Conclusions

This research focuses on the indoor ozone-initiated SOAs formation of d-limonene in the large environmental chamber. Based on the experimental results, the formation characteristics, effects of NH_3 existence, and potential pulmonary injuries from the ozonolysis were well investigated. Our results demonstrated that the presence of NH_3 indoor could significantly enhance the SOAs formation and increase the mean

particle diameters during the reactions between O_3 and d-limonene. Both of the nuclei coagulation and condensation take parts in the growth of SOAs. Besides, the NH_3 atmosphere could lead the formation of more toxic PM, evidencing with higher levels of TNF-α and IL-6 factors, while the anti-inflammatory cytokine of IL-10 was also reduced during the exposure to NH_3. Our results conclude that the SOAs formed from the ozonolysis of monoterpene in the presence of NH_3 could potentially induce the potent inflammatory reaction upon intratracheal instillation in mice lung. The finding is critical for further IAQ researches.

Acknowledgement

This research was financially supported by the National Science Foundation of China (41401567, 41573138) and the "Strategic Priority Research Program" of the Chinese Academy of Sciences (Grant No. XDB05000000), and was also partially supported by the Research Grants Council of the Hong Kong Special Administrative Region China (Project No. CUHK 412612). Yu Huang is also supported by the “Hundred Talent Program” of the Chinese Academy of Sciences.

References

Astort, F., Sittner, M., Ferraro, S.A., Orona, N.S., Maglione, G.A., De la Hoz, A., Tasat, D.R., 2014. Pulmonary Inflammation and Cell Death in Mice After Acute Exposure to Air Particulate Matter From an Industrial Region of Buenos Aires. Archives of Environmental Contamination and Toxicology 67, 87-96.

Bai, Z., Dong, Y., Wang, Z., Zhu, T., 2006. Emission of ammonia from indoor concrete wall and assessment of human exposure. Environment International 32, 303-311.

Bates, J.T., Weber, R.J., Abrams, J., Verma, V., Fang, T., Klein, M., Strickland, M.J., Sarnat, S.E., Chang, H.H., Mulholland, J.A., Tolbert, P.E., Russell, A.G., 2015. Reactive Oxygen Species Generation Linked to Sources of Atmospheric Particulate Matter and Cardiorespiratory Effects. Environmental Science & Technology 49, 13605-13612.

Breysse, P.N., Delfino, R.J., Dominici, F., Elder, A.C.P., Frampton, M.W., Froines, J.R., Geyh, A.S., Godleski, J.J., Gold, D.R., Hopke, P.K., Koutrakis, P., Li, N., Oberdorster, G., Pinkerton, K.E., Samet, J.M., Utell, M.J., Wexler, A.S., 2013. US EPA particulate matter research centers: summary of research results for 2005-2011. Air Quality Atmosphere and Health 6, 333-355.

Britigan, N., Alshawa, A., Nizkorodov, S.A., 2006. Quantification of ozone levels in indoor environments generated by ionization and ozonolysis air purifiers. Journal of the Air & Waste Management Association 56, 601-610.

Brown, S.K., Sim, M.R., Abramson, M.J., Gray, C.N., 1994. Concentrations of Volatile Organic Compounds in Indoor Air – A Review. Indoor Air 4, 123–134.

Cao, J.J., Xu, H.M., Xu, Q., Chen, B.H., Kan, H.D., 2012. Fine Particulate Matter Constituents and Cardiopulmonary Mortality in a Heavily Polluted Chinese City. Environmental Health Perspectives 120, 373-378.

Che, W.W., Frey, H.C., Lau, A.K.H., 2015. Comparison of Sources of Variability in School Age Children Exposure to Ambient PM2.5. Environmental Science & Technology 49, 1511-1520.

Chen, X., Hopke, P.K., 2010. A chamber study of secondary organic aerosol formation by limonene ozonolysis. Indoor Air 20, 320-328.

de Brito, J.M., Macchione, M., Yoshizaki, K., Toledo-Arruda, A.C., Saraiva-Romanholo, B.M., Andrade, M.d.F., Mauad, T., Rivero, D.H.R.F., Saldiva, P.H.N., 2014. Acute cardiopulmonary effects induced by the inhalation of concentrated ambient particles during seasonal variation in the city of Sao Paulo. Journal of applied physiology (Bethesda, Md. : 1985) 117, 492-499.

Delfino, R.J., Staimer, N., Tjoa, T., Arhami, M., Polidori, A., Gillen, D.L., George, S.C., Shafer, M.M., Schauer, J.J., Sioutas, C., 2010. Associations of Primary and Secondary Organic Aerosols With Airway and Systemic Inflammation in an Elderly Panel Cohort. Epidemiology 21, 892-902.

Destaillats, H., Lunden, M.M., Singer, B.C., Coleman, B.K., Hodgson, A.T., Weschler, C.J., Nazaroff, W.W., 2006. Indoor secondary pollutants from household product emissions in the presence of ozone: A bench-scale chamber study. Environmental Science & Technology 40, 4421-4428.

Diaz, E.A., Lemos, M., Coull, B., Long, M.S., Rohr, A.C., Ruiz, P., Gupta, T., Kang, C.M., Godleski, J.J., 2011. Toxicological Evaluation of Realistic Emission Source Aerosols (TERESA)-Power plant studies: assessment of breathing pattern. Inhalation Toxicology 23, 42-59.

Dick, C.A.J., Singh, P., Daniels, M., Evansky, P., Becker, S., Gilmour, M.I., 2003. Murine pulmonary inflammatory responses following instillation of size-fractionated ambient particulate matter. Journal of Toxicology and Environmental Health-Part A 66, 2193-2207.

Farina, F., Sancini, G., Mantecca, P., Gallinotti, D., Camatini, M., Palestini, P., 2011. The acute toxic effects of particulate matter in mouse lung are related to size and season of collection. Toxicology Letters 202, 209-217.
Godish, T.J., 1989. Indoor Air Pollution Control. MI, USA.
Godleski, J.J., Diaz, E.A., Lemos, M., Long, M., Ruiz, P., Gupta, T., Kang, C.M., Coull, B., 2011. Toxicological Evaluation of Realistic Emission Source Aerosols (TERESA)-power plant studies: assessment of cellular responses. Inhalation Toxicology 23, 60-74.
Goldring, S.R., Goldring, M.B., 2004. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clinical Orthopaedics and Related Research, S27-S36.
He, G.L., Ying, B., Liu, J., Gao, S.R., Shen, S., Balakrishnan, K., Jin, Y.L., Liu, F., Tang, N., Shi, K., Baris, E., Ezzati, M., 2005. Patterns of household concentrations of multiple indoor air pollutants in China. Environmental Science & Technology 39, 991-998.
Ho, K.F., Ho, S.S.H, Huang, R.J., Chuang, H.C., Cao, J.J., Han, Y.M., Lui, K.H., Ning, Z., Chuang, K.J., Cheng, T.J., Lee., S.C., Hu, D., Wang, B., Zhang, R.J., 2016. Chemical composition and bioreactivity of PM2.5 during 2013 haze events in China. Atmospheric Environment 126, 162-170.
Huang, W., Cao, J.J., Tao, Y.B., Dai, L.Z., Lu, S.E., Hou, B., Wang, Z., Zhu, T., 2012a. Seasonal Variation of Chemical Species Associated With Short-Term Mortality Effects of PM2.5 in Xi'an, a Central City in China. American Journal of Epidemiology 175, 556-566.
Huang, Y., Ho, K.F., Ho, S.S.H., Lee, S.C., Yau, P.S., Cheng, Y., 2011a. Physical parameters effect on ozone-initiated formation of indoor secondary organic aerosols with emissions from cleaning products. Journal of Hazardous Materials 192, 1787-1794.
Huang, Y., Ho, S.S.H., Ho, K.F., Lee, S.C., Gao, Y., Cheng, Y., Chan, C.S., 2011b. Characterization of biogenic volatile organic compounds (BVOCs) in cleaning reagents and air fresheners in Hong Kong. Atmospheric Environment 45, 6191-6196.
Huang, Y., Lee, S.C., Ho, K.F., Ho, S.S.H., Cao, N.Y., Cheng, Y., Gao, Y., 2012b. Effect of ammonia on ozone-initiated formation of indoor secondary products with emissions from cleaning products. Atmospheric Environment 59, 224-231.
Institute of Laboratory Animal Resources, Committee on Care Use of Laboratory Animals, National Institutes of Health, Resources, D.o.R., 1985. Guide for the care and use of laboratory animals. National Academies.
Jiang, R.T., Bell, M.L., 2008. A comparison of particulate matter from biomass-burning rural and non-biomass-burning urban households in northeastern China. Environmental Health Perspectives 116, 907-914.
Jones, N.C., Thornton, C.A., Mark, D., Harrison, R.M., 2000. Indoor/outdoor relationships of particulate matter in domestic homes with roadside, urban and rural locations. Atmospheric Environment 34, 2603-2612.
Kavouras, I.G., Mihalopoulos, N., Stephanou, E.G., 1999. Formation and gas/particle partitioning of monoterpenes photo-oxidation products over forests. Geophysical Research Letters 26, 55-58.
Koistinen, K., Kotzias, D., Kephalopoulos, S., Schlitt, C., Carrer, P., Jantunen, M., Kirchner, S., McLaughlin, J., Molhave, L., Fernandes, E.O., Seifert, B., 2008. The INDEX project: executive summary of a European Union project on indoor air pollutants. Allergy 63, 810-819.
Lamorena, R.B., Lee, W., 2008. Influence of ozone concentration and temperature on ultra-fine particle and gaseous volatile organic compound formations generated during the ozone-initiated

reactions with emitted terpenes from a car air freshener. Journal of Hazardous Materials 158, 471-477.
Lemos, M., Diaz, E.A., Gupta, T., Kang, C.M., Ruiz, P., Coull, B.A., Godleski, J.J., Gonzalez-Flecha, B., 2011. Cardiac and pulmonary oxidative stress in rats exposed to realistic emissions of source aerosols. Inhalation Toxicology 23, 75-83.
Leung, P.Y., Wan, H.T., Billah, M.B., Cao, J.J., Ho, K.F., Wong, C.K.C., 2014. Chemical and biological characterization of air particulate matter 2.5, collected from five cities in China. Environmental Pollution 194, 188-195.
Leungsakul, S., Jeffries, H.E., Kamens, R.M., 2005. A kinetic mechanism for predicting secondary aerosol formation from the reactions of d-limonene in the presence of oxides of nitrogen and natural sunlight. Atmospheric Environment 39, 7063-7082.
Lin, M., Stieb, D.M., Chen, Y., 2005. Coarse particulate matter and hospitalization for respiratory infections in children younger than 15 years in Toronto: A case-crossover analysis. Pediatrics 116, E235-E240.
Lin, W.-W., Karin, M., 2007. A cytokine-mediated link between innate immunity, inflammation, and cancer. Journal of Clinical Investigation 117, 1175-1183.
Madureira, J., Paciencia, I., Rufo, J., Ramos, E., Barros, H., Teixeira, J.P., Fernandes, E.D., 2015. Indoor air quality in schools and its relationship with children's respiratory symptoms. Atmospheric Environment 118, 145-156.
Magnani, N.D., Marchini, T., Tasat, D.R., Alvarez, S., Evelson, P.A., 2011. Lung oxidative metabolism after exposure to ambient particles. Biochemical and Biophysical Research Communications 412, 667-672.
Michael, S., Montag, M., Dott, W., 2013. Pro-inflammatory effects and oxidative stress in lung macrophages and epithelial cells induced by ambient particulate matter. Environmental Pollution 183, 19-29.
Mirowsky, J., Hickey, C., Horton, L., Blaustein, M., Galdanes, K., Peltier, R.E., Chillrud, S., Chen, L.C., Ross, J., Nadas, A., Lippmann, M., Gordon, T., 2013. The effect of particle size, location and season on the toxicity of urban and rural particulate matter. Inhalation Toxicology 25, 747-757.
Na, K., Song, C., Cocker, D.R., 2006. Formation of secondary organic aerosol from the reaction of styrene with ozone in the presence and absence of ammonia and water. Atmospheric Environment 40, 1889-1900.
Na, K., Song, C., Switzer, C., Cocker, D.R., 2007. Effect of ammonia on secondary organic aerosol formation from alpha-Pinene ozonolysis in dry and humid conditions. Environmental Science & Technology 41, 6096-6102.
Nazaroff, W.W., Weschler, C.J., 2004. Cleaning products and air fresheners: exposure to primary and secondary air pollutants. Atmospheric Environment 38, 2841-2865.
Ostachuk, A., Evelson, P., Martin, S., Dawidowski, L., Yakisich, J.S., Tasat, D.R., 2008. Age-related lung cell response to urban Buenos Aires air particle soluble fraction. Environmental Research 107, 170-177.
Parker, J.L., Larson, R.R., Eskelson, E., Wood, E.M., Veranth, J.M., 2008. Particle size distribution and composition in a mechanically ventilated school building during air pollution episodes. Indoor Air 18, 386-393.
Perez, I.R., Serrano, J., Alfaro-Moreno, E., Baumgardner, D., Garcia-Cuellar, C., del Campo, J.M.M., Raga, G.B., Castillejos, M., Colin, R.D., Vargas, A.R., 2007. Relations between PM10

composition and cell toxicity: A multivariate and graphical approach. Chemosphere 67, 1218-1228.
Pei, J. J., Yin, Y. H., Liu, J. J., 2016. Long-term indoor gas pollutant monitor of new dormitories with natural ventilation. Energy and Buildings 129, 514-523.
Rennick, D.M., Fort, M.M., 2000. Lessons from genetically engineered animal models - XII. IL-10-deficient (IL-10(-/-)) mice and intestinal inflammation. American Journal of Physiology-Gastrointestinal and Liver Physiology 278, G829-G833.
Rohr, A.C., Wilkins, C.K., Clausen, P.A., Hammer, M., Nielsen, G.D., Wolkoff, P., Spengler, J.D., 2002. Upper airway and pulmonary effects of oxidation products of (+)-alpha-pinene, d-limonene, and isoprene in BALB/c mice. Inhalation Toxicology 14, 663-684
Rossignol, S., Rio, C., Ustache, A., Fable, S., Nicolle, J., Meme, A., D'Anna, B., Nicolas, M., Leoz, E., Chiappini, L., 2013. The use of a housecleaning product in an indoor environment leading to oxygenated polar compounds and SOA formation: Gas and particulate phase chemical characterization. Atmospheric Environment 75, 196-205.
Sarigiannis, D.A., Karakitsios, S.P., Gotti, A., Liakos, I.L., Katsoyiannis, A., 2011. Exposure to major volatile organic compounds and carbonyls in European indoor environments and associated health risk. Environment International 37, 743-765.
Sarwar, G., Corsi, R., 2007. The effects of ozone/limonene reactions on indoor secondary organic aerosols. Atmospheric Environment 41, 959-973.
Seagrave, J., McDonald, J.D., Bedrick, E., Edgerton, E.S., Gigliotti, A.P., Jansen, J.J., Ke, L., Naeher, L.P., Seilkop, S.K., Zheng, M., Mauderly, J.L., 2006. Lung toxicity of ambient particulate matter from southeastern US sites with different contributing sources: Relationships between composition and effects. Environmental Health Perspectives 114, 1387-1393.
Singer, B.C., Coleman, B.K., Destaillats, H., Hodgson, A.T., Lunden, M.M., Weschler, C.J., Nazaroff, W.W., 2006a. Indoor secondary pollutants from cleaning product and air freshener use in the presence of ozone. Atmospheric Environment 40, 6696-6710.
Singer, B.C., Destaillats, H., Hodgson, A.T., Nazaroff, W.W., 2006b. Cleaning products and air fresheners: emissions and resulting concentrations of glycol ethers and terpenoids. Indoor Air 16, 179-191.
Tamas, G., Weschler, C.J., Toftum, J., Fanger, P.O., 2006. Influence of ozone-limonene reactions on perceived air quality. Indoor Air 16, 168-178.
USEPA, 1999. Large Chamber Test Protocol for Measuring Emissions of VOCs and Aldehydes. Research Triangle Park, North Carolina.
Verma, V., Ning, Z., Cho, A.K., Schauer, J.J., Shafer, M.M., Sioutas, C., 2009. Redox activity of urban quasi-ultrafine particles from primary and secondary sources. Atmospheric Environment 43, 6360-6368.
Waring, M.S., Siegel, J.A., 2011a. The effect of an ion generator on indoor air quality in a residential room. Indoor Air 21, 267-276.
Waring, M.S., Siegel, J.A., 2013. Indoor Secondary Organic Aerosol Formation Initiated from Reactions between Ozone and Surface-Sorbed D-Limonene. Environmental Science & Technology 47, 6341-6348.
Waring, M.S., Wells, J.R., Siegel, J.A., 2011b. Secondary organic aerosol formation from ozone reactions with single terpenoids and terpenoid mixtures. Atmospheric Environment 45, 4235-4242.

Wellenius, G.A., Diaz, E.A., Gupta, T., Ruiz, P.A., Long, M., Kang, C.M., Coull, B.A., Godleski, J.J., 2011. Electrocardiographic and respiratory responses to coal-fired power plant emissions in a rat model of acute myocardial infarction: results from the Toxicological Evaluation of Realistic Emissions of Source Aerosols Study. Inhalation Toxicology 23, 84-94.

Weschler, C.J., 2006. Ozone's impact on public health: Contributions from indoor exposures to ozone and products of ozone-initiated chemistry. Environmental Health Perspectives 114, 1489-1496.

Wilkins, C.K., Wolkoff, P., Clausen, P.A., Hammer, M., Nielsen, G.D., 2003. Upper airway irritation of terpene/ozone oxidation products (TOPS). Dependence on reaction time, relative humidity and initial ozone concentration. Toxicology Letters 143, 109-114.

Yu, J.Z., Griffin, R.J., Cocker, D.R., Flagan, R.C., Seinfeld, J.H., Blanchard, P., 1999. Observation of gaseous and particulate products of monoterpene oxidation in forest atmospheres. Geophysical Research Letters 26, 1145-1148.

Zhou, Z. H., Liu, Y. R., Yuan, J. J., Zuo, J., Chen, G. Y., Xu, L. Y., Rameezdeen, R.. 2016. Indoor PM2.5 concentrations in residential buildings during a severely polluted winter: A case study in Tianjin, China. Renewable & Sustainable Energy Reviews 64: 372-381.