Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

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Inflammatory responses to secondary organic aerosols (SOA) generated from biogenic and anthropogenic precursors

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Keywords: reactive oxygen/nitrogen species, inflammatory cytokines, particulate matter, secondary organic aerosol

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Atmospheric Chemistry and Physics Discussions

11 Abstract

12 Cardiopulmonary health implications resulting from exposure to secondary organic
13 aerosols (SOA), which comprise a significant fraction of ambient particulate matter (PM), have
14 received increasing interest in recent years. In this study, alveolar macrophages were exposed to
15 SOA generated from the photooxidation of biogenic and anthropogenic precursors (isoprene, α-
16 pinene, β-caryophyllene, pentadecane, *m*-xylene, and naphthalene) under different formation
17 conditions (RO_2 + HO_2 vs. RO_2 + NO dominant, dry vs. humid). Various cellular responses were
18 measured, including reactive oxygen/nitrogen species (ROS/RNS) production and secreted levels
19 of cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). SOA precursor identity
20 and formation condition affected all measured responses in a hydrocarbon-specific manner. With
21 the exception of naphthalene SOA, cellular responses followed a trend where TNF-α levels
22 reached a plateau with increasing IL-6 levels. ROS/RNS levels were consistent with relative levels
23 of TNF-α and IL-6, due to their respective inflammatory and anti-inflammatory effects. Exposure
24 to naphthalene SOA, whose aromatic ring-containing products may trigger different cellular
25 pathways, induced higher levels of TNF-α and ROS/RNS than suggested by the trend. Distinct
26 cellular response patterns were identified for hydrocarbons whose photooxidation products shared
27 similar chemical functionalities and structures, which suggests that the carbon backbone may be
28 important for determining cellular effects. A positive nonlinear correlation was also detected
29 between ROS/RNS levels and previously measured DTT activities for SOA samples. In the context
30 of ambient samples collected during summer and winter in the greater Atlanta area, all laboratory-
31 generated SOA produced similar or higher levels of ROS/RNS and DTT activities. These results
32 suggest that the health effects of SOA are important considerations for understanding the health
33 implications of ambient aerosols.

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Atmospheric Chemistry and Physics Discussions

Introduction

Particulate matter (PM) exposure is a leading global risk factor for human health (Lim et al., 2012) with numerous studies reporting associations between elevated PM concentrations and increases in cardiopulmonary morbidity and mortality (Li et al., 2008; Pope III and Dockery, 2006; Brunekreef and Holgate, 2002; Dockery et al., 1993; Hoek et al., 2013; Anderson et al., 2011; Pope et al., 2002). A possible mechanism for PM-induced health effects has been suggested by toxicology studies, wherein PM-induced oxidant production, including reactive oxygen and nitrogen species (ROS/RNS), initiates inflammatory cascades thus resulting in oxidative stress and cellular damage (Li et al., 2003a; Tao et al., 2003; Castro and Freeman, 2001; Gurgueira et al., 2002; Wiseman and Halliwell, 1996; Hensley et al., 2000). Furthermore, prolonged stimulation of these inflammatory cascades may lead to chronic inflammation, for which there is a recognized link to cancer (Philip et al., 2004). Together, these findings suggest that a possible relationship exists between PM exposure and observed health effects.

Various assays have been developed to study PM-induced oxidant production, including cell-free chemical assays that measure the oxidative potential of PM samples (Kumagai et al., 2002; Cho et al., 2005; Fang et al., 2015b) as well as cellular assays that measure intracellular ROS/RNS produced as a result of PM exposure (Landreman et al., 2008; Tuet et al., 2016). Cell-free assays simulate biologically relevant redox reactions using an anti-oxidant species (e.g. dithiothreitol, DTT; ascorbic acid, AA). The anti-oxidant is oxidized via electron transfer reactions catalyzed by redox-active species in the PM sample and its rate of decay serves as a measure of the concentration of redox-active species present (Fang et al., 2015b). On the other hand, cellular assays utilize a fluorescent probe (e.g. carboxy-H_2DCFDA) that reacts with ROS/RNS and the measured fluorescence is proportional to the concentration of ROS/RNS produced as a result of

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Discussion started: 28 March 2017

PM exposure (Landreman et al., 2008; Tuet et al., 2016). Both types of assays have been utilized extensively to characterize a variety of PM samples and identify sources that may be detrimental to health (Verma et al., 2015a; Saffari et al., 2015; Fang et al., 2015a; Bates et al., 2015; Li et al., 2003b; Tuet et al., 2016). In particular, numerous studies suggest that organic carbon constituents, especially humic-like substances (HULIS) and oxygenated polyaromatic hydrocarbons (PAH), may contribute significantly to PM-induced oxidant production (Li et al., 2003b; Kleinman et al., 2005; Hamad et al., 2015; Verma et al., 2015b; Lin and Yu, 2011). Furthermore, recent measurements of ROS/RNS production and DTT activity using ambient samples collected in summer and winter around the greater Atlanta area showed that there is a significant correlation between summertime organic species and intracellular ROS/RNS production, suggesting a possible role for secondary organic aerosols (SOA) (Tuet et al., 2016). The same study also reported a significant correlation between ROS/RNS production and DTT activity for summer samples, while a relatively flat ROS/RNS response was observed for winter samples spanning a similar DTT activity range (Tuet et al., 2016). These results highlight a need to consider multiple endpoints as a simple correlation may not exist between different endpoints, especially cellular responses that may result from complicated response networks.

Despite these findings, there are still many gaps in knowledge regarding PM-induced health effects. While field studies repeatedly showed that SOA often dominate over primary aerosols even in urban environments (Zhang et al., 2007; Jimenez et al., 2009; Ng et al., 2010), many prior health studies have focused on the effects of primary emissions (e.g. PM emitted directly from combustion engines) (Kumagai et al., 2002; Bai et al., 2001; McWhinney et al., 2013a; Turner et al., 2015) rather than those of SOA formed from the oxidation of emitted hydrocarbons (McWhinney et al., 2013b; Rattanavaraha et al., 2011; Kramer et al., 2016; Lund et

al., 2013; McDonald et al., 2010; McDonald et al., 2012; Baltensperger et al., 2008; Arashiro et al., 2016; Platt et al., 2014). The cellular exposure studies that do explore SOA focused on SOA formed from a single SOA precursor and include different measures of response (e.g. ROS/RNS, inflammatory biomarkers, gene expression, etc.) (Arashiro et al., 2016; Lund et al., 2013; McDonald et al., 2010; McDonald et al., 2012; Baltensperger et al., 2008). As a result, there is a lack of understanding in terms of the relative toxicity of individual SOA systems. Recently, Tuet et al. (2017) systematically investigated the DTT activities of SOA formed from different biogenic and anthropogenic precursors and demonstrated that intrinsic DTT activities were highly dependent on SOA precursor identity, with naphthalene SOA having the highest DTT activity. As a result, a systematic study on the cellular responses induced by these SOA systems may provide similar insights. Furthermore, cellular responses may complement these previously measured DTT activities to elucidate a more complete picture of the health effects of PM.

In the present study, alveolar macrophages were exposed to SOA generated under different formation conditions from various SOA precursors. Cellular responses induced by SOA exposure were measured, including intracellular ROS/RNS production and levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Intracellular ROS/RNS production serves as a general indicator of oxidative stress, whereas TNF-α and IL-6 are pro-inflammatory cytokines indicative of the inflammatory response (Henkler et al., 2010; Kishimoto, 2003; Wang et al., 2003). Precursors were chosen to include major classes of biogenic and anthropogenic compounds known to produce SOA upon atmospheric oxidation (Table S1). The selected biogenic precursors include: isoprene, the most abundant non-methane hydrocarbon (Guenther et al., 2006); α-pinene, a well-studied monoterpene with emissions on the order of global anthropogenic emissions (Guenther et al., 1993; Piccot et al., 1992); and β-caryophyllene, a representative sesquiterpene. Both

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Discussion started: 28 March 2017

103 monoterpenes and sesquiterpenes have been shown to contribute significantly to ambient aerosol
104 (Eddingsaas et al., 2012; Hoffmann et al., 1997; Tasoglou and Pandis, 2015; Goldstein and
105 Galbally, 2007). Similarly, the anthropogenic precursors include: pentadecane, a long-chain
106 alkane; *m*-xylene, a single-ring aromatic; and naphthalene, a poly-aromatic. These compounds are
107 emitted as products of incomplete combustion (Robinson et al., 2007; Jia and Batterman, 2010;
108 Bruns et al., 2016) and have considerable SOA yields (Chan et al., 2009; Ng et al., 2007b; Lambe
109 et al., 2011). In addition to precursor identity, the effects of humidity (dry vs. humid) and NO_x
110 levels (different predominant peroxy radical (RO_2) fates, $RO_2 + HO_2$ vs. $RO_2 + NO$) on SOA
111 cellular inflammatory responses were investigated, as different formation conditions have been
112 shown to affect aerosol chemical composition and mass loading, which could in turn result in a
113 different cellular response (Chhabra et al., 2010; Chhabra et al., 2011; Eddingsaas et al., 2012; Ng
114 et al., 2007b; Loza et al., 2014; Ng et al., 2007a; Chan et al., 2009; Boyd et al., 2015). Finally,
115 correlations between bulk aerosol composition, specifically elemental ratios, and cellular
116 inflammatory responses were investigated to determine whether there is a link between different
117 inflammatory responses and aerosol composition.

118 Methods

119 **Alveolar macrophage cell line.** Immortalized murine alveolar macrophages (MH-S,
120 ATCC®CRL-2019™) were cultured in RPMI-1640 media supplemented with 10% fetal bovine
121 serum (FBS, Quality Biological, InC.), 1% penicillin-streptomycin, and 50 µM β-mercaptoethanol
122 (BME) at 37°C and humid air containing 5% CO_2. For exposure experiments, MH-S cells were
123 seeded at a density of 2 x 10^4 cells $well^{-1}$ onto 96-well plates pre-treated with 10% FBS in
124 phosphate buffered saline (PBS, Cellgro). For seeding and all assay procedures thereon, FBS-

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
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Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

125 supplemented cell culture media without BME addition was used as BME is a reducing agent that
126 may interfere with inflammatory measurements.

127 **Chamber experiments.** SOA formed form the photooxidation of biogenic and
128 anthropogenic precursors were generated in the Georgia Tech Environmental Chamber (GTEC)
129 facility. Details of the facility have been described elsewhere (Boyd et al., 2015). Briefly, the
130 chamber facility consists of two 12 m^3 Teflon chambers suspended within a 21 x 12 ft temperature-
131 controlled enclosure. Black lights and natural sunlight fluorescent lamps surround the chambers,
132 and multiple sampling ports allow for injection of reagents, as well as gas- and aerosol-phase
133 measurements. Gas-phase O_3, NO_2, and NO_x concentrations were monitored using an O_3 analyzer
134 (Teledyne T400), a cavity attenuated phase shift (CAPS) NO_2 monitor (Aerodyne), and a
135 chemiluminescence NO_x monitor (Teledyne 200EU) respectively, while hydrocarbon decay was
136 monitored using a gas chromatography-flame ionization detector (GC-FID, Agilent 7890A).
137 Hydrocarbon decay was also used to estimate hydroxyl radical (OH) concentrations. For aerosol-
138 phase measurements, a Scanning Mobility Particle Sizer (SMPS, TSI) was used to measure aerosol
139 volume concentrations and distributions, while a High Resolution Time-of-Flight Aerosol Mass
140 Spectrometer (HR-ToF-AMS, Aerodyne; henceforth referred to as the AMS) was used to
141 determine bulk aerosol composition (DeCarlo et al., 2006). AMS data was analyzed using the data
142 analysis toolkit SQUIRREL (v. 1.57) and PIKA (v. 1.16G). Elemental ratios, including O:C, H:C,
143 and N:C, were obtained using the method outlined by Canagaratna et al. (2015) and used to
144 calculate the average carbon oxidation state ($\overline{OS}_c$) (Kroll et al., 2011). Temperature and relative
145 humidity (RH) were also monitored using a hydro-thermometer (Vaisala HMP110).

146 Experiments were designed to probe the effects of humidity, RO_2 fate, and precursor
147 identity on cellular inflammatory responses induced by different SOA formed under these

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Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

conditions (Table 1). All chamber experiments were performed at ~25 °C under dry (RH < 5%) or humid (RH ~ 45%) conditions. Chambers were flushed with pure air for ~24 hrs prior to each experiment. During this time, chambers were also humidified for humid experiments by means of a bubbler filled with deionized (DI) water. Seed aerosol was injected by atomizing a 15 mM $(NH_4)_2SO_4$ seed solution (Sigma Aldrich) to obtain a seed concentration of ~20 μg m^{-3}. It should be noted that experimental conditions deviate for experiment 7 (isoprene SOA under $RO_2 + HO_2$ dominant, "humid" conditions) due to low SOA mass yields. For this experiment, an acidic seed solution (8 mM $MgSO_4$ and 16 mM H_2SO_4) and a dry chamber were used to promote SOA formation via the isoprene epoxydiol (IEPOX) uptake pathway. This pathway has been shown to contribute significantly to ambient OA and has a higher SOA mass yield compared to the IEPOX + OH pathway (Surratt et al., 2010; Lin et al., 2012; Xu et al., 2015).

SOA precursor was then introduced by injecting a known amount of hydrocarbon solution [isoprene, 99%; α-pinene, ≥ 99%; β-caryophyllene, > 98.5%; pentadecane, ≥ 99%; *m*-xylene, ≥ 99%; naphthalene, 99% (Sigma Aldrich)] into a glass injection bulb and passing zero air over the solution until it fully evaporated. For pentadecane and β-caryophyllene, the glass bulb was also heated gently during hydrocarbon injection to ensure full evaporation (Tasoglou and Pandis, 2015). Naphthalene was injected by passing zero air over solid naphthalene flakes as described in previous studies (Chan et al., 2009). OH precursor was then introduced via injection of hydrogen peroxide (H_2O_2) for $RO_2 + HO_2$ experiments or nitrous acid (HONO) for RO_2 + NO experiments. For H_2O_2, a 50% aqueous solution (Sigma Aldrich) was injected using the same method described for hydrocarbon injection to achieve an H_2O_2 concentration of 3 ppm. This amount yielded OH concentrations on the order of 10^6 molec cm^{-3}. For HONO injections, HONO was first prepared by adding 10 mL of 1%wt aqueous $NaNO_2$ (VWR International) dropwise into 20 mL of 10%wt

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
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Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

171 H_2SO_4 (VWR International) in a glass bulb. Zero air was then passed over the solution to introduce
172 HONO into the chamber (Chan et al., 2009; Kroll et al., 2005). Photolysis of HONO yielded OH
173 concentrations on the order of 10^7 molec cm^{-3}. NO and NO_2 were also formed as byproducts of
174 HONO synthesis. Once all the H_2O_2 evaporated (RO_2 + HO_2 experiments) or NO_x concentrations
175 stabilized (RO_2 + NO experiments), the UV lights were turned on to initiate photooxidation.

176 **Aerosol collection and extraction.** Aerosol samples were collected onto 47 mm Teflon™
177 filters (0.45 μm pore size, Pall Laboratory). The total mass collected onto each filter was
178 determined by integrating the SMPS time-dependent volume concentration over the filter
179 collection period and multiplying by the total volume of air collected. To account for potential
180 H_2O_2 or HONO uptake, background filters were also collected. These filters were collected when
181 only seed particles and OH precursor (H_2O_2 or HONO) were injected into the chamber under
182 otherwise identical experimental conditions. All collected samples were placed in sterile petri
183 dishes, sealed with Parafilm M®, and stored at -20 °C until extraction and analysis (Fang et al.,
184 2015b). Collected particles were extracted following the procedure outlined in Fang et al. (2015a)
185 with modifications for cellular exposure. Briefly, filter samples were submerged in cell culture
186 media (RPMI-1640) and sonicated for two 30 min intervals (1 hr total) using an Ultrasonic
187 Cleanser (VWR International). In between sonication intervals, the water was replaced to reduce
188 bath temperature. After the final sonication interval, sample extracts were filtered using 0.45 μm
189 PTFE syringe filters (Fisherbrand™) to remove any insoluble material and supplemented with 10%
190 FBS (Fang et al., 2015b).

191 **Intracellular ROS/RNS measurement.** ROS/RNS were detected using the assay
192 optimized in Tuet et al. (2016). Briefly, the assay consists of five major steps: (1) pre-treatment of
193 96-well plates to ensure a uniform cell density, (2) seeding of cells onto pre-treated wells at 2 x

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
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Discussion started: 28 March 2017

194 10^4 cells $well^{-1}$, (3) incubation with ROS/RNS probe (carboxy-H_2DCFDA, Molecular Probes C-
195 400) diluted to a final concentration of 10 µM, (4) exposure of probe-treated cells to samples and
196 controls for 24 hrs, and (5) detection of ROS/RNS using a microplate reader (BioTek Synergy H4,
197 ex/em: 485/525 nm). Positive controls included bacterial cell wall component lipopolysaccharide
198 (LPS, 1 µg mL^{-1}), H_2O_2 (100 µM), and reference filter extract (10 filter punches mL^{-1}, 1 per filter
199 sample, from various ambient filters collected at the Georgia Tech site, while negative controls
200 included blank filter extract (2 punches mL^{-1}) and control cells (probe-treated cells exposed to
201 media only, no stimulants).

202 A previous study on the ROS/RNS produced induced by exposure to ambient PM samples
203 found that ROS/RNS production was highly dose-dependent and could therefore not be
204 represented by measurements taken at a single dose (Tuet et al., 2016). Here, we utilize the dose-
205 response curve approach described in Tuet et al. (2016). For each aerosol sample, ROS/RNS
206 production was measured over ten dilutions and expressed as a fold increase in fluorescence over
207 control cells. A representative dose-response curve is shown in Fig. 1. For comparisons to other
208 inflammatory endpoints and chemical composition, ROS/RNS production was represented using
209 the area under the dose-response curve (AUC), as AUC has been shown to be the most robust
210 metric for comparing PM samples (Tuet et al., 2016).

211 **Cytokine measurement.** Secreted levels of TNF-α and IL-6 were measured post-exposure
212 (24 hrs) using enzyme-linked immunosorbent assay (ELISA) kits following manufacturer's
213 specifications (ThermoFisher). All measurements were carried out using undiluted cell culture
214 supernatant. For each aerosol sample, TNF-α and IL-6 were measured over seven dilutions and
215 represented as a fold increase over control. Similarly, the AUC was used to represent each endpoint
216 for comparison purposes.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

Cellular metabolic activity. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Biotium) was used to assess cellular metabolic activity. Briefly, supernatants containing sample extracts were removed after the exposure period and replaced with media containing MTT. Cells were then returned to the incubator for 4 hrs, during which the tetrazolium dye was reduced by cellular NAD(P)H-dependent oxidoreductases to produce an insoluble purple salt (formazan). Dimethyl sulfoxide was then used to solubilize the salt and the absorbance at 570 nm was determined using a microplate reader (BioTek Synergy H4).

Statistical analysis. Linear regressions between bulk aerosol composition and cellular inflammatory responses were evaluated using Pearson's correlation coefficient, and the significance of each correlation coefficient was determined using multiple imputation, which calculated the total variance associated with the slope of each regression. Details of this method are described in Pan and Shimizu (2009). Briefly, response parameters (i.e. AUCs for each endpoint) were assumed to follow a normal distribution. Ten "estimates" were obtained for each response using the average and standard deviation determined from the dose-response curve fit. These estimates were then plotted against bulk aerosol composition (e.g. O:C, H:C, and N:C) to obtain ten fits, and the slopes and variances generated from these fits were used to calculate the between and within variance. Finally, a Student's *t*-test was used to calculate and evaluate the associated *p*-values using a 95% confidence interval.

Results and Discussion

Effect of SOA precursor and formation condition on SOA inflammatory response. To investigate whether SOA formed from different precursors elicited different inflammatory responses, levels of ROS/RNS, TNF-α, and IL-6 were measured after exposing alveolar

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

239 macrophages to SOA generated from six VOCs generated under three formation conditions (Table
240 1). The AUC per mass of SOA (μg) in the extract for ROS/RNS, TNF-α and IL-6 are shown in
241 Fig. 2, shaped by SOA formation condition. It should be noted that all responses were normalized
242 to probe-treated control cells to account for differences between endogenous levels of ROS/RNS
243 produced in cells (Henkler et al., 2010). Uncertainties associated with AUC were determined by
244 averaging the AUCs obtained by fitting dose-response data with each point removed
245 systematically, following the methodology described in Tuet et al. (2016). ROS/RNS production
246 was also measured for background filters and found to be within the uncertainty of control cells,
247 indicating that there was no evidence for significant H_2O_2 or HONO uptake onto seed particles
248 (Fig. S1). Furthermore, exposure to filter extract did not result in decreases in metabolic activity
249 as measured by the MTT assay for all SOA systems investigated (Fig. S2). Since results from MTT
250 may represent the number of viable cells present, changes in inflammatory endpoints did not likely
251 result from changes in the number of cells exposed (i.e. decreases in response cannot be attributed
252 to cell death).

253 For all inflammatory responses measured (levels of ROS/RNS, TNF-α, and IL-6), SOA
254 precursor identity and formation condition influenced the level of response, as demonstrated by
255 the range of values obtained from different SOA precursors and different formation conditions
256 (Fig. 2). Despite having a clear effect, no obvious trends were observed for each variable (precursor
257 or formation condition) on individual responses. This is in contrast to that observed for the
258 oxidative potential as measured by DTT ($OP^{WS\text{-}DTT}$) for these samples, where only precursor
259 identity influenced $OP^{WS\text{-}DTT}$ substantially (Tuet et al., 2017). However, this may not be surprising
260 as DTT is a chemical assay, which only accounts for the potential of species to participate in redox
261 reactions (Cho et al., 2005), whereas cellular assays account for many complicated cellular events

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
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Discussion started: 28 March 2017

involved in intricate positive and negative feedback loops. Due to the considerably different classes of compounds chosen as SOA precursors, aerosol compositional changes between different precursors were generally larger than those between different formation conditions of the same precursor (see Fig. 3a) (Tuet et al., 2017). DTT may only be sensitive to larger differences arising from different precursors (i.e. a different carbon backbone), whereas cellular assays could also be sensitive to differences between different formation conditions and chemical composition of the same precursor. Moreover, while Tuet et al. (2017) showed that the intrinsic $OP^{WS\text{-}DTT}$ spanned a wide range, with isoprene and naphthalene SOA generating the lowest and highest $OP^{WS\text{-}DTT}$, these bounds were less clear for cellular responses. While isoprene and naphthalene SOA still generated the lowest and highest inflammatory responses in general, a few exceptions exist (e.g. ROS/RNS levels induced by pentadecane SOA formed under dry, $RO_2 + HO_2$ dominant conditions, Fig. 2).

Though no apparent trends in individual inflammatory responses were observed as a function of SOA precursor identity or formation condition, several patterns among all three inflammatory responses were observed for SOA precursors with similar carbon backbones. Exposure to isoprene SOA induced the lowest levels of TNF-α and IL-6 among the aerosol systems studied (Fig. 2). Furthermore, isoprene SOA generated from different pathways (i.e. photooxidation under different RO_2 fates and reactive uptake of IEPOX) (Surratt et al., 2010; Xu et al., 2014; Chan et al., 2010) produced similar responses for each inflammatory endpoint. These results suggest that different isoprene SOA products (Surratt et al., 2010; Xu et al., 2014; Chan et al., 2010) may induce similarly low inflammatory responses and are consistent with the intrinsic $OP^{WS\text{-}DTT}$ obtained for these SOA samples, where isoprene SOA generated the lowest $OP^{WS\text{-}DTT}$ of all SOA systems studied and the $OP^{WS\text{-}DTT}$ was similar for all SOA formation conditions explored (Tuet et al., 2017). This finding is in contrast to a previous study by Lin et al. (2016),

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

where methacrylic acid epoxide (MAE)-derived SOA was found to be substantially more potent than IEPOX-derived SOA. However, while exposure to MAE-derived SOA induced the upregulation of a larger number of oxidative stress response genes than IEPOX-derived SOA, the fold change of several genes reported in Lin et al. (2016) are actually similar. Thus, it is possible that the inflammatory cytokines measured in this study are involved in pathways concerning those genes, resulting in a similar response level regardless of SOA formation condition.

Similarly, exposure to SOA generated from the photooxidation of α-pinene and *m*-xylene resulted in similar inflammatory responses for all three formation conditions (Fig. 2). These cellular assay results are consistent with results from the DTT assay where the $OP^{WS\text{-}DTT}$ was not significantly different between SOA formed under different formation conditions (Tuet et al., 2017). Response levels induced by these two SOA systems are also similar across all three inflammatory measurements investigated (Fig. 2). This suggests that products from both precursors may induce similar cellular pathways resulting in the production of similar levels of inflammatory markers. Indeed, there are several similarities between products formed from the photooxidation of α-pinene and *m*-xylene. For instance, a large portion of α-pinene and *m*-xylene oxidation products under both $RO_2 + HO_2$ and $RO_2 + NO$ pathways are ring-breaking products with a similar carbon chain length (Eddingsaas et al., 2012; Vivanco and Santiago, 2010; Jenkin et al., 2003). As a result of this similarity, products from both SOA systems may interact with the same cellular targets and induce similar cellular pathways, resulting in a similar response regardless of precursor identity and formation condition. These observations further imply that the chemical structures of oxidation products may be important regardless of PM source/precursor.

A different pattern was observed for β-caryophyllene and pentadecane SOA, where the IL-6 response spanned a much larger range than ROS/RNS and TNF-α (Fig. 2). This is in contrast to

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
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Discussion started: 28 March 2017

the trends observed for the $OP^{WS\text{-}DTT}$ for β-caryophyllene and pentadecane SOA, where $OP^{WS\text{-}DTT}$ was similar regardless of formation condition (Tuet et al., 2017). This suggests that there are differences between organic peroxides and organic nitrates formed from certain precursors that influence cellular responses, but are not captured by redox potential measurements. Less is known about the effects of humidity on SOA formation and chemical composition for all SOA systems investigated, as most laboratory chamber studies in literature have been conducted under dry conditions. Specifically here, very high levels of IL-6 were observed post-exposure to pentadecane SOA formed under humid conditions. Prior studies reported opposing findings with some showing a significant effect of water on aerosol formation and chemical composition (Nguyen et al., 2011; Wong et al., 2015; Healy et al., 2009; Stirnweis et al., 2016), while others found little influence (Edney et al., 2000; Boyd et al., 2015; Cocker III et al., 2001). It is clear that humidity effects are highly hydrocarbon-dependent and further studies into the specific products formed under humid conditions are required to understand how these differences in chemical composition may translate to different cellular endpoints. Nonetheless, the known products formed from the photooxidation of these hydrocarbons may provide some insight into the inflammatory responses observed. While there are no prior studies involving pentadecane oxidation products, it is expected that the oxidation products will be similar to those reported in the oxidation of dodecane (i.e. same functionalities with a longer carbon chain) (Loza et al., 2014). It is therefore likely that pentadecane oxidation products resemble long chain fatty acids and could potentially insert into the cell membrane (Loza et al., 2014). This insertion could potentially affect membrane fluidity, which is known to affect cell function substantially although the specific effect depends strongly on the particular modification and cell type of interest (Baritaki et al., 2007; Spector and Yorek, 1985). In some cases, these alterations lead to the induction of apoptosis, which involves pathways

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

leading to the production of TNF-α (Baritaki et al., 2007; Wang et al., 2003). TNF-α can then induce the production of IL-6, which once produced can also inhibit the production of TNF-α in a feedback loop (Kishimoto, 2003; Wang et al., 2003). These cellular events are consistent with the observed inflammatory response induced by pentadecane SOA exposure, where there is a high IL-6 response and a lower TNF-α response. The low ROS/RNS response observed is also in line with these cellular events, as IL-6 exhibits anti-inflammatory functions, which can neutralize ROS/RNS production. These responses are less pronounced for β-caryophyllene aerosol, which may be due to the shorter carbon chain observed in known products (Chan et al., 2011). While β-caryophyllene and pentadecane are both C15 precursors, β-caryophyllene is a bicyclic compound and many SOA products retain the 4-membered ring, resulting in a shorter carbon backbone (Chan et al., 2011). As a result, fewer products may insert into the cell membrane, leading to a lesser response compared to pentadecane SOA exposure.

Naphthalene exhibits a completely different pattern from the rest of the SOA systems investigated, with a large range observed for both TNF-α and IL-6 under different formation conditions (Fig. 2). Higher levels of ROS/RNS were also observed as a result of exposure to naphthalene aerosol irrespective of SOA formation condition. Similarly, the $OP^{WS\text{-}DTT}$ of naphthalene SOA previously measured by Tuet et al. (2017) was an outlier among all SOA systems investigated, as the measured $OP^{WS\text{-}DTT}$ was at least twice that of the next highest SOA system. These observations are consistent with the formation of specific SOA products such as naphthoquinones, which are known to induce redox-cycling in cells and are formed under both $RO_2 + HO_2$ and $RO_2 + NO$ pathways (Henkler et al., 2010; Kautzman et al., 2010). Consequently, aerosol generated from naphthalene may induce higher levels of inflammatory responses than other SOA due to this process (Henkler et al., 2010; Lorentzen et al., 1979). However, as shown

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

by the high levels of IL-6, exposure to naphthalene SOA may also induce anti-inflammatory pathways not captured by $OP^{WS\text{-}DTT}$ measurements. Moreover, a clear increasing trend is apparent for TNF-α and IL-6 produced upon naphthalene SOA exposure, with a higher level of both cytokines observed for aerosol formed under RO_2 + NO dominant and humid conditions. Previously, the effect of different RO_2 fates on SOA $OP^{WS\text{-}DTT}$ was attributed to the different products known to form under both pathways (Tuet et al., 2017). The same explanation applies for cellular measurements as SOA products that promote electron transfer reactions with anti-oxidants can result in redox imbalance as measured by $OP^{WS\text{-}DTT}$ and the induction of related cellular pathways such as ROS/RNS and cytokine production (Tuet et al., 2017). Finally, naphthalene SOA induced cellular responses outside of those observed for other aerosol systems, with higher levels of all inflammatory markers than other SOA systems. As shown previously for $OP^{WS\text{-}DTT}$, naphthalene may be an outlier due to aromatic ring-containing products, which may then induce different cellular pathways compared to other aerosol systems investigated, the products of which do not contain aromatic rings. Additionally, many known aerosol products formed from the photooxidation of naphthalene have functionalities that resemble those of dinitrophenol, which is known to decouple phosphorylation from electron transfer (Terada, 1990). It is therefore possible that the aromatic functionality present in the majority of naphthalene SOA products results in the involvement of very different cellular pathways, leading to outlier inflammatory endpoint responses. Various products of naphthalene oxidation such as nitroaromatics and polyaromatics are known to have mutagenic properties and may induce the formation of DNA adducts (Baird et al., 2005; Helmig et al., 1992). As such, it is possible that these products may induce health effects via other pathways as well and naphthalene SOA exposure may have effects beyond redox imbalance and oxidative stress.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

Bulk aerosol elemental ratios (O:C, H:C, and N:C) were determined for each SOA system investigated. Different types of organic aerosol are known to span a wide range of O:C, which may be utilized as an indication of oxidation, and the van Krevelen diagram was used to visualize whether changes in O:C and H:C ratios corresponded to changes in levels of inflammatory response (Fig. 3a, S3) (Chhabra et al., 2011; Lambe et al., 2011; Ng et al., 2010). Changes in the slope within the van Krevelen space provide information on SOA functionalization (Heald et al., 2010; Van Krevelen, 1950; Ng et al., 2011). Beginning from the precursor hydrocarbon, a slope of 0 indicates alcohol group additions, a slope of -1 indicates carbonyl and alcohol additions on separate carbons or carboxylic acid additions, and a slope of -2 indicates ketone or aldehyde additions.

As seen in Fig. 3a, the laboratory-generated aerosols span a large range of O:C and H:C ratios. Both SOA formation condition and precursor identity influenced elemental ratios, however, precursor identity generally had a larger effect as evident by the clusters observed for different SOA precursors. Despite these differences in chemical composition, there were no obvious trends between O:C or H:C and any inflammatory endpoint measured. This is similar to that observed for chemical oxidative potential as measured by DTT, where a higher O:C did not correspond to a higher oxidative potential for both laboratory-generated and ambient aerosols (Tuet et al., 2017). This is likely due to the different formation conditions used to generate SOA, which may not be directly comparable. Nevertheless, a significant correlation was observed between ROS/RNS and $\overline{OS}_c$ (Fig. 3b). This positive correlation is not surprising, as a higher average oxidation state would likely correspond to a better oxidizing agent. Future studies should evaluate the effect of the degree of oxidation for SOA formed from the same SOA precursor under the same formation condition to investigate whether atmospheric aging of aerosol (which typically leads to increases in the

degree of oxidation) affects inflammatory responses. Finally, the N:C ratio was also determined for SOA systems formed under conditions that favor the RO_2 + NO pathway (Fig. S4) and were found to span a large range. Similarly, there was no obvious trend between N:C ratios and the inflammatory endpoints measured.

Relationship between inflammatory responses. To visualize whether there exists a relationship between inflammatory markers measured, levels of TNF-α and IL-6 are shown in Fig. 4, sized by ROS/RNS. With the exception of naphthalene SOA, the inflammatory cytokine responses for all aerosol systems investigated follow an exponential curve (Fig. 4, shown in black) where there appears to be a plateau for TNF-α levels. Along this curve, ROS/RNS levels also appear to increase with increasing inflammatory cytokine levels to a certain point, after which ROS/RNS levels decrease. These observations are in line with the interconnected effects of both cytokines. While both TNF-α and IL-6 have pro-inflammatory effects that may lead to the increase of ROS/RNS production, the individual pathways are also involved in many complicated stimulation and inhibition loops and there is extensive cross-talk between both pathways. For instance, TNF-α induces the production of glucocorticoids, which in turn inhibits both TNF-α and IL-6 production (Wang et al., 2003). IL-6 also directly inhibits the production of TNF-α and other cytokines induced as a result of TNF-α (e.g. IL-1) and stimulates pathways that lead to the production of glucocorticoids (Kishimoto, 2003). As a result, increases in IL-6 may be accompanied by decreases in TNF-α, resulting in the observed plateau. Furthermore, ROS/RNS levels may represent a fine balance between anti-inflammatory and pro-inflammatory effects. Both cytokines are involved in the acute phase reaction and can affect ROS/RNS levels via pro-inflammatory pathways. IL-6 also exhibits some anti-inflammatory functions and may thus lower ROS/RNS levels as well. These interconnected pathways could account for the observed parabolic

423 pattern for ROS/RNS production. Exposure to naphthalene SOA resulted in responses outside of
424 those observed for other aerosol systems, likely due to the formation of aromatic ring-retaining
425 products as discussed in the previous section.

426 **Comparison with ambient data.** To evaluate how the oxidative potential and ROS/RNS
427 production of the SOA systems investigated compare in the context of ambient samples, the
428 measurements obtained in this study were plotted with those obtained in our previous study
429 involving ambient samples collected around the greater Atlanta area (Fig. 5) (Tuet et al., 2016).
430 These ambient samples were analyzed using the same methods for determining oxidative potential
431 (DTT assay (Cho et al., 2005; Fang et al., 2015b)) and ROS/RNS production (cellular carboxy-
432 H_2DCFDA assay (Tuet et al., 2016)). Furthermore, the same extraction protocol (water-soluble
433 extract) was followed in both studies (Tuet et al., 2016). Results from both studies are therefore
434 directly comparable. Previously, a significant correlation between ROS/RNS production and
435 oxidative potential as measured by DTT was observed for summer ambient samples. In the same
436 study, correlations between ROS/RNS production and organic species were also observed for
437 summer ambient samples, and it was suggested that these correlations may reflect contributions
438 from photochemically produced SOA (Tuet et al., 2016).

439 Fig. 5 shows that laboratory-generated SOA oxidative potential is comparable to that
440 observed in ambient samples, with the exception of naphthalene SOA, which produced higher
441 DTT activities due to its aromatic ring retaining products (Tuet et al., 2017; Kautzman et al., 2010).
442 Laboratory-generated SOA also induced similar or higher levels of ROS/RNS compared to
443 ambient samples. There are many possible explanations for the observed higher response for some
444 SOA samples. For instance, individual, single precursor SOA systems were considered in this
445 study, whereas ambient aerosol contains SOA from multiple precursors as well as other species

that are not considered in this study (e.g. metals). Interactions between SOA from different precursors is likely to occur and may result in different response levels. Complex interactions between SOA and other species present in the ambient (e.g. metals or other organic species) are also likely involved (Tuet et al., 2016). Previous studies have also suggested the possibility of metal-organic complexes. For instance, Verma et al. (2012) showed that certain metals were retained on a C-18 column, which is utilized to remove hydrophobic components, suggesting that these metals were likely complexed and removed in the process. Further chamber studies involving photochemically generated SOA and metals may elucidate these interactions. Furthermore, there are likely species present in the ambient that do not contribute to ROS/RNS production. That is, while certain species contribute to the mass of PM, there is little to no ROS/RNS production associated with these species. Ambient samples where these species comprise a significant fraction will have a low per mass ROS/RNS production level. Finally, only three SOA formation conditions were investigated in this study. There are multiple other possible oxidation mechanisms that lead to the formation of SOA in the ambient, which were not accounted for in this study. Nonetheless, despite the low ROS/RNS levels observed post SOA exposure, there is an association between ROS/RNS production and DTT activity (Fig. 5). These results suggest that our previous findings based on ambient filter samples may be extended to SOA samples. That is, while the relationship between ROS/RNS production and DTT activity is complex, DTT may serve as a useful screening tool as samples with low DTT activities are likely to produce low levels of RNS/RNS (Tuet et al., 2016).

Implications. Levels of ROS/RNS, TNF-α, and IL-6 were measured after exposing cells to the water-soluble extract of SOA generated from the photooxidation of six SOA precursors under various formation conditions. Although previous epidemiological and ambient studies have

found correlations between metals and various measures of health effects (Verma et al., 2010; Pardo et al., 2015; Burnett et al., 2001; Huang et al., 2003; Akhtar et al., 2010; Charrier and Anastasio, 2012), the measured levels of TNF-α, IL-6, and ROS/RNS obtained in this study demonstrate that organic aerosols alone can induce a cellular response. This was previously observed for the oxidative potential as measured by DTT activity as well, where the same laboratory-generated organic aerosol samples catalyzed redox reactions and resulted in measureable DTT decay in the absence of metal species (Tuet et al., 2017).

Results from this study also show that SOA precursor identity and formation condition influenced response levels, with naphthalene SOA producing the highest cellular responses of the SOA systems investigated. As discussed previously, the aromatic functionality present in many naphthalene photooxidation products may be an important consideration for health effects. It may therefore be worthwhile to investigate other anthropogenic aromatic ring-containing precursors as well and to closely study the cellular effects of naphthalene SOA products given its high response. Several patterns were also noted for SOA systems whose products shared similar functionalities and chemical structures. For instance, photooxidation productions from pentadecane and β-caryophyllene share similarities with long chain fatty acids and may participate in membrane insertions, whereas many known products of naphthalene photooxidation are mutagens capable of inducing cellular pathways beyond those that affect cellular redox balance (Baird et al., 2005; Helmig et al., 1992). Given these observations, it may be possible to roughly predict responses based on known SOA products as SOA systems whose products share similar functionalities and carbon chain length (i.e. similar carbon backbone) are likely to induce similar cellular pathways and produce similar levels of various inflammatory endpoints. Exposure studies involving individual classes of SOA products may elucidate further details as to whether these types of

predictions would be plausible. Moreover, such studies could be used to determine whether the hypothesized cellular pathways are indeed involved and whether certain cellular functions are indeed affected by specific products (e.g. membrane insertion by pentadecane photooxidation products and oxidative phosphorylation decoupling by naphthalene photooxidation products).

Mixture effects may be another important consideration as ambient PM contains SOA formed from multiple SOA precursors. As a result, precursor emissions and their corresponding SOA formation potential must be considered to fully assess PM health effects. Furthermore, it may be worthwhile to investigate various prediction models for multi-component mixtures to bridge the gap between laboratory studies and real ambient exposures. For instance, concentration addition may not apply as ambient aerosol is formed in the presence of multiple precursors and the SOA produced may induce response levels completely different from those observed for single precursor SOA systems that comprise the mixture. Interactions between organic components and metal species have also been suggested in previous studies (Verma et al., 2012; Tuet et al., 2016) and may influence responses significantly. While these interactions were not considered in the current study, there may be evidence to support the plausibility of mixture effects as ambient PM samples produced lower levels of ROS/RNS than that of any single SOA system investigated. Laboratory chambers can serve as an ideal platform to investigate mixture effects, as experiments can be conducted under well-controlled conditions where the aerosol chemical composition and health endpoints can be determined.

Finally, this study confirms that while there is not one simple correlation between oxidative potential and cellular responses for different PM samples, the DTT assay may serve as a useful screening tool as a low DTT activity will likely correspond to a low cellular response. Furthermore, while ROS/RNS may serve as a general indicator of oxidative stress, there may be instances where

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

515 a low level of ROS/RNS does not necessary indicate a lack of cellular response. In the current
516 study, ROS/RNS levels were associated with levels of inflammatory cytokines for the majority of
517 SOA systems investigated. However, aerosol formed from the photooxidation of pentadecane
518 induced low levels of ROS/RNS production and relatively high levels of both cytokines (i.e. higher
519 than expected given the ROS/RNS level measured). These results suggest that at least one
520 additional measure (e.g. inflammatory cytokines) may be required to fully interpret ROS/RNS
521 measurements.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

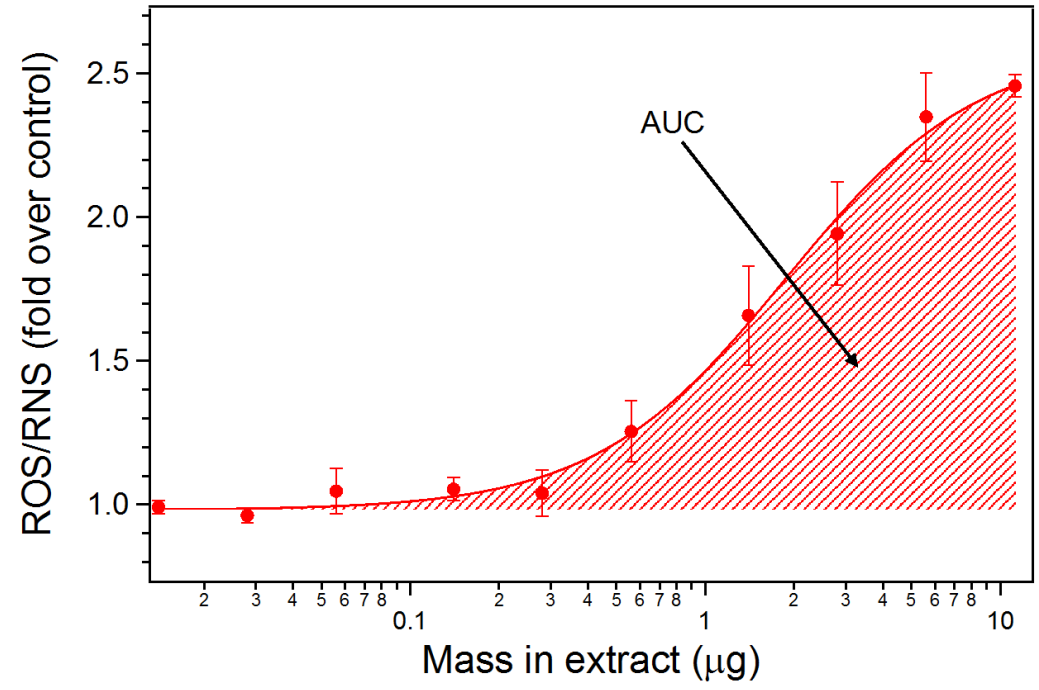

Figure 1. Representative dose-response curve of ROS/RNS produced as a result of filter exposure (naphthalane SOA formed under dry, RO_2 + NO dominant conditions). ROS/RNS is expressed as a fold increase over control cells, defined as probe-treated cells incubated with stimulant-free media. Dose is expressed as mass in extract (μg). Data shown are means ± standard error of triplicate exposure experiments. The Hill equation was used to fit the dose-response curve and the area under the dose-response curve (AUC) is shown.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

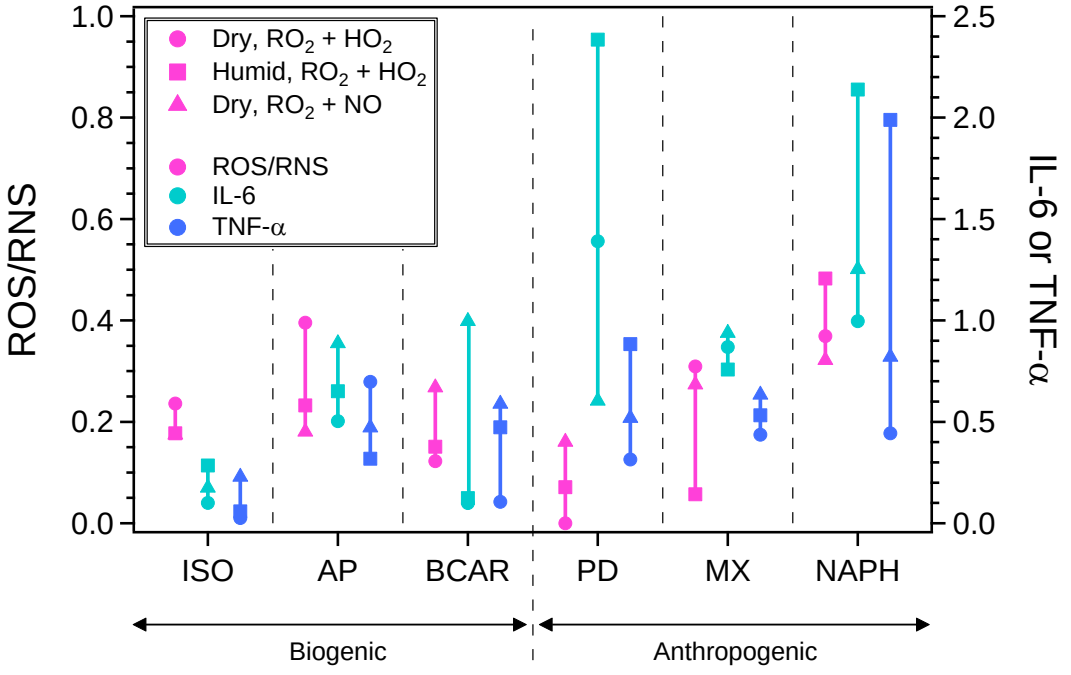

Figure 2. Area under the dose-response curve for various inflammatory responses induced as a result of SOA exposure: **ROS/RNS**, **IL-6**, and **TNF-α**. SOA were generated from various precursors (ISO: isoprene, AP: α-pinene, BCAR: β-caryophyllene, PD: pentadecane, MX: *m*-xylene, and NAPH: naphthalene) under various conditions (circles: dry, RO_2 + HO_2; squares: humid, RO_2 + HO_2; and triangles: dry, RO_2 + NO). Lines connecting the same inflammatory response for SOA generated from the same precursor under different formation conditions are also shown.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
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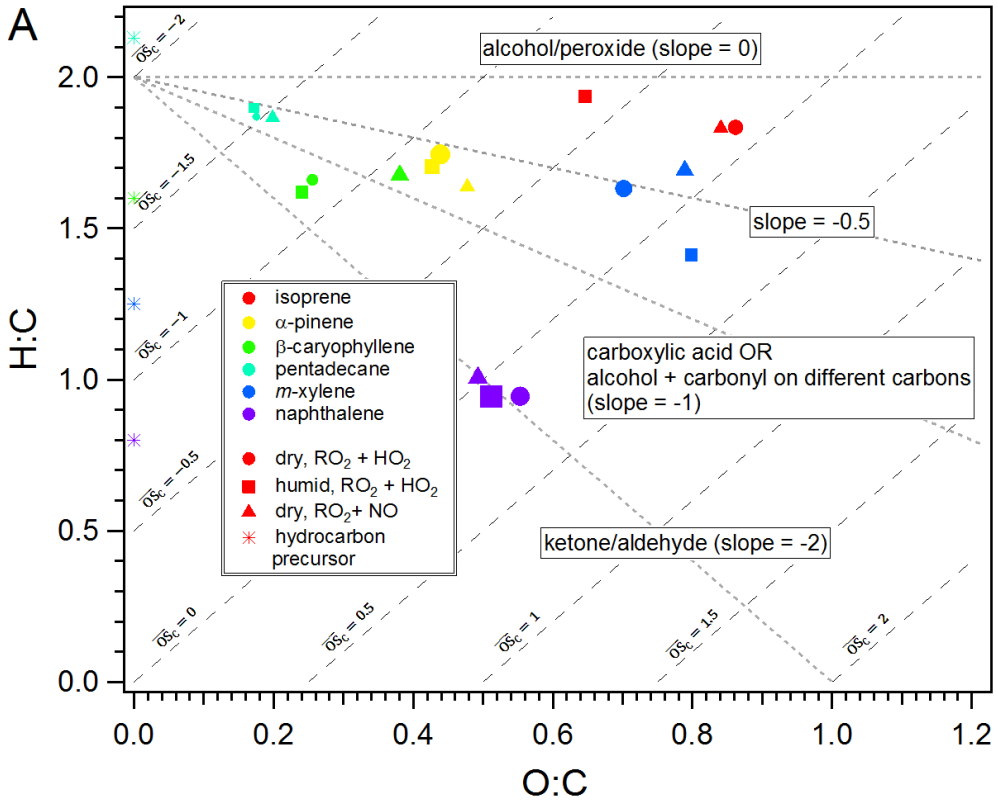
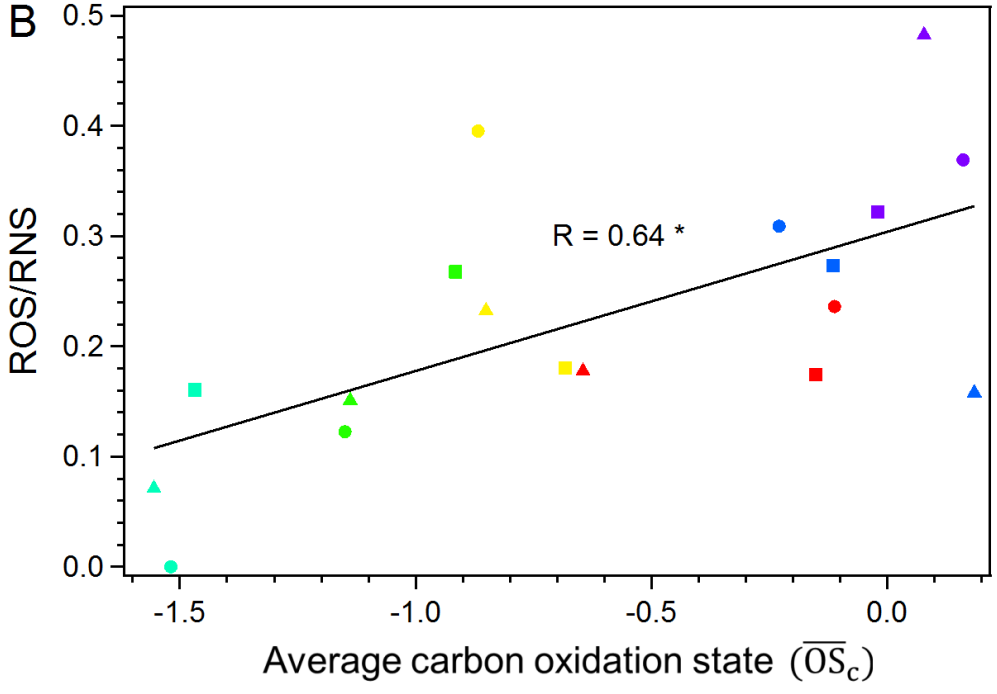
Discussion started: 28 March 2017





Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

539 **Figure 3.** van Krevelen plot for various SOA systems sized by ROS/RNS levels (panel A) and
540 correlation between ROS/RNS levels and average carbon oxidation state (panel B). Data points
541 are colored by SOA system (red: isoprene, yellow: α-pinene, green: β-caryophyllene, light blue:
542 pentadecane, blue: *m*-xylene, and purple: naphthalene), shaped according to formation conditions
543 (circle: dry, $RO_2 + HO_2$; square: humid, $RO_2 + HO_2$; and triangle: dry, $RO_2 + NO$). SOA precursors
544 are shown as stars, colored by SOA system.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

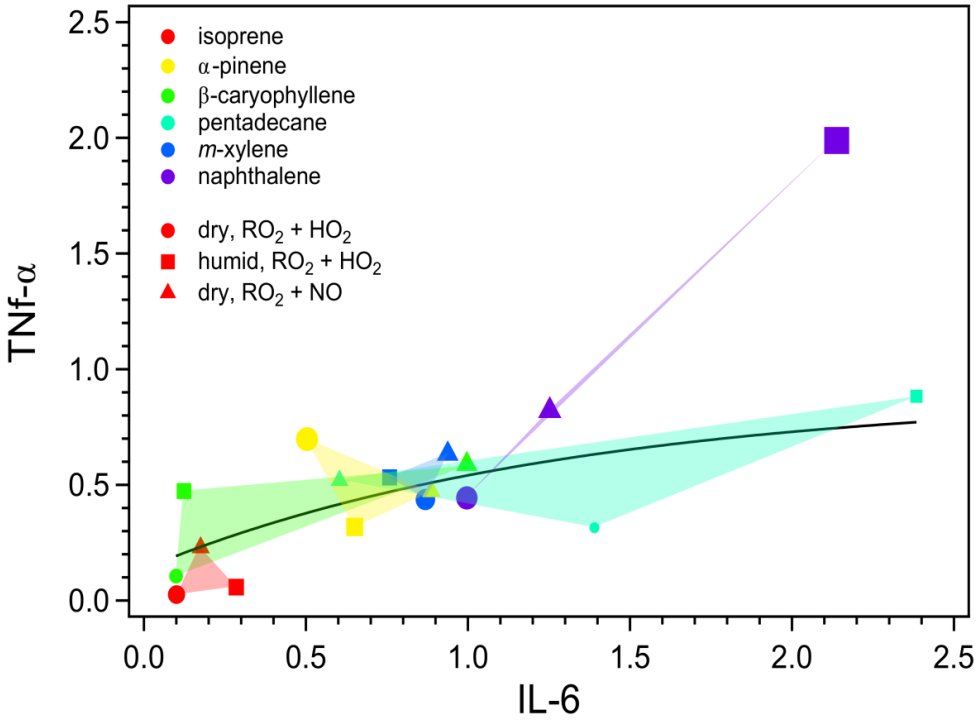
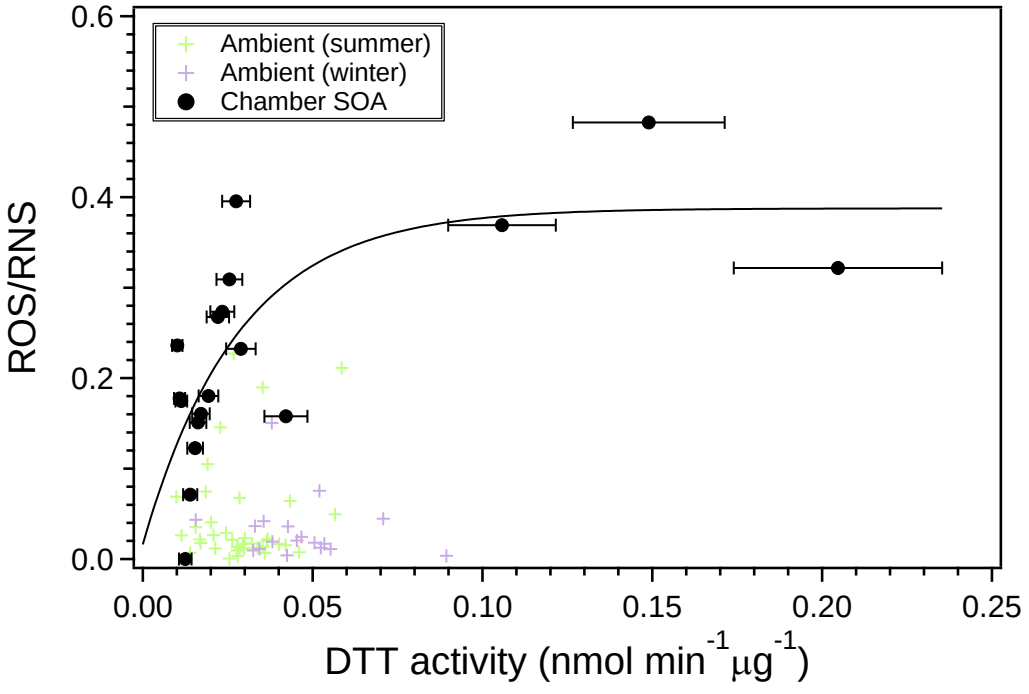


Figure 4. Area under the dose-response curve per mass of SOA for various inflammatory responses induced as a result of SOA exposure. Data points are sized according to ROS/RNS level. SOA were generated from various SOA precursors (red: isoprene, yellow: α-pinene, green: β-caryophyllene, light blue: pentadecane, blue: *m*-xylene, and purple: naphthalene) under various conditions (circles: dry, $RO_2 + HO_2$; squares: humid, $RO_2 + HO_2$; and triangles: dry, $RO_2 + NO$). A fitted curve excluding naphthalene data is shown as a guide. Shaded regions for each system, colored by SOA precursor, are also shown to show the extent of clustering and provide a visualization for the different patterns observed.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017



554

555 **Figure 5.** ROS/RNS production and intrinsic DTT activities for chamber SOA and ambient
556 samples collected around the greater Atlanta area. All samples were analyzed using the method
557 outlined in Cho et al. (2005) and Tuet et al. (2016). Ambient samples are colored by season as
558 determined by solstice and equinox dates between June 2012 and October 2013 (Tuet et al.,
559 2016). A fitted curve for laboratory-generated samples is shown as a guide.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2017-262, 2017
Manuscript under review for journal Atmos. Chem. Phys.
Discussion started: 28 March 2017

Atmospheric Chemistry and Physics Discussions

560 **Table 1.** Experimental conditions.

Experiment	SOA precursor	OH precursor	Relative humidity (%)	$[HC]_0$ (ppb)
1	isoprene	H_2O_2	<5%	97
2	α-pinene	H_2O_2	<5%	191
3	β-caryophyllene	H_2O_2	<5%	36
4	pentadecane	H_2O_2	<5%	106
5	*m*-xylene	H_2O_2	<5%	450
6	naphthalene	H_2O_2	<5%	178
7	isoprene	H_2O_2	<5%[a]	97
8	α-pinene	H_2O_2	40%	334
9	β-caryophyllene	H_2O_2	42%	63
10	pentadecane	H_2O_2	45%	106
11	*m*-xylene	H_2O_2	45%	450
12	naphthalene	H_2O_2	44%	431
13	isoprene	HONO	<5%	970
14	α-pinene	HONO	<5%	174
15	β-caryophyllene	HONO	<5%	21
16	pentadecane	HONO	<5%	74
17	*m*-xylene	HONO	<5%	431
18	naphthalene	HONO	<5%	145

561 [a] Acidic seed (8 mM $MgSO_4$ and 16 mM H_2SO_4) was used instead of 8 mM $(NH_4)_2SO_4$

562 ACKNOWLEDGMENT

563 This work was supported by the Health Effects Institute under research agreement No. 4943-
564 RFA13-2/14-4. Wing Y. Tuet acknowledges support by the National Science Foundation
565 Graduate Research Fellowship under Grant No. DGE-1650044.

566 ABBREVIATIONS

567 PM: particulate matter; SOA: secondary organic aerosol; ROS/RNS: reactive oxygen/nitrogen
568 species; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6

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