Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2016-371, 2016
Manuscript under review for journal Atmos. Chem. Phys.
Published: 17 May 2016

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In Vitro Exposure to Isoprene-Derived Secondary Organic Aerosol by Direct Deposition and its Effects on *COX-2* and *IL-8* Gene Expression

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Abstract

Atmospheric oxidation of isoprene, the most abundant non-methane hydrocarbon emitted into Earth's atmosphere primarily from terrestrial vegetation, is now recognized as a major contributor to the global secondary organic aerosol (SOA) burden. Anthropogenic pollutants significantly enhance isoprene SOA formation through acid-catalyzed heterogeneous chemistry of epoxide products. Since isoprene SOA formation as a source of fine aerosol is a relatively recent discovery, research is lacking on evaluating its potential adverse effects on human health. The objective of this study was to examine the effect of isoprene-derived SOA on inflammation-associated gene expression in human lung cells using a direct deposition exposure method. We assessed altered expression of inflammation-related genes in human bronchial epithelial cells (BEAS-2B) exposed to isoprene-derived SOA generated in an outdoor chamber facility. Measurements of gene expression of known inflammatory biomarkers interleukin 8 (IL-8) and cyclooxygenase 2 (COX-2) in exposed cells, together with complementary chemical measurements, showed that a dose of 0.067 μg cm^{-2} of SOA from isoprene photooxidation leads to statistically significant increases in *IL-8* and *COX-2* mRNA levels. Resuspension exposures using aerosol filter extracts corroborated these findings, supporting the conclusion that isoprene-derived SOA constituents induce the observed changes in mRNA levels. Future studies are needed to systematically examine the molecular mechanisms of toxicity.

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1. Introduction

Recent work has shown that isoprene (2-methyl-1,3-butadiene) is an important precursor of secondary organic aerosol (SOA), which has potential impacts on climate change and public health (Lin et al., 2013b; Rohr, 2013). Current understanding of isoprene SOA formation is based on laboratory studies showing that gas-phase photooxidation of isoprene generates key SOA precursors, including isomeric isoprene epoxydiols (IEPOX), methacrylic acid epoxide (MAE), hydroxymethyl-methyl-α-lactone (HMML), and isoprene hydroxyhydroperoxides (ISOPOOH) (Paulot et al., 2009; Surratt et al., 2010; Lin et al., 2012; Lin et al., 2013b; Nguyen et al., 2015; Krechmer et al., 2015). The formation of SOA from these precursors is influenced by controllable anthropogenic emissions such as oxides of nitrogen (NO_x) and sulfur dioxide (SO_2). Atmospheric oxidation of SO_2 contributes to particle acidity, which enhances isoprene SOA formation through acid-catalyzed reactive uptake and multiphase chemistry of IEPOX and MAE (Surratt et al., 2007; Surratt et al., 2010; Lin et al., 2012; Gaston et al., 2014; Riedel et al., 2015), while NO_x determines whether the oxidation pathway leading to IEPOX or MAE/HMML predominates (Lin et al., 2013b; Surratt et al., 2010; Nguyen et al., 2015). Isoprene SOA comprises a large portion of global atmospheric fine particles ($PM_{2.5}$, aerosol with aerodynamic diameters ≤ 2.5 μm) (Carlton et al., 2009; Henze et al., 2008) but few studies have focused on its health implications. Evaluating the health effects of SOA from isoprene oxidation is important from a public health perspective, not only because of its atmospheric abundance, but also because the anthropogenic contribution is the only component amenable to control (Pye et al., 2013; Gaston et al., 2014; Xu et al., 2015; Riedel et al., 2015).

Many studies have shown that particulate matter is closely linked to health effects ranging from exacerbation of asthma symptoms to mortality associated with lung cancer and

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cardiopulmonary disease (Dockery et al., 1993; Schwartz et al., 1993; Samet et al., 2000). $PM_{2.5}$, in particular, has been linked to negative health outcomes with an estimated contribution of 3.2 million premature deaths worldwide as reported in the Global Burden of Disease Study 2010 (Lim et al., 2012). Despite evidence that particle composition affects toxicity, fewer studies focus on the link between chemical composition and health/biological outcomes (Kelly and Fussell, 2012). Prior work on complex air mixtures has shown that gaseous volatile organic compounds (VOC) alter the composition and ultimately the toxicity of particles (Ebersviller et al., 2012a, b). SOA resulting from natural and anthropogenic gaseous precursors, such as α-pinene and 1,3,5-trimethylbenzene, have been shown to affect cellular function (Gaschen et al., 2010; Jang et al., 2006), but no data on the cellular effects of isoprene-derived SOA exist.

The objective of this study is to examine the toxicity of isoprene-derived SOA through *in vitro* exposures using a direct deposition device. Compared to exposure of cells in culture media to resuspended particles, direct particle deposition likely provides a more biologically relevant exposure model and enhances sensitivity of cells to air pollution particle exposures (Volckens et al., 2009; Lichtveld et al., 2012; Hawley et al., 2014a; Hawley et al., 2014b; Zavala et al., 2014). The Electrostatic Aerosol *in vitro* Exposure System (EAVES) used in this study deposits particles generated in our outdoor photochemical chamber directly onto lung cells by electrostatic precipitation (de Bruijne et al., 2009). Similar techniques and devices have been used to expose cells to diesel exhaust particles (Lichtveld et al., 2012; Hawley et al., 2014b), but our study is the first to utilize the EAVES to explore the potential adverse effects of isoprene SOA on human lung cells.

We have recently demonstrated that isoprene-derived SOA has the potential for inducing reactive oxygen species (ROS) (Kramer et al., 2016), which are linked to oxidative stress and

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inflammation (Li et al., 2003; Reuter et al., 2010). In this study we chose to examine the gene expression levels of interleukin-8 (*IL-8*) and cyclooxygenase-2 (*COX-2*), not only for their links to inflammation and oxidative stress (Koji, 2008; Kunkel et al., 1991), but because both have been examined in previous studies using the EAVES for fresh and aged diesel exhaust (Lichtveld et al., 2012). We compared the gene expression levels in cells exposed to SOA generated in an outdoor chamber from photochemical oxidation of isoprene in the presence of NO and acidified sulfate seed aerosol to cells exposed to a dark control mixture of isoprene, NO, and acidified sulfate seed aerosol to isolate the effects of the isoprene-derived SOA on the cells using the EAVES. In addition, we collected SOA onto filters for subsequent resuspension exposure to ensure that effects observed from EAVES were mostly attributable to particle-phase organic products.

2. Experimental Section

2.1 Generation of SOA in the Outdoor Chamber Facility. SOA were generated by photochemically oxidizing a mixture of acidified sulfate seed aerosol, isoprene, and NO injected into an outdoor smog chamber facility. The outdoor chamber is a 120-m^3 triangular cross-section Teflon chamber located on the roof of the Gillings School of Global Public Health, University of North Carolina at Chapel Hill. The chamber facility has been described in detail elsewhere by Lichtveld et al. (2012). The outdoor chamber facility is equipped with sampling lines that allow direct deposition exposure of cells, online chemical measurements, and filter collection for offline chemical analysis. Sampling lines run from the underside of the chamber directly to the chemistry lab below where online measurement instruments and the direct deposition exposure device are located. Injection ports are also located on the underside of the chamber.

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To generate isoprene-derived SOA, the chamber was operated on sunny days to allow natural sunlight to trigger photochemical reactions. Acidified sulfate seed aerosols were generated by nebulizing an aqueous solution containing 0.06 M $MgSO_4$ + 0.06 M H_2SO_4 into the chamber to a particle concentration of approximately 170 μg m^{-3}, which was allowed to stabilize for 30 min to ensure a well-mixed condition. After stabilization, 3.5 ppmv isoprene (Sigma-Aldrich, 99%) and 200 ppbv NO (AirGas, 1.00%) were injected into the chamber. Photochemical aging was allowed for approximately one hour to reach the desired exposure conditions of 30-40 μg m^{-3} growth of isoprene-derived SOA on the pre-existing 170 μg m^{-3} of acidified sulfate aerosol. This chamber experiment was replicated on three separate sunny days.

2.2 Control Chamber Experiments. As a dark chamber control, to isolate the effect of SOA on exposed cells, mixtures of isoprene, NO, and 170 μg m^{-3} of acidified sulfate seed aerosol were injected into the chamber in the dark (after sunset). Conducting the chamber experiments in the dark ensured no photochemical oxidation of isoprene. The dark control was replicated on three different nights. Except for the absence of solar radiation (no SOA), all chamber operations and exposure conditions were similarly maintained.

As an added control to ensure that the device itself and the cell handling had no significant effect on cell cytotoxicity, cells were exposed in the EAVES to a clean chamber and compared to unexposed cells kept in an incubator for the same duration as the exposure. The cytotoxicity results ensured that there is no effect of chamber conditions and device operation on the cells.

2.3 Cell Culture. Human bronchial epithelial (BEAS-2B) cells were maintained in keratinocyte growth medium (KGM BulletKit; Lonza), a serum-free keratinocyte basal medium (KBM) supplemented with bovine pituitary extract, human epidermal growth factor, insulin,

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hydrocortisone, and GA-1000 (gentamicin, amphotericin B), and passaged weekly. Passage number for both photochemical exposures and dark control exposures varied between 52 and 60. Because BEAS-2B are an immortalized line of human bronchial epithelium, there are limitations with its use such as it being genetically homogeneous, being a single cell type, and being SV-40 transformed (Reddel et al., 1988). However, BEAS-2B is a stable, proliferative cell line shown to be useful in airway inflammation studies such as ours (Devlin et al., 1994).

2.4 Direct Deposition Exposure. In preparation for air-liquid interface exposures, cells were seeded onto collagen-coated Millicell cell culture inserts (30 mm diameter, 0.4-μm pore size, 0.6 cm^2 filter area; Millipore, Cambridge, MA) at a density of 50,000 cells/well 24 hours prior to exposure. At the time of exposure, cells reached ~80% confluence, confirmed through microscopy. Immediately before exposure, cell medium was removed from the apical and basolateral sides of 2 seeded Millicell cell culture inserts. One insert was transferred to a titanium dish containing 1.5 mL of keratinocyte basal medium (KBM; Lonza), supplying cells with nutrients from the basolateral side and constant moisture while allowing exposure to be performed at an air-liquid interface. The other insert was transferred into a 6 well plate with 2 mL of KBM and placed in the incubator as an unexposed control.

Cells were exposed to chamber-generated isoprene SOA using the EAVES located in the laboratory directly beneath the outdoor chamber (de Bruijne et al., 2009; Lichtveld et al., 2012). The EAVES, located in an incubator at 37°C, sampled chamber air at 1.5 L min^{-1}. The target relative humidity (RH) in the chamber during EAVES exposures was approximately 70%. Exposure time was one hour commencing when target exposure conditions were achieved in the outdoor chamber for both photochemical and dark control experiments. Detailed description of the EAVES can be found in de Bruijne et al. (2009).

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Following exposure, the cell culture insert was transferred to a 6-well tissue culture plate containing 2 mL of fresh KBM. The control Millicell was also transferred to 2 mL of fresh KBM. Nine hours post-exposure, extracellular medium was collected and total RNA was isolated using Trizol (Life Technologies), consistent with past studies (de Bruijne et al., 2009). Extracellular medium and the extracted RNA samples were stored at -20°C and -80°C, respectively, until further analysis.

2.5 Filter Resuspension Exposure. Chamber particles were collected, concurrently with EAVES sampling, onto Teflon membrane filters (47 mm diameter, 1.0 µm pore size; Pall Life Science) for photochemical (light) and dark chamber experiments to be used for chemical analysis and resupsension exposures. The resuspension experiments served as a control for possible effects of gaseous components such as ozone (O_3) and NO_x present in the direct deposition experiments; however, prior studies have shown that gaseous components do not yield cellular responses within the EAVES device (de Bruijne et al., 2009; Ebersviller et al., 2012a, b). Mass loadings of SOA collected on the filters were calculated from sampling volumes and average aerosol mass concentrations in the chamber during the sampling period. A density correction of 1.6 g cm^{-3} (Riedel et al., 2016) and 1.25 g cm^{-3} (Kroll et al., 2006) was applied to convert the measured volume concentrations to mass concentrations for the acidified sulfate seed and SOA growth, respectively. The particles collected on Teflon filter membranes for resuspension cell exposure were extracted by sonication in high-purity methanol (LC/MS CHROMASOLV, Sigma-Aldrich). Filter samples from multiple experiments were combined and the combined filter extract was dried under a gentle stream of nitrogen (N_2). KBM medium was then added into the extraction vials to re-dissolve SOA constituents.

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In preparation for filter resuspension exposures, cells were seeded in 24-well plates at a density of 2.5×10^4 cells/well in 250 µL of KGM 2 days prior to exposure. At the time of exposure when cells reached ~80% confluence, cells were washed twice with phosphate buffered saline (PBS) buffer, and then exposed to KBM containing 0.01 and 0.1 mg mL^{-1} isoprene SOA extract from photochemical experiment and seed particles from dark control experiments.

Following a 9-hour exposure, extracellular medium was collected and total RNA was isolated using Trizol (Life Technologies). Extracellular medium and the extracted RNA samples were stored at -20°C and -80°C, respectively, until further analysis.

2.6 Chemical and Physical Characterization of Exposures. Online and offline techniques were used to characterize the SOA generated in the chamber. The online techniques measured the gas-phase species NO, NO_x and O_3 and the physical properties of the aerosol continuously throughout the chamber experiments. Offline techniques measured aerosol-phase species collected onto Teflon membrane filters (47 mm diameter, 1.0 µm pore size; Pall Life Science) from photochemical and dark chamber experiments. Filter samples were stored in 20 mL scintillation vials protected from light at -20°C until analyses.

Real-time aerosol size distributions were measured using a Differential Mobility Analyzer (DMA, Brechtel Manufacturing Inc.) coupled to a Mixing Condensation Particle Counter (MCPC, Model 1710, Brechtel Manufacturing Inc.) located in the laboratory directly underneath the chamber. O_3 and NO_x were measured with a ML 9811 series Ozone Photometer (Teledyne Monitor Labs, Englewood, CO) and ML 9841 series NO_x Analyzer (American Ecotech, Warren RI), respectively. Data were collected at one-minute intervals using a data acquisition system (ChartScan/1400) interfaced to a computer. The presence of isoprene in the

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chamber was confirmed and quantified using a Varian 3800 gas chromatograph (GC) equipped with a flame ionization detector (FID).

Chemical characterization of SOA constituents was conducted offline from extracts of filters collected from chamber experiments by gas chromatography interfaced with an electron ionization quadrupole mass spectrometer (GC/EI-MS) or by ultra performance liquid chromatography interfaced with a high-resolution quadrupole time-of-flight mass spectrometer equipped with electrospray ionization (UPLC/ESI-HR-QTOFMS). Detailed operating conditions for the GC/EI-MS and UPLC/ESI-HR-QTOFMS analyses as well as detailed filter extraction protocols have been described previously by Lin et al. (2012). For GC/EI-MS analysis, filter extracts were dried under a gentle stream of N_2 and trimethylsilylated by the addition of 100 μL of BSTFA + TMCS (99:1 v/v, Supelco) and 50 μL of pyridine (anhydrous, 99.8%, Sigma-Aldrich) and heated at 70 ºC for 1 h. For UPLC/ESI-HR-QTOFMS analysis, residues of filter extracts were reconstituted with 150 μL of a 50:50 (v/v) solvent mixture of high-purity water and methanol.

The isoprene-derived SOA markers: 2-methyltetrols, isomeric 3-methyltetrahydrofurans-3,4-diols (3-MeTHF-3,4-diols), and 2-methylglyceric acid, synthesized according to the published procedures (Lin et al., 2013b; Zhang et al., 2012), were available in-house as authentic standards to quantify the major components of isoprene SOA. 2-Methyltetrol organosulfates, synthesized as a mixture of tetrabutylammonium salts, were also available as a standard. Purity was determined to be >99% by ^{1}H NMR and UPLC/ESI-QTOFMS analysis (Budisulistiorini et al., 2015). The C_5-alkene triols and IEPOX dimer were quantified using the response factor obtained for the synthetic 2-methyltetrols.

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A representative ambient $PM_{2.5}$ sample collected from the rural southeastern U.S. (Yorkville, GA) (Lin et al., 2013a) during the summer of 2010 was analyzed to confirm atmospheric relevance of the chamber-generated SOA constituents.

2.6 Cytotoxicity Assay. Cytotoxicity was assessed through measurement of lactate dehydrogenase (LDH) released into the extracellular medium from damaged cells using the LDH cytotoxicity detection kit (Takara). To ensure that the EAVES device itself and operation procedure had no effect on cytotoxicity, the LDH release from cells exposed to clean chamber air was measured. LDH release by cells exposed via the EAVES to the photochemically aged (light) and non-photochemically aged (dark) particles was compared to unexposed cells maintained in the incubator for the same duration. For the resuspension exposures, LDH release by cells exposed to SOA through resuspended extract of photochemically aged and non-photochemically aged particles was compared to cells maintained in KBM only. Additionally, LDH release from the light exposures, dark control, and resuspension exposures were compared to positive controls exposed to 1% Triton X-100 to ensure that cell death would not affect gene expression results.

2.7 Gene Expression Analysis. Changes in *IL-8* and *COX-2* mRNA levels were measured in BEAS-2B cells exposed to isoprene-derived SOA generated in our outdoor chamber facility using QuantiTect SYBR Green RT-PCR Kit (Qiagen). All mRNA levels were normalized against β-actin mRNA, which was used as a housekeeping gene. The relative expression levels (i.e., fold change) of *IL-8* and *COX-2* were calculated using the comparative cycle threshold ($2^{-\Delta\Delta CT}$) method (Livak and Schmittgen, 2001). For EAVES exposures, changes in *IL-8* and *COX-2* from isoprene-derived SOA exposed cells were compared to cells exposed in dark controls. Similarly, for resuspension exposures changes in *IL-8* and *COX-2* from isoprene-derived SOA exposed cells were compared to cells exposed to particles collected under dark conditions.

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256 **2.8 Statistical Analysis**

257 The software package GraphPad Prism 4 (GraphPad) was used for all statistical analyses. All
258 data were expressed as mean ± SEM. Comparisons between data sets for cytotoxicity and gene
259 expression analysis were made using unpaired *t*-test with Welch's correction. Significance was
260 defined as $p < 0.05$.

261 **3. Results and Discussion**

262 **3.1 Physical and Chemical Characterization of Exposure.** Figure 1 shows the change in
263 particle mass concentration and gas (O_3, NO, NO_x) concentration over time during typical
264 photochemical and dark control experiments. Under dark control conditions (Fig. 1a) there is no
265 increase in aerosol mass concentration following isoprene injection. Average total aerosol mass
266 concentration was 155.0±2.69 µg m^{-3} (1 standard deviation) with no particle mass attributable to
267 organic material.

268 In contrast, Fig. 1b shows an increase in aerosol mass concentration after 1 h post
269 isoprene injection, which can be attributed to the photochemical oxidation of isoprene and
270 subsequent production and reactive uptake of its oxidation products. The average increase in
271 aerosol mass concentration attributable to SOA formation for three daylight chamber
272 experiments conducted on separate days was 44.5±5.7µg m^{-3}. Average total aerosol mass
273 concentration during particle exposure was 173.1± 4.2 µg m^{-3}.

274 O_3 and NO_x concentrations measured during EAVES exposure were approximately 270
275 ppb and 120 ppb for photochemical experiments. For dark control experiments (e.g., Fig. 1a), the
276 O_3 and NO_x concentrations were approximately 15 ppb and 180 ppb. Previous studies
277 characterizing the EAVES device show definitively that gas-phase products do not induce cell
278 response (de Bruijne et al., 2009; Ebersviller et al., 2012a, b). However, resuspension exposures

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279 were conducted in addition to EAVES exposure to ensure that biological effects were
280 attributable to only particle-phase constituents and not gas-phase products such as O_3 and NO_x.
281 The chemical composition of aerosol, collected onto filters concurrently with cell
282 exposure and characterized by GC/EI-MS and UPLC/ESI-HR-QTOFMS, are shown in Fig. 2.
283 The dominant particle-phase products of the isoprene-SOA collected from photochemical
284 experiments include 2-methyltetrols, C_5-alkene triols, isomeric 3-MeTHF-3,4-diols, IEPOX-
285 derived dimers, and IEPOX-derived organosulfates. The sum of the IEPOX-derived SOA
286 constituents quantified by the available standards accounted for ~80% of the observed SOA
287 mass. The MAE-derived SOA constituents, 2-methylglyceric acid and the organosulfate
288 derivative of MAE, accounted for 1.4% of the observed SOA mass, confirming that particle-
289 phase products generated were predominantly formed from the reactive uptake of IEPOX onto
290 acidic sulfate aerosols. All the same particle-phase products are measured in the $PM_{2.5}$ sample
291 collected in Yorkville, GA, demonstrating that the composition of the chamber-generated SOA is
292 atmospherically relevant. Aerosol collected from the dark control experiments contained no
293 IEPOX- or MAE-derived SOA constituents or other measurable organic constituents.

294 **3.2 Cytotoxicity**

295 LDH release for cells exposed using the EAVES device is expressed as a fold-change relative to
296 the unexposed incubator control. For resuspension exposures, LDH release is expressed as fold-
297 change relative to cells exposed to KBM only. Results shown in Fig. 3a confirm that there is no
298 effect of chamber conditions and device operation on the cells when comparing LDH release
299 from cells exposed to a clean air chamber and cells unexposed in an incubator. Additionally,
300 LDH release from all exposure conditions in EAVES exposed cells (Fig. 3b) and resuspension
301 exposed cells (Fig. 3c) is negligible relative to positive controls exposed to 1% Triton X-100,

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confirming that the exposure concentration of isoprene-derived SOA utilized in this study was not cytotoxic. All cytotoxicity results ensured that exposure conditions were not adversely affecting the cells nor their gene expression.

3.3 Pro-inflammatory Gene Expression. Changes in the mRNA levels of *IL-8* and *COX-2* from cells exposed to isoprene-derived SOA using the EAVES are shown as fold-changes relative to dark controls in Fig. 3. This comparison, as well as the results of the resuspension experiment discussed below, ensure that all effects seen in the cells are attributable to the isoprene-derived SOA and no other factors. A one-hour exposure to a mass concentration of approximately 45 µg m^{-3} of organic material was sufficient to significantly alter gene expression of the inflammatory biomarkers in bronchial epithelial cells. Based on deposition efficiency characterized by de Bruijne et al. (2009), the estimated dose was 0.29 µg cm^{-2} of total particle mass with 23% attributable to organic material formed from isoprene photooxidation (0.067 µg cm^{-2} of SOA).

Changes in the mRNA levels of *IL-8* and *COX-2* from cells exposed to resuspended isoprene-derived SOA collected from photochemical experiments are shown as fold-changes relative to cells exposed to resuspended particles from dark control experiments in Fig. 4. At a low dose of 0.01 mg mL^{-1} of isoprene SOA extract there is no significant increase in *IL-8* and *COX-2* mRNA expression. The isoprene SOA extract, however, induces a response at a dose of 0.1 mg mL^{-1}. The statistically significant increase in mRNA expression from the resuspension exposure at 0.1 mg mL^{-1} confirms that similar fold changes observed for both *IL-8* and *COX- 2* from the EAVES exposures are not attributable to gaseous photooxidation products, such as O_3, and support the characterization of the EAVES as a particle exposure device (de Bruijne et al., 2009).

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The similar fold change observed in both the EAVES exposure and resupsension exposure, in addition to confirming that the biological effects can be attributed to the particle-phase photochemical products (isoprene-derived SOA), suggests that exposure by resuspension is appropriate for isoprene-derived SOA and may yield results similar to direct deposition exposures. Unlike diesel particulate extracts, which agglomerate during resuspension exposures, isoprene-derived SOA constituents are water-soluble based on reverse-phase LC separations (Surratt et al., 2006; Lin et al., 2012) and remain well mixed in the cell medium used for exposure. Therefore, resuspension exposures do not appear to be a limitation for toxicological assessments of isoprene SOA.

3.4 Biological Implications. The goal of this study was to initially identify potential hazards associated with exposure to isoprene-derived SOA by using a direct exposure device as a model that has both atmospheric and physiological relevance. With this model, a dose of 0.067 μg cm^{-2} isoprene SOA, induced statistically significant increases in *IL-8* and *COX-2* mRNA levels in exposed BEAS-2B cells. By choosing *IL-8* and *COX-2* as our genes of interest, we are able to compare our results to other studies of known harmful particle exposures. In a similar study using the EAVES, normal human bronchial epithelial (NHBE) cells exposed to 1.10 μg cm^{-2} diesel particulate matter showed less than a 2-fold change over controls in both *IL-8* and *COX-2* mRNA expression (Hawley et al., 2014b). In another study, A549 human lung epithelial cells were exposed by direct deposition for 1 hour to photochemically-aged diesel exhaust particulates at a dose of 2.65 μg cm^{-2} from a 1980 Mercedes or a 2006 Volkswagen (Lichtveld et al., 2012). Exposure to aged Mercedes particulates induced a 4-fold change in IL-8 and ~2-fold change in *COX-2* mRNA expression, while exposure to aged Volkswagen particulates induced a change of ~1.5-fold in *IL-8* and 2-fold in *COX-2* mRNA expression (Lichtveld et al., 2012). Although the

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differences in cell types preclude direct comparisons, the finding of significant increases in *COX-2* and *IL-8* expression at doses much lower than reported for comparable increases in inflammatory gene expression levels induced by photochemically-aged diesel particulates indicates that isoprene SOA may be a public health concern warranting further toxicological investigation. In particular, recent work from our laboratory using the dithiothreitol (DTT) assay demonstrated that isoprene-derived SOA have equal or greater ROS generation potential than diesel exhaust PM (Rattanavaraha et al., 2011; Kramer et al., 2016), highlighting the importance of a more comprehensive investigation of the toxicity of isoprene-derived SOA, by *in vitro* exposure experiments, which may in turn justify further extension to *in vivo* work.

Our findings are consistent with other studies showing that photochemical oxidation of similar chemical mixtures increases toxicity in cell culture models and elevates expression of inflammatory biomarker genes (Lichtveld et al., 2012; Rager et al., 2011). Previous *in vitro* studies using a gas-phase only exposure system have shown that gas-phase products of isoprene photooxidation significantly enhance cytotoxicity and *IL-8* expression (Doyle et al., 2004; Doyle et al., 2007). Consistent with the reports that *IL-8* and *COX-2* play important roles in lung inflammation (Nocker et al., 1996; Li et al., 2013), *in vivo* studies have shown that isoprene oxidation products cause airflow limitation and sensory irritation in mice (Rohr et al., 2003). However, this is the first report of inflammatory response in lung epithelial cells exposed to SOA from isoprene photooxidation by direct deposition. *In vitro* studies such as this one serve as a necessary part of hazard characterization, particularly for a complex air mixture that has not been fully studied (Hayashi, 2005).

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4. Conclusions

This study indicates that an atmospherically relevant composition of isoprene-derived SOA is capable of increasing the expression of *IL-8* and *COX-2* in human bronchial epithelial cells. The SOA were generated as NO levels approached zero, which represents conditions characteristic of urban locales downwind of rural isoprene sources. As shown in Fig. 2, the aerosol generated for exposures in this study are chemically similar to fine aerosol samples collected from the Southeastern U.S., which indicates that the chamber exposures are representative of exposures that may be encountered by populations in regions where isoprene emissions interact with anthropogenic pollutants. The same particle-phase products found in our photochemical experiments have been measured in significant quantities (accounting on average for 33% of fine organic aerosol mass) in ambient fine organic particles collected in the Southeastern U.S. (Lin et al., 2013b; Budisulistiorini et al., 2013; Rattanavaraha et al., 2016; Budisulistiorini et al., 2016) and in other isoprene-rich environments (Hu et al., 2015). Under such conditions, the compounds that may contribute to toxicity or inflammatory effects in exposed cells include 2-methyltetrols, C_5-alkene triols, isomeric 3-MeTHF-3,4-diols, dimers, and organosulfates. Since the compounds identified and quantified in this study do not form independently (Paulot et al., 2009; Surratt et al., 2010; Lin et al., 2012; Lin et al., 2013b) toxicological effects are best assessed by exposure to the total mixture as in this study. Taken together, this study demonstrates that atmospherically relevant compositions of isoprene-derived SOA can induce adverse effects, suggesting that anthropogenically-derived acidic sulfate aerosol may drive the generation and toxicity of SOA.

Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2016-371, 2016
Manuscript under review for journal Atmos. Chem. Phys.
Published: 17 May 2016

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Acknowledgements

Research described in this article was conducted under contract to the Health Effects Institute (HEI), an organization jointly funded by the United States Environmental Protection Agency (EPA) (Assistance Award No. R-82811201), and certain motor vehicle and engine manufacturers. The contents of this article do not necessarily reflect the views of HEI, or its sponsors, nor do they necessarily reflect the views and policies of the EPA or motor vehicle and engine manufacturers. M. A. was supported by a graduate fellowship provided by the National Science Foundation (DGE-0646083), from the Center for Faculty Excellence, University of North Carolina at Chapel Hill, and in part by a grant from the National Institute of Environmental Health Sciences (T32-ES007018).

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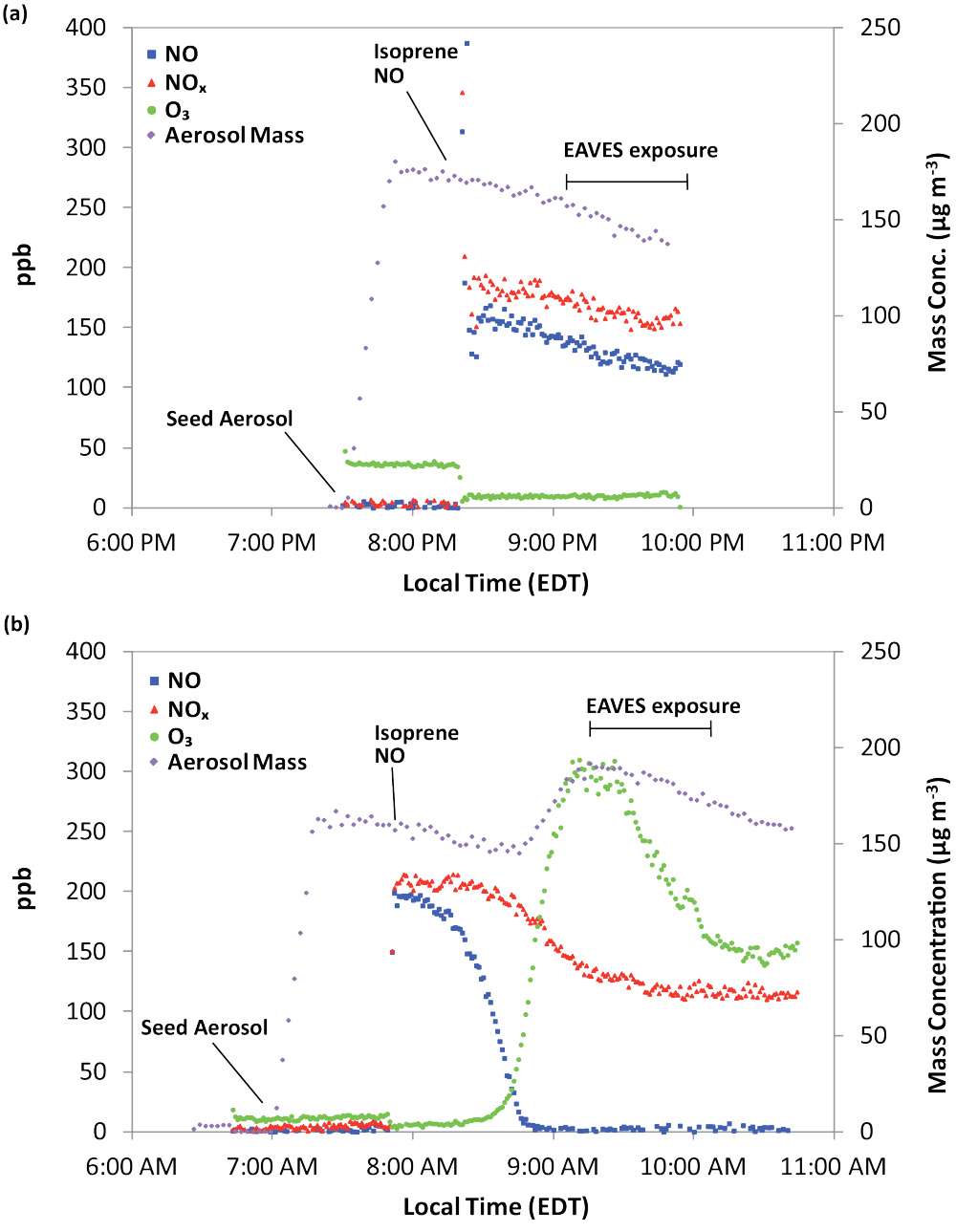
Manuscript under review for journal Atmos. Chem. Phys.
Published: 17 May 2016



641

642 **Figure 1.** Aerosol mass concentration and gas-phase product concentrations over time for (a)
643 dark control chamber experiment and (b) photochemically produced isoprene-derived SOA
644 exposure chamber experiment.

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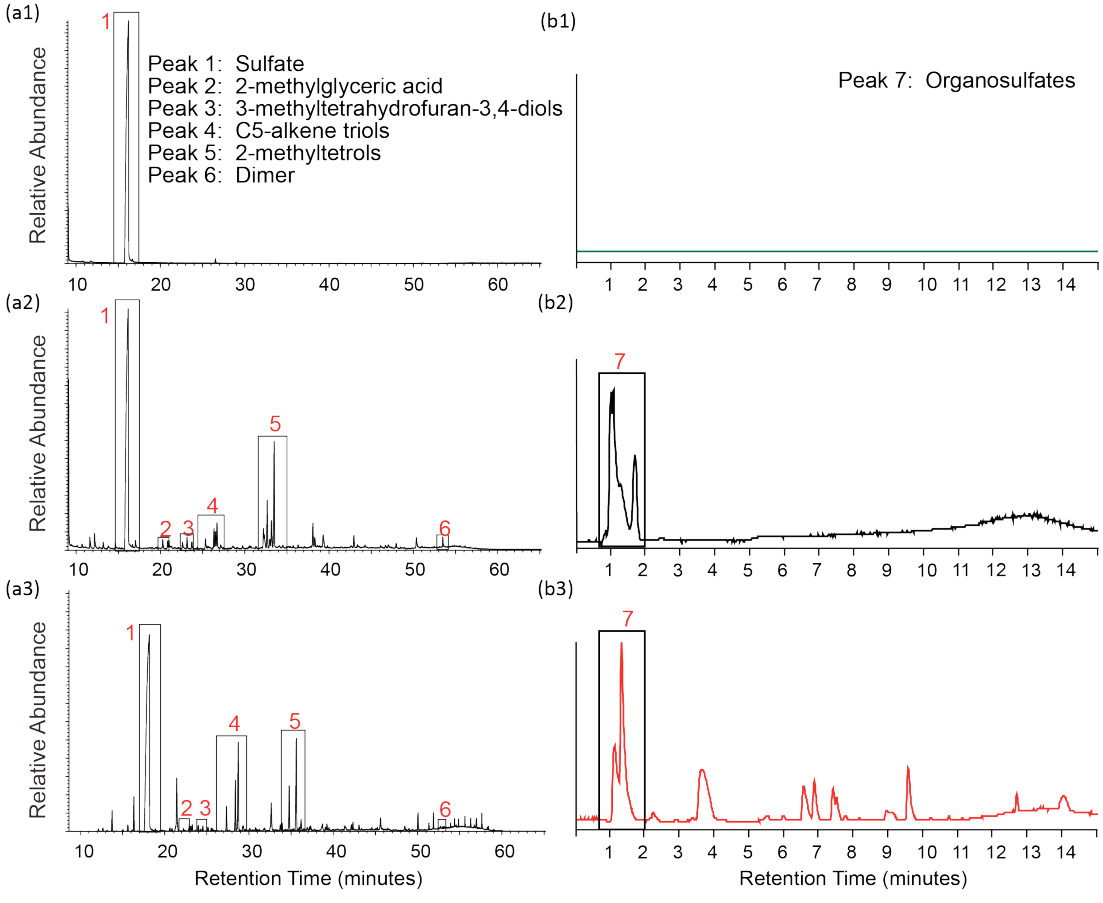

Figure 2. (a) GC/EI-MS total ion chromatograms (TICs) and (b) UPLC/ESI-HR-QTOFMS base peak chromatograms (BPCs) from a (1) dark control chamber experiment, (2) isoprene-derived SOA exposure chamber experiment, and (3) $PM_{2.5}$ sample collected from Yorkville, GA during summer 2010.

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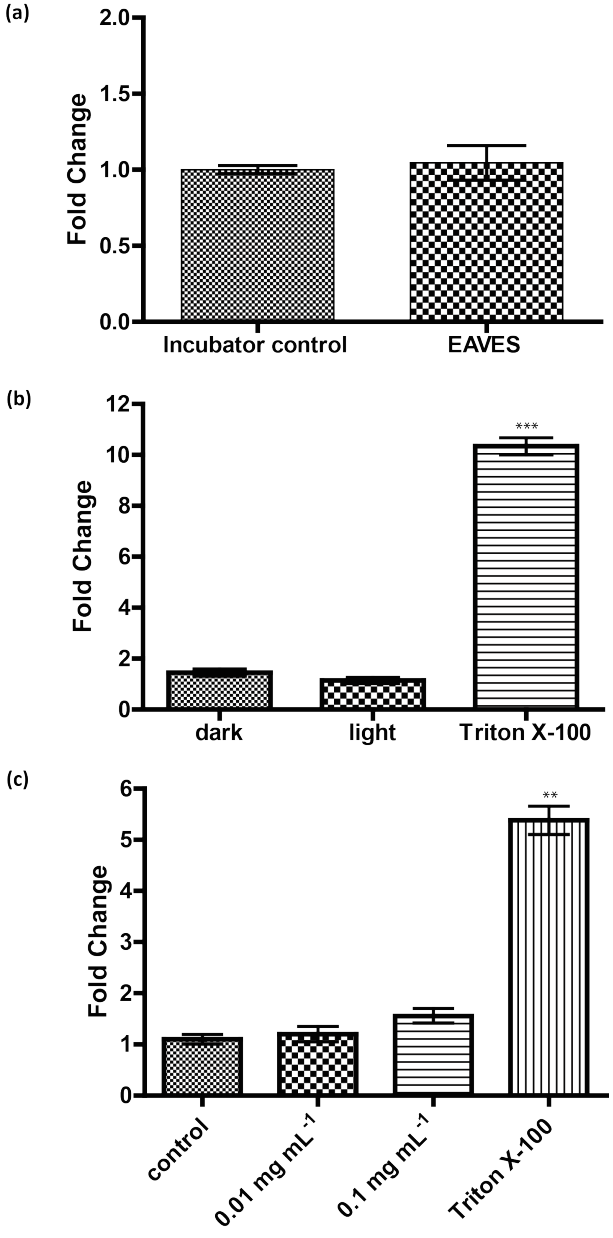

650

651 **Figure 3.** LDH release for (a) clean air controls, (b) EAVES exposures, normalized to incubator
652 control, and (c) resuspension exposures, normalized to KBM only control. **p<0.005 and
653 ***p<0.0005.

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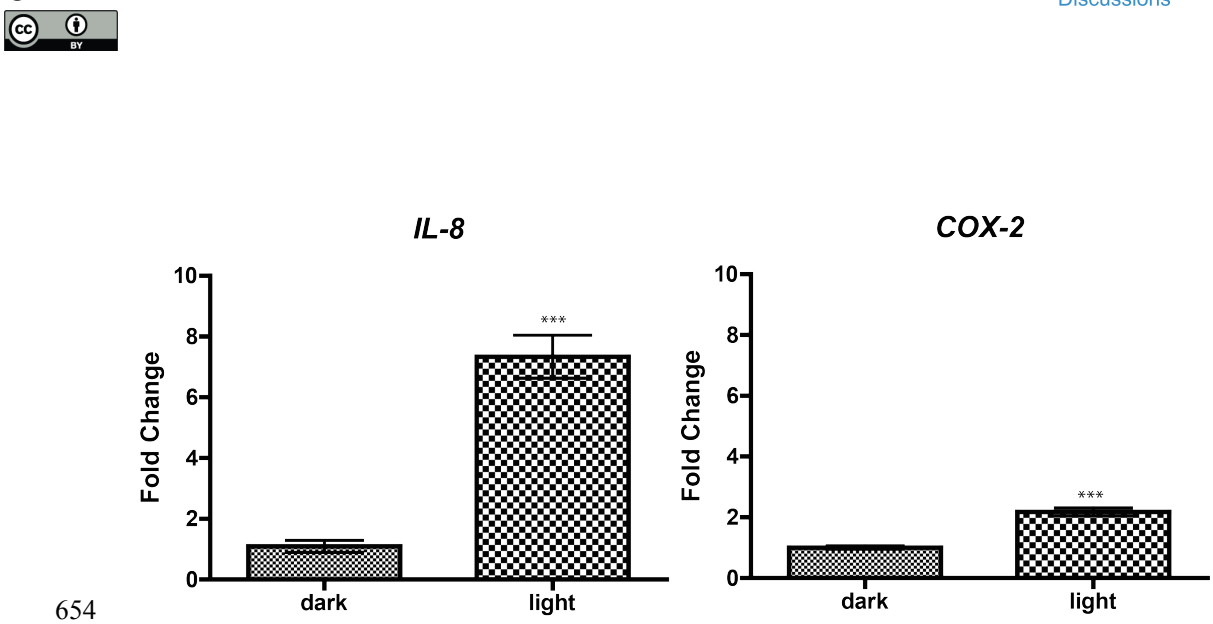

654

655 **Figure 4.** *IL-8* and *COX-2* mRNA expression induced by exposure to isoprene-derived SOA

656 using EAVES device all normalized to dark control experiments. All experiments conducted in

657 triplicate. ***p<0.0005.

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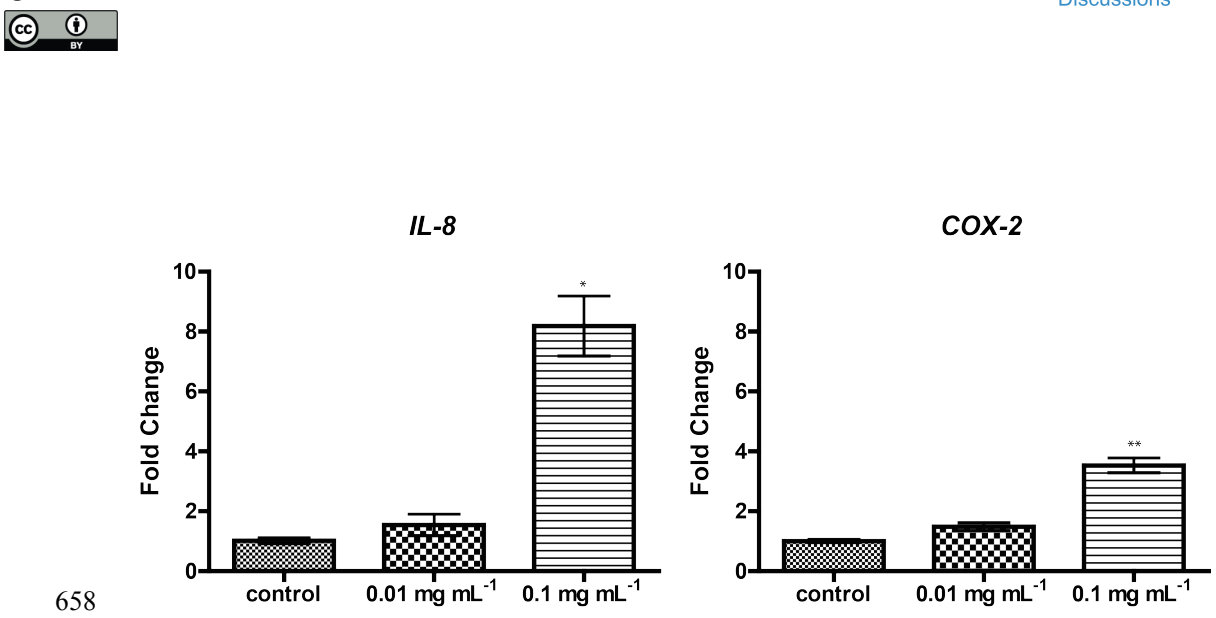

658

659 **Figure 5.** *IL-8* and *COX-2* expression induced by exposure to isoprene-derived SOA using

660 resuspension method all normalized to dark control experiments. All experiments conducted in

661 triplicate. *p<0.05 and **p<0.005.