Gaseous VOCs rapidly modify particulate matter and its biological effects – Part 1: Simple VOCs and model PM

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Abstract

This is the first of a three-part study designed to demonstrate dynamic entanglements among gaseous organic compounds (VOC), particulate matter (PM), and their subsequent potential biological effects. We study these entanglements in increasingly complex VOC and PM mixtures in urban-like conditions in a large outdoor chamber. To the traditional chemical and physical characterizations of gas and PM, we added new measurements of gas-only- and PM-only-biological effects, using cultured human lung cells as model indicators. These biological effects are assessed here as increases in cellular damage or expressed irritation (i.e., cellular toxic effects) from cells exposed to chamber air relative to cells exposed to clean air. The exposure systems permit gas-only- or PM-only-exposures from the same air stream containing both gases and PM in equilibria, i.e., there are no extractive operations prior to cell exposure.

Our simple experiments in this part of the study were designed to eliminate many competing atmospheric processes to reduce ambiguity in our results. Simple volatile and semi-volatile organic gases that have inherent cellular toxic properties were tested individually for biological effect in the dark (at constant humidity). Airborne mixtures were then created with each compound and PM that has no inherent cellular toxic properties for another cellular exposure. Acrolein and p-tolualdehyde were used as model VOCs and mineral oil aerosol (MOA) was selected as a surrogate for organic-containing PM. MOA is appropriately complex in composition to represent ambient PM, and it exhibits no inherent cellular toxic effects and thus did not contribute any biological detrimental effects on its own.

Chemical measurements, combined with the responses of our biological exposures, clearly demonstrate that gas-phase pollutants can modify the composition of PM (and its resulting detrimental effects on lung cells) – even if the gas-phase pollutants are not considered likely to partition to the condensed phase: the VOC-modified-PM showed significantly more damage and inflammation to lung cells than did the original PM. Because gases and PM are transported and deposited differently within the atmosphere...
and the lungs, these results have significant consequences. For example, current US policies for research and regulation of PM do not recognize this “effect modification” phenomena (NAS, 2004).

These results present an unambiguous demonstration that – even in these simple mixtures – physical and thermal interactions alone can cause a modification of the distribution of species among the phases of airborne pollution mixtures and can result in a non-toxic phase becoming toxic due to atmospheric thermal processes only. Subsequent work extends the simple results reported here to systems with photochemical transformations of complex urban mixtures and to systems with diesel exhaust produced by different fuels.

1 Introduction

In its most-recent document summarizing the state of the science related to PM, the US National Research Council (NRC) stated that “a finding that the effect of particles depends on the concentration of another pollutant – that is, “effect modification” – would have implications for setting NAAQS [National Ambient Air Quality Standards] independently for the various criteria pollutants” (NAS, 2004).

This study was designed to demonstrate the existence of “effect modification” in a highly simplified system, and to increase the recognition of the atmosphere itself as a significant source of detrimental biological effects that are typically associated with PM. Researchers have long known that gases and particles interact in important and dynamic processes during the ageing of particulate matter (PM) in the atmosphere. Volatile organic compounds (VOCs) are constantly shifting between the gas and particle phases of ambient air and at the same time can be modified by chemical reactions in each phase. Partitioning theory has evolved over decades, and has more recently been coupled to atmospheric chemistry models in an attempt to capture and characterize these interactions in a quantitative way (Kamens et al., 1981; Pankow et al., 1997; Kamens and Jaoui, 2001; Lee et al., 2004; Donahue et al., 2006; Hu and Ka-
mens, 2007). What has remained uncharacterised to this point is if – and how – these interactions affect the actual toxicity of each phase. While toxicological co-pollutant effects such as synergism, antagonism and the effects of pre-exposures have been investigated, much less is known about the direct effects of gas/particle interactions on the resulting toxicological risks resulting from changes in composition and dosimetry of both phases. Inclusion of these processes is necessary if the contribution of the atmosphere as a source of toxicity is to be modelled accurately in a laboratory setting.

Currently, most studies of health implications of PM exposures focus primarily on the toxicity of extracted PM itself or direct primary PM (e.g., Steenhof et al., 2011; Farina et al., 2011). This approach, while attractive to laboratory researchers, ignores the complexity of the air surrounding PM in the ambient environment, thereby missing the contribution of the atmosphere itself as a source of toxicity. The NRC states that “such approaches are likely to over-simplify the underlying biological processes and how the mixture of air pollutants that is inhaled adversely affects health” (NAS, 2004).

The absorbed dose of a VOC from the air is determined by both its lipophility and its ability to diffuse through water (McClellan and Henderson, 1995; Schwarzenbach et al., 2003; Salem and Katz, 2006). If airway cells are to absorb VOCs, the VOC must be transported to the air-liquid interface in the lung, where diffusion processes limit transfer of the VOC to the liquid layer. Compounds with high aqueous solubility will usually be taken out of the airway early. Conversely, compounds with low aqueous solubility may have very low absorption by the airway, and the bulk of the inhaled gas will be exhaled immediately. Dissolution into PM will not only deliver both of these classes of compounds to a region of the lung they would not normally enter, it will also hold them at an elevated concentration for an extended period of time, thereby increasing their overall dose to that region of the lung (and possibly to the body as a whole).

PM is able to bypass the diffusion-limited transfer of species across the aqueous layer above the surface of the cells in the airway. Bypassing this transfer brings partitioned VOCs into direct contact with the cellular membrane, thereby altering the
dosimetry of the VOCs. When species are delivered to the cell surface in this manner, simple diffusion will push the VOCs from the particle into the cellular membrane, where they can reside until they are able to diffuse into the cell body (Becker et al., 2003; Schwarzenbach et al., 2003). Once apolar and low-polarity organics become infused in the lipid layer of the cell membrane, they can resist cellular clearance processes. When VOCs enter the cell in this manner, they will most likely be free of endosomal membranes – giving them free access to all compartments of the cell, including the nucleus (McClellan and Henderson, 1995; Becker et al., 2003). If the VOCs are metabolised by cellular processes or diffuse across the capillary membrane into the bloodstream, it will simply shift the partitioning equilibrium to cause more of the toxicant to diffuse into the cell’s interior, thereby maintaining an elevated dose (Foster and Costa, 2005).

The purpose of this work is to demonstrate that physical/thermal processes among gases and particles can alter the delivery of air toxics to lung cells. This and subsequent studies (Ebersviller, 2012a) and (Ebersviller, 2012b) describe an integrated, step-wise approach to move from very simple systems to systems that approach the complexity of the ambient atmosphere. Altogether, these studies reveal the existence of inherent and acquired gas and particulate cellular toxic effects, and their dynamic entanglement, that is, they provide both chemical and biological effects data that demonstrate and defend the existence of PM “effect modification” as called for by the NRC.

2 Methods

2.1 Experimental design

Our total experimental design is divided into “simple” systems (systems in which no photochemistry occurs) and “complex” systems (in which species were photochemically oxidized in situ). The latter also include chamber injections of real emissions from a diesel engine operating with different fuels.
The results presented in Part 1 are based on the simplest system in which particle uptake of VOCs can be investigated. This simplicity is by design, and the main motivation behind creating such a highly simplified system is the desire to assign causality unambiguously. Thus, many competing processes have been deliberately removed to allow observations of the detrimental effects responses to PM exposure to be assigned to a very limited number of chemical and physical processes.

To reduce the number of factors that had to be investigated to explain observed effects on PM effects properties, a model PM material was selected. To be suitable for this study, the material had to produce a particle of sufficient complexity to be representative of atmospheric PM, but lack any inherent biological effects properties of its own. This approach is not an attempt to simulate the actual atmosphere, but to create a model with its inherent generalizations, distortions, and deletions, but still suitable to illustrate contributions to the acquired biological effects properties of both the gas and particle phases.

Our experiments are only performed when the minimum outdoor temperature is forecast to be at least 15.0°. In the work presented here, the measured temperature range that occurred across all experiments was 16.5–28.0°. For experiments in which the chamber needed to be humidified, the chamber was first flushed with the output of a clean air generator to purge background VOCs, and then humidified with HPLC-grade water to a dew point (DP) of at least 16.0°, but less than 19.0°. While this narrow range of DP may not represent the entire range of relative humidities that may be encountered in the ambient environment, it does limit variability in the system caused by humidity effects while still avoiding false-positives from biological effects endpoints caused by desiccation of the cells. To remove the possibility that either the VOCs or the PM used in these experiments might be modified by photochemical processes, all experiments were performed in the large outdoor chamber at night.

In vitro cell cultures were exposed to six types of air mixtures: clean air, clean air containing mineral oil aerosol (MOA), a single volatile organic compound (VOC) only (with no aerosol present), and a single VOC mixed with MOA. To explore the degree to
which volatility alters the uptake of VOCs by PM, single VOCs in both the volatile and semi-volatile range were selected (bringing the total number of air mixtures to six). No cells were exposed to both VOCs at one time. The goal of this work is not to compare the toxicity of specific VOCs and, as such, the biological responses of the lung cells will not be cross-compared between VOCs.

2.2 Chamber

UNC’s atmospheric chemistry groups use environmental irradiation chambers (smog chambers) to study the dynamics of the chemical and physical processes that occur naturally in the atmosphere (Jeffries et al., 1976; Kamens et al., 1981; Jeffries et al., 1985; Sexton et al., 2004; Doyle et al., 2007). The chambers allow complex atmospheres to be generated repeatedly in a controlled environment, but still aged in a more-realistic manner than has previously been available to toxicologists. More recently, the chambers have been coupled directly to two in vitro exposure systems capable of evaluating the relative toxicities of both the gas and particle phases of air pollution mixtures while maintaining the equilibria between the gas and particle phases throughout exposure (Doyle et al., 2004, 2007; Sexton et al., 2004; de Bruijne et al., 2009). Due to the dynamic nature of the atmospheric processes involved, the retention of these equilibria is vital to an accurate estimate of the effects of exposure to airborne pollutants. These characteristics, when combined, lend themselves to a very simple approach for investigating the direct modification of particle toxicity by the gas phase. The UNC smog chambers are, therefore, uniquely suited to explore the processes of interest here.

The studies described here used the Gillings Innovation Laboratory 120 m$^3$ chamber on the roof of the School of Global Public Health at UNC and the sampling and biological analysis laboratories located directly below it (Fig. 1). The chamber is equipped with a high-volume vent fan connected to a HEPA filter that removes PM from ambient air during large-volume flushing of the chamber. In addition, the chamber is connected to a clean air generator that scrubs moisture, VOCs and PM from air before it enters the
chamber. Pollutants to be studied (VOCs, PM, etc.) can be injected into the chamber through its floor. Chamber contents are monitored continuously via sample lines that pass through the floor of the chamber, the roof of the building, and into the laboratory below. The sample lines are made of a variety of materials to accommodate both the analytical and toxicological needs of the laboratory. The laboratory is equipped with a suite of analytical instruments that sample the chamber directly, allowing detailed analysis of its contents to be performed in real-time. For analytes that may be lost in long transfer lines, samples are collected directly below the chamber on the roof of the building.

2.3 Choice of gases and particles to test

Several volatile organic compounds were considered for inclusion in this study of gas/particle interactions. The VOC species chosen have been detected in our own chamber experiments involving complex VOC systems, and were also reported by Grosjean et al. in a study of carbonyl compounds measured at the inlet and outlet of a highway tunnel (Grosjean et al., 2001). Candidate species that were reported in multiple studies, or known to be of particular interest to air pollution researchers, were given preference in selection (Grosjean et al., 2001; EPA, 2012).

We selected p-tolualdehyde (TOLALD) to represent a semi-volatile species likely to be in both the gas and particle phases in the ambient environment (96 %; Sigma-Aldrich, St. Louis, MO). While p-tolualdehyde may not seem an obvious choice, it has several qualities to recommend it for this study. Along with its tendency to be present in both phases, it is a first generation oxidation product of vinyl-aromatic compounds and has an aldehyde function while retaining its aromatic function. We selected acrolein (ACRO) to represent a more-volatile species generally considered unlikely to be in the particle phase (95 %; Sigma-Aldrich, St. Louis, MO). In addition to ACRO being on the EPA’s “Dirty Thirty” list of toxicants, we have extensive experience using acrolein for both chemical and toxicological tests.
Mineral oil aerosol (MOA) was used as a surrogate for organic-containing ambient PM (pharmaceutical grade, 100%). Exposure to mineral oil elicits no acute biological effects from lung cells, making mineral oil an ideal PM material for this study. While most ambient PM has a mixture of elemental and organic carbon (EC/OC), the EC fraction is at the core of the particle and is (generally) completely surrounded by an OC layer. What this means is that any interactions between the PM and the gas phase happens via the OC fraction. In addition, mineral oil aerosolised with a large Collison nebuliser (May, 1973) has a size distribution similar to diesel exhaust particles observed in other chamber studies. For this study, MOA is a convenient and sufficiently complex surrogate for ambient PM that contains organic material.

Prior to use, all mineral oil was steri-filtered in the laboratory to remove any particulate or biological contaminants. In addition, the very large negative pressure required to pull the mineral oil through the small pores of the filter apparatus served as a mechanism by which to remove any dissolved VOCs which may alter the toxicity of the aerosol. The steri-filtered mineral oil was kept sealed and stored in a sterile laboratory to minimize the possibility that the filtered stock would absorb gaseous VOCs from laboratory air in our chemistry/exposure laboratory. For experiments in which particles were needed, a large-volume Collison nebuliser was used to aerosolise mineral oil directly into the chamber, to a concentration of ca. 1.4 mg m$^{-3}$, as determined by SMPS (described below).

### 2.4 Gas phase compositional analysis

The gas-phase composition and dilution rate of the chamber were monitored continuously by two Varian 3800 gas chromatographs (GCs), one with an electron capture detector (ECD) and the other with a flame ionization detector (FID). As stated above, the sample lines feeding the GCs travel from the floor of the chamber, through the roof of the building, and directly to the GCs in the laboratory below. The GC-FID has dual packed columns (one for light hydrocarbons, the other for heavy and aromatic hydrocarbons) and uses helium as a carrier gas. The GC-ECD has a single packed...
column, and is used for measurement of electron-accepting species. The GC-ECD uses a blend of argon and methane as a carrier gas. Each GC was equipped with an automated valve system that sampled the chamber contents every 30 min, and was run at a static temperature throughout sampling.

A Varian 3800 GC with Saturn 2200 mass spectrometer (MS) was used to analyse condensed-phase samples (such as function-specific or filter samples). The GCMS is also equipped with an FID, uses an RTX-5 fused-silica capillary column (60 m long, 0.32 mm ID, 0.5 µm film thickness; Restek US, Bellefonte, PA) and was operated under a temperature-optimized elution program with helium as the carrier gas. Initially, the MS was operated in EI mode, and the ion trap was set to collect all mass fragments from 40–650 mass units. When increased sensitivity was desired, the ion trap was programmed to retain fragments with a more-focused range of sizes.

2.5 Particle phase analysis

2.5.1 Scanning mobility particle sizer

During all experiments, the size distribution and mass concentration of the aerosols in the test atmospheres were monitored with a Scanning Mobility Particle Sizer (SMPS – TSI 3081 Differential Mobility Analyser (DMA) with TSI 3022A Condensation Particle Counter; TSI Inc., St. Paul, MN). The instrument quantified particles from 19 to 882 nm in diameter. The SMPS was set to scan continually on a 3 min-per-sample cycle (150 s up-scan, 30 s down-scan). The particle size distributions, as well as the number and mass concentrations, were logged continually for each experiment.

2.5.2 Filters

Teflon membrane filters (2 µm pore size, 47 mm diameter: Pall Corporation, New York) were used to collect particles at an average flow rate of 17.5 l m\(^{-1}\) for 1–4 h. Filters were collected concurrently with exposure periods. Static was removed from the filters
prior to weighing both before and after sampling. These procedures were carried out efficiently so that the filter was exposed to laboratory air as little as possible to minimize the loss of VOCs during weighing. After each filter was weighed, it was placed in a certified-clean sample jar, and spiked with Storage Internal Standard (IS) to characterize the loss of VOC with storage. Then each jar was sealed with Parafilm and placed immediately into a −20 °C freezer for storage until extraction and analysis. The aerosol mass concentration in the air was calculated by dividing the mass collected on the filter by the total volume of air sampled during collection. This value was used as a check against the value calculated from SMPS data.

2.5.3 Species-specific analysis of carbonyls

Filter samples were analysed by a modified protocol using o-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine chloride (PFBHA). PFBHA is used to selectively sample for carbonyl-containing organic compounds (Yu et al., 1995, 1997; Liu et al., 1999b,a; Healy et al., 2008). PFBHA selectively derivatises carbonyl-containing functional groups of organic species, with preference given to aldehydes and ketones over organic acids and esters. Mass spectra of PFBHA-derivatised carbonyl-containing compounds display a signature peak at \( m/z = 181 \) that is usually the base peak of the spectrum. In this study, filter samples were incubated under a PFBHA solution (after sample collection) to collect carbonyl-containing species from the condensed phase. Sample processing and analysis were then carried out as described before (Yu et al., 1995, 1997; Liu et al., 1999b,a; Healy et al., 2008). All filter samples from this study were analysed by GCMS (as stated above), using a large-volume injection liner to increase the sensitivity of the instrument (Termonia et al., 1988).

2.6 Biological effects model and measurements of response

Cultured human epithelial lung cells, type A549, were used as the biological receptor model in this study. A549 human respiratory cells are a commercially available cell...
line that has been shown to react in a manner that is representative of the human airway in vivo (Lieber et al., 1976; ATCC, 2011). While we conced that, in certain circumstances, primary lung cells may be a more desirable model than an immortalized cell line, A549 cells (which lack the individual-to-individual susceptibility variations of the primary lines) are used worldwide, allowing for easier replication of experiments, and are perfectly suited for this demonstration-of-principle work. The A549 cells were cultured and prepared for exposure as explained previously (Jaspers et al., 1997; Doyle et al., 2004; Sexton et al., 2004; de Bruijne et al., 2009).

The Gas In Vitro Exposure System (GIVES) and Electrostatic Aerosol in Vitro Exposure System (EAVES), described in more detail below, allow the cellular toxicity of the gaseous and particle phase components of the same air pollution mixture to be evaluated independently of one another, and with no substantive modification to the sample prior to exposure. This allows cells to be exposed to pollutants as they might be found in the ambient environment. These exposure systems, as models of the human deep lung, have their own generalizations, distortions, and deletions. These models are intended to be biosensors – a means by which to further characterize the distribution of species among the gas and particle phases, and to indicate which systems warrant further evaluation for harmful biological effects. This is measured by gauging cellular responses to treatment air exposures relative to clean air exposures.

Following exposure, all cell culture samples were allowed to express their responses for 9 h in clean air in an incubator. After the 9 h of expression, basolateral supernatants (media below the cells) were collected. All liquid samples were stored at −20 °C for later analysis of toxicological endpoints (see below).

### 2.6.1 Gas in Vitro Exposure System (GIVES)

Gas exposures from the chamber occur in air-tight, eight litre, modular cell-exposure chamber (Billups-Rothenberg, MIC-101TM, Del Mar, CA), as described before (Jaspers et al., 1997; Doyle et al., 2004; Sexton et al., 2004). Briefly, the modular chamber holds tissue culture plates containing Transwells (Costar, Cambridge, MA) for...
concurrent exposures. The inlet of the exposure chamber is connected to a sampling manifold coupled directly to the smog chamber on the roof (Doyle et al., 2004; Sexton et al., 2004; Doyle et al., 2007). Gravitational settling alone does not deliver sufficient PM to the surface of the cells to elicit a response (for the exposure durations used in this study). As such, only gas-phase toxicants are able to induce a response from cells exposed in the GIVES – even though the particles are never removed from the air stream prior to entry into the cell-exposure chamber. Exposure of cell cultures in the GIVES lasted 4 h.

2.6.2 Electrostatic Aerosol in Vitro Exposure System (EAVES)

The Electrostatic Aerosol in Vitro Exposure System (EAVES) is a necessary and sufficient sampler to test the basic hypothesis in this study. The EAVES is designed to expose human lung cells directly to particles without prior collection in media, thereby providing an efficient and effective alternative to more conventional particle in vitro exposure methods (de Bruijne et al., 2009; Lichtveld et al., 2012). Thus, although the air stream is not altered prior to entry into the EAVES, exposure to air pollution mixtures containing gas-phase toxic species, but that lack PM, do not elicit a response from cell cultures in this sampler (for the exposure durations used in this study). When PM that has cellular toxic properties is present in the air stream, however, there is a significant increase in response from exposed cells (relative to clean air controls) (de Bruijne et al., 2009). Cells exposed in the EAVES device are grown on Millicell membranes (Costar, Cambridge, MA) as described before (de Bruijne et al., 2009). The durations of exposure for membranes in the EAVES device were determined by the particle mass concentration in the air for each experiment, and lasted between 30 min and one hour. The exposure duration was varied to ensure equal deposition of PM on the cellular surface, while still providing ample time for a dose of PM to be delivered to the cells that was sufficient to elicit a response. For experiments in which no PM was present, the duration of exposure in the device was matched to the corresponding experiment with PM (i.e., TOLALD only was matched to the duration of exposure for TOLALD with PM).
2.6.3 Control exposures

In addition to each pollutant mixture listed above, cells were exposed to clean chamber air with each exposure system, as well as to incubator air. The response of the clean air exposure was compared to incubator controls to ensure that they were equal (as determined by a t-test, $p = 0.05$). The response of cells to clean air exposure was then used as a normalizing factor for the response of cells to each of the pollution mixtures.

2.6.4 Biological effects endpoints (cytotoxicity and inflammation)

Cytotoxicity is expressed as increase of lactate dehydrogenase (LDH) levels over clean air controls. LDH is only released by cells when the integrity of their cellular membranes have been compromised through either damage or failure (apoptosis). LDH levels were measured using a Cytotoxicity Detection Kit (TaKaRa Bio Inc., Tokyo, Japan). Interleukin 8 (IL-8) cytokine is an inter-cellular signalling protein that is released by cells in response to stimulus by an irritant. IL-8 release was evaluated using an ELISA kit (BD Biosciences, San Diego, CA). Though it has been noted previously that the presence of PM interferes with the proper functioning of ELISA kits (Seagrave et al., 2004), it was determined that this interference was not an issue with the PM used in this study. Inflammatory response is reported as increase of IL-8 levels over clean air controls.

Tables of response ratios accompany bar charts of endpoint measurements. Response ratios were calculated by dividing the cellular response to the exposure listed in the column heading of the table by the response to the exposure listed in the first column. For example, in Table 3, the gas-only response to TOLALD by itself (Exp. C) was 3.21 times higher than the clean air exposure (Exp. A). The gas-only response to TOLALD with MOA, however, was 0.83 (Exp. D). Therefore, in Table 3, the ratio of their responses is given by $(0.82/3.21) = 0.26$ meaning that the response of cells exposed to TOLALD with MOA by the gas-only method was roughly one quarter the response observed from cells exposed to TOLALD alone (or, the response for TOLALD was...
3.85 times greater than was observed for TOLALD with MOA). All results and further discussion is given below.

### 2.7 Statistical analysis

All calibration curves were tested by fitting a linear least squares line through the data. Calibration factors were calculated for each species to be analysed, with appropriate propagation of uncertainty. All instrument measurements are reported as the mean ±95% confidence interval, unless otherwise stated.

For toxicity endpoints, replicate tests of the same sample were averaged, and cell samples exposed to the same air mixture (i.e., incubator control, clean air control, GIVES exposure, etc.) were grouped for one-way analysis of variance (ANOVA - GraphPad Prism Software, La Jolla, CA). ANOVA analysis was used to determine whether there were statistically-significant differences between the cellular responses to each exposure mixture and their corresponding controls. Cellular response to insult is expressed as the mean ± standard error. The independent variable is exposure. Pair wise comparison (treatment vs. control) was performed as a subtest of the overall ANOVA. Group differences were considered significant if the test statistical type 1 error \( p \leq 0.05 \).

### 3 Results

#### 3.1 Gas phase compositional analysis

The gas-phase conditions during exposure, as well as particle mass concentrations, are summarized in Table 1. There were no VOCs detected in the chamber during either clean air exposure (with or without MOA). There was a slight variation in the concentration of TOLALD between exposures, though a difference of 178 ppbV should not be enough to cause a significant discrepancy in response from the cells. The ACRO...
exposures were more closely matched (a difference of 8 ppbV). Relative humidities (RH) are presented for each exposure mixture in the chamber air (where it might impact partitioning dynamics) as well as in the incubator (where variations in RH might affect cellular response). The uncertainty reported in Table 1 represents the 95 % confidence interval.

### 3.2 Particle phase characterization

The particle mass concentrations across all of the exposures in which no particles were added were close to the limit of detection for the SMPS instrument (as determined by the averages and 95 % confidence intervals reported in Table 3). The particle mass concentrations for the exposures in which MOA was added to the chamber were ca. 1500 times higher than the exposures without PM, well above the level of uncertainty (Table 1). No change in mode particle diameter was observed when VOCs and MOA were mixed in the chamber (relative to MOA in clean air; Fig. 2).

Analysis of filter samples was only possible for the portion of the study that used ACRO. Filters taken from the chamber contained no detectable ACRO when (1) MOA was tested in clean air and, (2) when ACRO was tested without PM. Filter samples taken from the chamber when it contained ACRO mixed with MOA, however, yielded an average ACRO mass of 1.87 ng per filter (corresponding to 1.06 ng ACRO per mg PM). Further discussion of these results is given below.

### 3.3 Toxicological response

Figure 3 is the key for the colour coding and annotations used in Figs. 4–7.

The relative inflammatory responses to the mixtures that contained TOLALD are given in Fig. 4. The only significant increases in response from cells exposed by the gas-only method were observed when TOLALD was placed in the chamber without MOA (Exp. C; Fig. 4a). This exposure resulted in 3.21 and 2.70 times more IL-8 production than the clean air and clean air with MOA (Table 3). The only significant
increase in response observed from cells exposed by the particle-only method was when TOLALD was mixed with MOA in the chamber (Exp. D; Fig. 4b, Table 3). The responses observed from our measure of cellular damage (LDH) resembled the inflammatory response, but with a greater magnitude in measured response (Fig. 5, Table 4).

Figures 6 and 7 show the relative response of lung cells to insult by the four air mixtures in which acrolein (ACRO) was used as the VOC of interest. As with TOLALD, a significant response was only observed when ACRO was present in the chamber (Exps. E and F). Likewise, the only significant response from cells exposed with the particle-only method was observed when ACRO was mixed with MOA (Exp. F; Fig. 6b). A similar trend was observed with the measure of cellular damage (Fig. 7). The only difference between the responses observed for the two endpoints (IL-8 and LDH) was that the cellular damage observed following exposure to ACRO with MOA was not significantly greater than the control (Exp. F; Fig. 7b). Response ratios are summarized in Table 5 and Table 6. Further discussion of results follows.

4 Discussion

This demonstration study is an effort to create a simplified and abstract model of processes that might occur in the ambient environment and human body. While simple, it provides us with results that would be difficult to detect directly in the ambient environment with human subjects. The latter conditions are subject to competing processes that, without insight, would likely mask the true causes of observed effects in these complex and open systems. The findings here permit us to apply the same causal reasoning resulting from this study to complex, photochemical systems with urban VOC mixtures and systems with real diesel PM; these additional findings are described in our two subsequent studies (Ebersviller, 2012a,b).

Even at the relatively high particle concentration in the MOA exposure in clean air, neither of the in vitro exposure systems registered an increase in toxicity from the clean air exposure with MOA present, demonstrating that MOA itself is not inherently toxic for
the endpoints we used (Exp. B; Figs. 4–7). This was true even though ca. 0.2 mg m$^{-3}$ more MOA than the average of the exposures with VOCs was present (Table 1). This lack of inherent acute toxicity will allow us to draw more concrete conclusions about the source of observed cellular responses to particle-phase exposures by removing the chance of false-positive responses due to any inherent toxicity of the particle phase. Moreover, the particle size distribution of each exposure atmosphere was continually monitored (Fig. 2), so we are able to rule out the effects of particle size variation on toxicity.

Efforts were made to exclude many transformative processes from this test system (i.e., the experiments were carried out at night to prevent the particles themselves from having their toxicity altered by direct reaction with sunlight). As stated above, this was by design, so that causation of observed effects could be assigned relatively unambiguously. As there was no chemistry occurring in the chamber, only physical (thermodynamic) processes were present. In addition, any effect that variations in RH might have had on the cells was minimal, as the lowest RH encountered in the study was observed during the Clean Air with MOA exposure. No response was observed from the cells in this exposure, so the likelihood of RH causing the increase in response observed from the VOC-containing exposures is negligible. Any increase in the toxicity of the particle phase was, therefore, solely due to the dynamic uptake of gas-phase toxics. This is a significant distinction to make, since the interplay between gases and particles occurs constantly in the ambient environment.

The increase in response (inflammation, as well as, cytotoxicity) observed in the particle exposure for the TOLALD with MOA system was consistent with theoretical expectations, as TOLALD was selected for its likelihood to partition favourably to the condensed phase (Exp. D; Figs. 4b and 5b, Tables 3 and 4). When MOA and TOLALD were both added to the chamber the response from the cells exposed with the gas-only method decreased by 71% (relative to TOLALD alone). We can infer from this decrease in gas-only response that enough gas-phase TOLALD has partitioned to the PM in the system to diminish the gas-phase toxicity.
The strong biological response from the cells exposed with the particle-only in vitro method clearly demonstrates that the toxicity of the MOA has been modified by gas phase TOLALD (Exp. D; Figs. 4b and 5b). In addition, the decrease in gas-phase response observed for TOLALD with MOA (relative to TOLALD alone), when coupled with this dramatic increase in response from cells exposed to the mixture of TOLALD and MOA with the PM-only in vitro system, indicates that the observed ‘transfer’ of toxicity between the phases is most likely due to the uptake of gaseous TOLALD by MOA. Any further discussion of the particle uptake of TOLALD is, however, hampered by the loss of the filter samples taken for chemical analysis. We can attempt to compensate for the loss of the filter samples by calculating an estimate of the TOLALD loading of the MOA using $K_p$ equations from Pankow (1997) and $K_{oa}$ equations from Schwarzenbach (2003). Thus, the amount of TOLALD we have calculated to be taken up by the MOA would be on the order of 1.87–2.77 ng TOLALD per µg MOA (summarized in Table 2). This means that, using measurements of collection efficiency for the EAVES device (de Bruijne et al., 2009), the amount of TOLALD that would be delivered to each cell culture insert in the particle-only exposure is calculated to be between 2.82 ng (using $K_p$) and 4.18 ng (using $K_{oa}$). As TOLALD is not considered hazardous (it is used as an aroma additive in food), we do not consider that the amount calculated to have been delivered to the cells should have been sufficient to cause such a large increase in the particle-phase toxicity. Likewise, we do not expect that removing the calculated 2.82–4.18 ng of TOLALD from the gas-phase should have been enough of a loss to diminish the response of the gas-phase exposures to the level of the clean air control. The loss of the filter samples has diminished our ability to explain our observations with chemical measurements, yet the biological response measurements support significant uptake of gaseous toxicity by the initially non-toxic PM.

To summarize: we have demonstrated that (1) MOA in clean air does not cause a response from exposed cells; (2) MOA, when mixed with TOLALD induced a large response from cells exposed with the particle-only method; and (3) we observed a significant decrease in response from cells exposed to TOLALD with MOA by the gas-only method.
method (relative to TOLALD alone). All of this suggests that, in an environment with realistic levels of humidity (such as we established in the chamber) theoretical calculations likely underestimate the uptake of TOLALD by PM (discussed further below).

Given that ACRO is targeted by the EPA for control on its “Dirty Thirty” list (EPA, 2012), the large response from the cells in the gas-phase exposure method is consistent with expectations (Exp. E; Figs. 6a and 7a). Also, given the volatility of acrolein, the lack of change in inflammatory response from cells in the gas-only exposure system when MOA was added was also consistent with expectations (Exp. F; Fig. 6a). The most intriguing results are apparent in the dramatic increase in response from the particle-only exposure method when MOA was mixed with ACRO in the chamber (Exp. F; Fig. 6b). Such a large increase in response (> 5×, Table 5) was unexpected, as ACRO is (according to partitioning theory) even less likely to be found in the particle phase than TOLALD. A similar, though somewhat less dramatic, trend was observed with our measure of cellular damage (LDH in Fig. 7). The one difference between the inflammatory response and cellular damage measurement was observed for cells in the gas-only exposure system when challenged with the mixture of ACRO and MOA. For this exposure, there was a significant inflammatory response, but no response from cells for the cellular damage marker (see Exp. F in Figs. 6a and 7a). In general, inflammatory responses occur at lower exposure levels than cellular damage, so this apparent incongruity could merely indicate that, while the insult was sufficient to elicit an inflammatory response from the cells, it was not enough to result in failure of the cellular membrane.

This outcome is analogous to our observations involving TOLALD (above), in which enough TOLALD partitioned to the MOA in the mixture to diminish the cellular response from the gas-only exposure to the level of clean air (Fig. 4a). Partitioning theory predicts that more ACRO persists in the gas-phase than TOLALD (ACRO is more volatile than TOLALD). Therefore, the persistence of the significant gas-only inflammatory response when MOA was added to the chamber agrees with theoretical predictions. It appears that enough ACRO partitioned to the PM present, however, to diminish the cellular
damage response relative to ACRO alone.

While it is possible that there is another explanation for the lack of cellular damage from the ACRO mixture with MOA (i.e., the PM altering the manner in which ACRO affects cells), the large inflammatory response observed for the same exposure makes our explanation highly probable. Future investigations into these phenomena will be able to look at the mechanisms of action for the observed toxicity and RH in greater detail.

The analysis of filter samples taken from the air mixtures of MOA and ACRO yielded surprising results. The mass of ACRO measured on the filters (0.93 ± 0.59 ng ACRO per mg PM) was ca. 1.8–2.5 times greater than predicted by partitioning theory (0.37–0.50 ng ACRO per mg PM; Pankow, Schwarzenbach; summarized in Table 2). One explanation for the higher-than-predicted concentration of ACRO in the particle phase is given by recent evidence that realistic levels of humidity can facilitate particle uptake for water soluble compounds (Parikh et al., 2011; Lim et al., 2010). While there is always the concern that a higher-than-expected concentration for an analyte might be from contamination, the filter samples were handled in such a way as to completely segregate them from the possibility of absorbing ACRO from the laboratory air. ACRO has, to our knowledge, never entered the laboratory in which all filter handling and processing was performed. Recent studies have reported detecting species with higher-than-expected volatilities in ambient PM, where they can undergo polymerization processes (Liggio and McLaren, 2003; Hu and Kamens, 2007; Cao and Jang, 2008; Zhou et al., 2011). ACRO, while not included in the study by Liggio and McLaren, has a volatility that falls within the range of those tested. Furthermore, ACRO is known to polymerize readily, especially in an acidic environment. Polycroine itself has been associated with negative human health outcomes, such as Alzheimer’s disease (Seidler and Yeargans, 2004; Liu et al., 2005; Seidler et al., 2006). Moreover, whatever the form acrolein may be taking in the condensed phase, the acute inflammatory response observed indicates that it is still capable of eliciting a biological effect.
If we assume that particles are more capable of picking up volatile species than expected, it does not necessarily indicate that current partitioning theory is invalid. For instance, if small molecules are easier to dissolve in the condensed phase than currently believed, the calculation of activity coefficients may simply need to be adjusted to account for these effects. In addition, recent findings indicate that humidity may play a larger role in affecting the interactions of PM and water soluble compounds than was previously expected (Parikh et al., 2011; Li et al., 2000; Lim et al., 2010). These statements are not meant to be an assertion that solubility will be found to account for the observed uptake, but simply as an illustrative example. This work is meant to be an indication of the direction that future work may go, rather than a definitive answer to the problem. The determination of the causes of these observations, as well as the adjustment to the calculation of activity coefficients, is therefore outside the scope of this work.

Traditionally, the toxicity of PM is estimated by collecting particles and then resuspending them in a liquid medium that can be instilled onto in vitro cells or into the lungs of test animals (Steenhof et al., 2011; Farina et al., 2011). Additionally, in toxicology studies, filter samples are routinely left exposed to laboratory air for extended periods of time and/or are autoclaved. These sample handling practices will result in the loss of volatile species from the particle phase and, by extension, most likely diminish effects such as those that we observed during this study.

5 Conclusions

These results have important ramifications across the air pollution field. This study has shown that atmospheric gases that are toxic can, through physical/thermal processes, cause non-toxic PM to become toxic. Due to deposition and clearance mechanisms in the human body, the “newly toxic” PM can act as a pre-concentrator and delivery mechanism to deliver a dose of the atmospheric gas to a region of the lung that might not normally encounter it. Atmospheric gases delivered to the lung in this way may have...
an increased biological effect relative to that of the gas-phase toxic species. This may help explain the inability of laboratory-based toxicologists to replicate effects seen in near-roadway epidemiology studies that has confounded the risk assessment field and policy makers alike (Dreher, 2000; Schlesinger et al., 2006). The time scale for these inter-phase dynamics is on the order of seconds. What this means in the real world is that vehicle emissions (and other PM) may not be exceptionally toxic as they enter the atmosphere but, when mixed into an aged or ageing air mass, can change dramatically in composition and biological effect by the time they drift off-road and reach the general population. Thus, an important consideration is the role of PM as a pre-concentrator and carrier of gas-phase toxics into regions of the lungs they may not normally reach, where the toxic load can be released even if the PM mass is mostly expelled.

While the creation of oxidized daughter products has long been investigated for its potential to produce ozone and PM, toxicological work has remained largely focused on single pollutants or primary sources of PM (e.g., Weldy et al., 2011; Tsukue et al., 2010). This work demonstrates that the dynamic shifting of toxicity from the gas phase to the PM present in the mixture is likely to be an important component to understanding the response of individuals exposed in the ambient environment. This dynamic shift is an example of what the NRC was referring to when asking for evidence of “effect modification” (NAS, 2004). Further, it identifies an important, currently neglected, source of PM toxicity, the reactive atmosphere itself. To incorporate the reactive atmosphere into toxicological studies fully, researchers must have a mechanism by which to photochemically age species in situ. Currently, very few biological exposure facilities have these kind of transformative capabilities available to them, resulting in exposure studies that miss the full complexity of the entanglements encountered in the ambient environment. What this means for their exposure studies is that they not only miss the full complexity of these entanglements, they likely miss important biological effects associated with PM that has been fully exposed to, and processed by, the atmosphere. It is these dynamic entanglements that we have shown here to be so important for cellular effects. Further demonstrations of these “effect modifications”, in more complex
and realistic atmospheric systems, are provided in Part 2 and Part 3 of our studies (Ebersviller, 2012a,b).

Acknowledgements. This research was supported in part by a grant from the National Institute of Environmental Health Sciences (P30ES010126) and in part by support from the Gillings School of Global Public Health’s Gillings Innovation Laboratory Program for Research and Innovation Solutions.

References


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Table 1. Exposure Conditions. All measurements represent the mean value ±95% confidence interval.

<table>
<thead>
<tr>
<th>Experimental Condition(^a)</th>
<th>Avg. VOC Conc. (^a) (ppmV)</th>
<th>PM Conc. (^a) (mgm(^{-3}))</th>
<th>RH Chamber(^c) (%)</th>
<th>RH Exposure(^d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Clean Air</td>
<td>ND(^b)</td>
<td>0.001 ± 0.001</td>
<td>47.8 ± 0.3</td>
<td>31.7 ± 0.1</td>
</tr>
<tr>
<td>B. Clean Air w/MOA</td>
<td>ND(^b)</td>
<td>1.561 ± 0.027</td>
<td>69.2 ± 1.0</td>
<td>30.1 ± 0.4</td>
</tr>
<tr>
<td>C. Clean Air TOLALD</td>
<td>2.012 ± 0.102</td>
<td>0.001 ± 0.003</td>
<td>73.8 ± 2.3</td>
<td>34.3 ± 1.0</td>
</tr>
<tr>
<td>D. Clean Air TOLALD w/MOA</td>
<td>1.834 ± 0.057</td>
<td>1.317 ± 0.007</td>
<td>68.6 ± 2.2</td>
<td>30.8 ± 0.8</td>
</tr>
<tr>
<td>E. Clean Air ACRO</td>
<td>0.937 ± 0.053</td>
<td>0.007 ± 0.001</td>
<td>70.9 ± 0.8</td>
<td>39.4 ± 0.1</td>
</tr>
<tr>
<td>F. Clean Air ACRO w/MOA</td>
<td>0.929 ± 0.095</td>
<td>1.415 ± 0.027</td>
<td>92.6 ± 0.4</td>
<td>32.3 ± 0.3</td>
</tr>
</tbody>
</table>

\(^a\) MOA is mineral oil aerosol, ACRO is acrolein, TOLALD is p-tolualdehyde, and RH is relative humidity

\(^b\) none detected

\(^c\) the RH of the air in the outdoor chamber (ambient temp.)

\(^d\) the RH of the air in the in vitro exposure systems (37.0°).
**Table 2.** Theoretical and observed values for partitioning of TOLALD and ACRO to PM.

<table>
<thead>
<tr>
<th>VOC</th>
<th>Theoretical Concentration</th>
<th>Observed Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_p^a$</td>
<td>$K_{oa}^b$</td>
</tr>
<tr>
<td></td>
<td>(ng VOC per mg PM)</td>
<td></td>
</tr>
<tr>
<td>TOLALD</td>
<td>1.87</td>
<td>2.77</td>
</tr>
<tr>
<td>ACRO</td>
<td>0.37</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>missing$^c$</td>
<td>0.93 ± 0.59</td>
</tr>
</tbody>
</table>

$^a$ Calculated from equations in Pankow 1997

$^b$ Calculated from equations in Schwarzenbach (2003)

$^c$ All samples were lost
Table 3. Ratios of measured inflammatory responses (IL-8) for in vitro human lung cell exposures to TOLALD mixtures, (Table 1 and Fig. 4).

<table>
<thead>
<tr>
<th>Gas-Only Exposure</th>
<th>Reference Condition*</th>
<th>A.</th>
<th>B.</th>
<th>C.</th>
<th>D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Clean Air</td>
<td>1.00</td>
<td>1.19</td>
<td>3.21</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>B. Clean Air w/MOA</td>
<td>1.00</td>
<td>2.70</td>
<td>0.70</td>
<td></td>
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<tr>
<td>C. Clean Air TOLALD</td>
<td>1.00</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Clean Air TOLALD w/MOA</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PM-Only Exposure</th>
<th>Reference Condition*</th>
<th>A.</th>
<th>B.</th>
<th>C.</th>
<th>D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Clean Air</td>
<td>1.00</td>
<td>1.00</td>
<td>1.17</td>
<td>2.63</td>
<td></td>
</tr>
<tr>
<td>B. Clean Air w/MOA</td>
<td>1.00</td>
<td>1.17</td>
<td>2.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Clean Air TOLALD</td>
<td>1.00</td>
<td>2.25</td>
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</tr>
<tr>
<td>D. Clean Air TOLALD w/MOA</td>
<td>1.00</td>
<td></td>
<td></td>
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</tbody>
</table>

* MOA is mineral oil aerosol, TOLALD is p-tolualdehyde.
### Table 4. Ratios of measured cellular damage (LDH) for in vitro human lung cell exposures to TOLALD mixtures, (Table 1 and Fig. 5).

<table>
<thead>
<tr>
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</thead>
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<tr>
<td></td>
<td>Reference Condition*</td>
<td>A.</td>
<td>B.</td>
<td>C.</td>
</tr>
<tr>
<td>A. Clean Air</td>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>5.55</td>
</tr>
<tr>
<td>B. Clean Air w/MOA</td>
<td></td>
<td>1.00</td>
<td>5.53</td>
<td>1.59</td>
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<tr>
<td>C. Clean Air TOLALD</td>
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<td>1.00</td>
<td>0.29</td>
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<td>D. Clean Air TOLALD w/MOA</td>
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<table>
<thead>
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<tr>
<td></td>
<td>Reference Condition*</td>
<td>A.</td>
<td>B.</td>
<td>C.</td>
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<tr>
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<td>1.37</td>
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<tr>
<td>B. Clean Air w/MOA</td>
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<td>1.00</td>
<td>1.11</td>
<td>3.20</td>
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<td>C. Clean Air TOLALD</td>
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<td>1.00</td>
<td>2.90</td>
<td></td>
</tr>
<tr>
<td>D. Clean Air TOLALD w/MOA</td>
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<td></td>
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<td>1.00</td>
</tr>
</tbody>
</table>

* MOA is mineral oil aerosol, TOLALD is p-tolualdehyde.
Table 5. Ratios of measured inflammatory responses (IL-8) for in vitro human lung cell exposures to ACRO mixtures, (Table 1 and Fig. 6).

<table>
<thead>
<tr>
<th></th>
<th>Reference Condition*</th>
<th>A</th>
<th>B</th>
<th>E</th>
<th>F</th>
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<tr>
<td>A. Clean Air</td>
<td>1.00</td>
<td>1.19</td>
<td>2.91</td>
<td>3.37</td>
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<tr>
<td>B. Clean Air w/MOA</td>
<td>1.00</td>
<td>2.45</td>
<td>2.83</td>
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<tr>
<td>E. Clean Air ACRO</td>
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<td></td>
<td></td>
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<tr>
<td>F. Clean Air ACRO w/MOA</td>
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<td></td>
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<tr>
<td><strong>PM-Only Exposure</strong></td>
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<tr>
<td>A. Clean Air</td>
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<td>0.98</td>
<td>5.45</td>
<td></td>
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<tr>
<td>B. Clean Air w/MOA</td>
<td>1.00</td>
<td>0.89</td>
<td>4.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Clean Air ACRO</td>
<td>1.00</td>
<td>5.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. Clean Air ACRO w/MOA</td>
<td>1.00</td>
<td></td>
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</tbody>
</table>

* MOA is mineral oil aerosol, ACRO is acrolein.
Table 6. Ratios of measured cellular damage (LDH) for in vitro human lung cell exposures to ACRO mixtures, (Table 1 and Fig. 7).

<table>
<thead>
<tr>
<th></th>
<th>Gas-Only Exposure</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Condition*</td>
<td>A. Clean Air</td>
<td>B. Clean Air w/ MOA</td>
<td>E. Clean Air ACRO</td>
<td>F. Clean Air ACRO w/ MOA</td>
</tr>
<tr>
<td></td>
<td>1.00 1.00 3.33 1.61</td>
<td>1.00 3.31 1.60</td>
<td>1.00 0.48</td>
<td>1.00</td>
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<table>
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<th>PM-Only Exposure</th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Condition*</td>
<td>A. Clean Air</td>
<td>B. Clean Air w/ MOA</td>
<td>E. Clean Air ACRO</td>
<td>F. Clean Air ACRO w/ MOA</td>
</tr>
<tr>
<td></td>
<td>1.00 1.22 1.08 2.20</td>
<td>1.00 0.88 1.79</td>
<td>1.00 2.04</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* MOA is mineral oil aerosol, ACRO is acrolein.
Fig. 1. Chamber schematic. Sample lines pass through the floor of the chamber, the roof, and then directly into the laboratory below. Filter sampling is performed on the roof of the building to minimize loss to the sample lines.
Fig. 2. MOA particle size distributions for Clean Air and single-VOC experiments. Particle mass concentrations reported in the inset were determined from TSP filter measurements.
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Fig. 3. Key for toxicology response plots. MOA is mineral oil aerosol, ACRO is acrolein, TOLALD is p-tolualdehyde.

* Indicates a statistically significant difference in response relative to clean air and the VOC w/ MOA
# indicates a statistically significant difference in response between the VOC alone and the VOC w/ MOA
φ indicates a statistically significant difference in response relative to MOA in clean air.
Fig. 4. The IL-8 inflammatory response of lung cells following exposure to four air mixtures. The response induced by exposure to (a) the gaseous components of each mixture and (b) the particle phase components of each mixture.
Fig. 5. Cellular damage following exposure to four air mixtures. The response induced by exposure to (a) the gaseous components of each mixture and (b) the particle-phase components of each mixture.
Fig. 6. The IL-8 inflammatory response of lung cells following exposure to four air mixtures. The response induced by exposure to (a) the gaseous components of each mixture and (b) the particle phase components of each mixture.
Fig. 7. Cellular damage following exposure to four air mixtures. The response induced by exposure to (a) the gaseous components of each mixture and (b) the particle-phase components of each mixture.