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In Vitro Exposures in Diesel Exhaust Atmospheres: Resuspension of PM from Filters Verses Direct Deposition of PM from Air

Kim M. Lichtveld[a,†], **Seth M. Ebersviller**[a], **Kenneth G. Sexton**[a], **William Vizuete**[a], **Ilona Jaspers**[b], and **Harvey E. Jeffries**[a,*]

[a]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[b]Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Abstract

One of the most widely used *in vitro* particulate matter (PM) exposures methods is the collection of PM on filters, followed by resuspension in a liquid medium, with subsequent addition onto a cell culture. To avoid disruption of equilibria between gases and PM, we have developed a direct *in vitro* sampling and exposure method (DSEM) capable of PM-only exposures. We hypothesize that the separation of phases and post-treatment of filter-collected PM significantly modifies the toxicity of the PM compared to direct deposition, resulting in a distorted view of the potential PM health effects.

Controlled test environments were created in a chamber that combined diesel exhaust with an urban-like mixture. The complex mixture was analyzed using both the DSEM and concurrently-collected filter samples. The DSEM showed that PM from test atmospheres produced significant inflammatory response, while the resuspension exposures at the same exposure concentration did not. Increasing the concentration of resuspended PM sixteen times was required to yield measurable IL-8 expression. Chemical analysis of the resuspended PM indicated a total absence of carbonyl compounds compared to the test atmosphere during the direct-exposures. Therefore, collection and resuspension of PM into liquid modifies its toxicity and likely leads to underestimating toxicity.

Introduction

The purpose of this study is to illustrate potential problems with a widely used atmospheric particulate matter (PM) sampling and *in-vitro* exposure method for assessing a particular PM's ability to elicit toxicant-induced biological effects in cultured human cells. The majority of *in vitro* studies examining the effects of PM exposure have used an extractive technique based on collecting ambient PM on filters, resuspending the particles in a liquid medium, and then adding them to a cell culture.[1–7] This method is referred to here as the "resuspended PM method". Yet, many studies of physical and thermal processes among PM

Address correspondence to Dr. Harvey Jeffries, University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Dept. Environmental Science and Engineering, 166 Rosenau Hall, CB #7431, |Chapel Hill, NC 27599-7431 USA. harvey@unc.edu.
†née de Bruijne

Supporting Information
In the supporting information section of this manuscript, the identifications of peaks and concentrations are listed in the tables for Figures 2 and 3. Table S1 is the hydrocarbon data corresponding to Figure 2 and Table S2 is the carbonyl data corresponding to Figure 3. This information is available free of charge via the Internet at http://pubs.acs.org.

and volatile and semi-volatile oxidized organic gases have shown that, in a reactive organic gas and PM system like the urban atmosphere, the exchange of mass between the gas and PM phases can be rapid and dynamic.[8–12] The understanding that PM can gain mass (and acquire toxic properties) from gas-to-particle processes seems to be accepted by toxicologists and health-researchers.[13–21] The concept that this mass is likely labile and can be lost from the PM when the gaseous environment is changed is missing from these studies.[22, 23] As an example of how fast mass may leave SOA aerosols, Kamens and Coe [24] measured effective rate constants for off-gassing of fluorene and phenanthrene for diesel soot particles of 0.51 s^{-1} and 0.37 s^{-1} (loss of 95% of on-particle mass in 6–8 s if gas removed). For pinonaldehyde, a seven carbon product of alphapinene oxidation, a rate constant for off-gassing from PM of 1.8 s^{-1} was used to successfully model SOA in outdoor chamber studies (loss of 95% of on-particle mass in < 2 s).[12]

Previously, we have developed a direct-sampling system capable of producing PM-only-exposures to cultured human lung cells while maintaining the equilibria among gases and particles.[25–28] This direct-sampling system has been used to demonstrate the evolution of gas-phase toxicity in photochemically aged urban-like environments[26–32] and recently has been used to demonstrate the rapid transfer of gaseous toxicity to initially non-toxic PM, making this PM then toxic to exposed lung cells.[33, 34] Other researchers have begun to develop direct methods for PM exposures.[35–41]

In this study, we compare *in vitro* responses produced by exposure to resuspended PM, which was collected on a filter from a reactive gas-PM air stream with responses produced by exposure to the PM that was directly deposited on cells, while still in equilibria, from the same reactive gas-PM air stream. We hypothesize that the separation of phases and post-treatment of filter-collected PM significantly modifies the composition and therefore the toxicity of the collected PM, resulting in a distorted view of the potential PM health effects.

Approach

To test the hypothesis, we created controlled test environments in an outdoor sunlit chamber by combining whole diesel exhaust (gases and PM) from either of two vehicles (old and new) with an urban-like, complex volatile organic carbon compound (VOC) mixture. To modify the injected primary PM to be like urban atmospheric PM by permitting secondary organic aerosol (SOA) growth, these mixtures were exposed to natural sunlight from sunrise to sunset. Each test environment was sampled with both of our direct-exposure systems, and filter samples of PM were taken at the same time for processing by the resuspension technique and subsequent exposure of lung cells to the resuspended PM. We have shown previously that gas-phase oxidative chemistry is a major source of gas-phase toxicity in these environmental systems. We have also demonstrated that this gas-phase toxicity can and does move to the particle-phase, increasing any inherent or primary PM toxicity.[33, 34] This study, therefore, focuses only on the PM-only DEM to compare with a comparable exposure for the PM-only resuspension method.

Materials and Methods

Overview

The schematic in Figure 1 shows the experimental and exposure setup. The Gillings Outdoor Irradiation Chamber is located on the roof of the Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC. The triangular-cross-section chamber has a volume of 120 m^3 (7.4 m by 6.0 m by 5.4 m high) enclosed in Teflon film walls. The chamber is coupled to the chemistry and biology laboratory on the top floor via parallel thermally-insulated sample lines through the roof.[25, 33, 34] In addition, provisions for filter

collection and for difficult-to-sample species (such as carbonyls) are available immediately under the chamber floor (on the roof of the building). In the top-floor laboratories, analytical instruments and *in vitro* exposure system are connected to the chamber, preserving both gases and particles together as they are conveyed directly to the cells during exposure.

The rooftop chamber also has a stainless steel exhaust-transfer manifold that can be connected to the tailpipes of vehicles operated in the parking lot adjacent to the building. A venturi-driven dilution blower as described by McDonald *et al.*[42] entrains, dilutes, and delivers hot tailpipe emissions directly into the rooftop chambers.

Diesel Exhaust and Urban VOC Injections into Outdoor Chamber

To generate two types of PM in complex VOC-reactive atmospheres, diesel exhaust (DE) was injected into the chamber in the dark from either a 1980 Mercedes-Benz model 300SD that lacked emission controls or a 2006 Volkswagen Beetle compliant with EPA's vehicle emission standards. Both vehicles were operated with commercially available low sulfur diesel fuel. Given the differences in age and emission controls, the two vehicles produced exhausts with different compositions. These vehicles represent real on-road sources that would be encountered in the real world.

Each vehicle was allowed to idle until the vehicles' temperature gauges reached normal operating conditions. The engines were then throttled to approximately 2700 revolutions per minute and the venturi-driven dilution blower was used to deliver the DE to the roof-top chamber. DE was injected into the chamber until the particle concentration reached ca.1.2 mg/m^3, as measured by a scanning mobility particle sizer (SMPS - model 3936L25, TSI, Inc., St Paul, MN). During the exposure and sampling period following the generation of the urban-like complex atmosphere (described below), particles in the range of 0.02 to 1.0 Om were measured with the SMPS. Diesel-exhaust-only chemical conditions are not very reactive, and thus urban-like VOCs must be added to create a more typical oxidative environment. A VOC mixture comprised of 54 VOCs (SynUrb54; based on an average of EPA's analysis of air samples from 39 cities) was added at a total concentration of 2.0 parts per million carbon (ppmC).[28, 33]

Photochemically reacting the DE+VOC mixtures creates multiple generations of oxidized daughter products *in situ*, many of which are unavailable commercially. For each vehicle, the contents of the chamber were allowed to react in natural sunlight from sunrise to sunset to create oxidized urban-like test atmospheres *in situ*. All filter sampling and direct exposures described here took place in the dark, after sunset.

Analytical Methods

The chemical and physical monitoring of the chamber was the same as that described by Ebersviller *et al.*[33] and will only be briefly summarized here.

Ozone was measured with a ML9811 series Ozone Photometer (Monitor Labs, Englewood, CO). Nitrogen oxides were measured with a ML9841 series NO_x Oxides of Nitrogen Analyzer (Monitor Labs, Englewood, CO). All chamber data were recorded with one-minute resolution using a data acquisition system connected to a computer. Prior to each experiment, the O_3 and NO_x meters were calibrated by gas-phase titration using a NIST standard NO tank and stable O_3 source.

A Varian 3400/2000 GC/MS, with both a MS and flame ionization detector to identify and quantify the species in each mixture, was used to continually monitor gas-phase hydrocarbon compositions of the exposure atmospheres. A non-reactive tracer, carbon tetra-chloride, was injected (2.5 μl) at the beginning of each experiment to permit analysis of the

dilution rate of the chamber. A Varian 3800 GC, with an electron capture detector, was used to monitor the gaseous tracer concentration.

Modified mister samplers, similar to those described by Seaman *et al.*[43], were used to determine the carbonyl content of the test atmospheres. Sampled carbonyls were detected with a O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) method.[43–46] The PFBHA derivatives were analyzed by gas chromatography/mass spectrometry (GCMS) on a Varian 3800 GC/Saturn 2200 Ion Trap MS.

Growth of Cell Cultures and Exposure Pretreatment

For both *in vitro* methods, the A549 cell line, which is derived from a non-small-cell adenocarcinoma of the human lung, was used to assess the biological effects of the ambient PM mixture. [47, 48] The A549 human epithelial cells were grown on collagen coated membrane supports (Millicell R-CM; Millipore, Cambridge, MA) in complete media (F12, 10% fetal bovine serum with antibiotics [0.01% penicillin/streptomycin] (Invitrogen, Carlsbad, CA) as previously described. [25] Several hours before exposure (after the cells reached confluency), the complete media was exchanged and replaced with serum-free medium (BSA) (F12K, 1.5 μg/ml bovine serum albumin with 0.01% penicillin/ streptomycin; Invitrogen, Carlsbad, CA). Immediately before exposure apical media was removed from all exposure and controls.[25–28, 33, 34, 49] All exposures with the direct sampler were performed at air liquid interface. Due to the method itself, ALI exposures are never possible using resuspended PM since you have to instill the medium plus PM to conduct the exposure.

We recognize that an immortalized cell line lacks the individual-to-individual susceptibility variations of primary lines. The goal of this work was not to compare inter-personal variation in responses, but to focus on the comparison of the two exposure methods. The immortalized cell line was, therefore, ideally suited for this work.

Resuspension Exposure Method

Chamber air was drawn through pre-weighed Teflon membrane filters (2.0 Om pore size, 47 mm diameter; Pall Corporation, Port Washington, NY) at 17.5 liters/min for three hours to collect particles. After collection, the filters were re-weighed on a microbalance (Mettler Toledo MX5, Columbus, OH) and then placed in a 50 mL centrifuge tube containing BSA media.[3] The tube was vortexed and sonicated to remove PM from the filter (as determined by visual inspection). Filters were then dried in clean petri dishes at room temperature, and re-weighed. To determine the total particle mass that was resuspended in BSA, the initial mass of the filter was subtracted from the difference between the post-collection filter weight and the dry weight after extraction in BSA. Using these methods, approximately 67% of the PM was removed from the filter surface. The mass removed from each filter was used to calculate doses for *in vitro* exposures. Resuspension controls were created by treating blank filters with the extraction procedure. All tubes containing resuspension solutions were covered with foil and stored at −20°C. All resuspended-PM exposures were conducted two months after collection.

To start the resuspension-PM *in vitro* exposures, the resuspended PM was added to the apical side of the cellular membrane and allowed to incubate for nine hours prior to isolation of RNA. All solutions were prepared to ensure that the volume instilled was the same for all resuspension exposures (274 μl; controls were exposed to the same volume of medium without the particles present). Resuspended-PM exposures were performed at 2.6 $\mu g/cm^2$ (the same amount delivered directly to the cells by the direct exposure sampler)[25]. An

additional resuspended-PM exposure was performed at 42.5 μg/cm^2 for a comparison with exposure concentrations used by other researchers.[1, 3, 4, 7]

Direct Deposition Exposure Method

Direct deposition of PM (ranging from 0.02 to 10 μm in diameter) on cultured human lung cells is accomplished by applying a charge to the PM in a flowing air stream and then using a repelling electric field above the cells to rapidly, but gently, deposit the PM directly on to the air-liquid interface of the cells.[25] The direct-exposure sampler used in this study is called the Electrostatic Aerosol in Vitro Exposure System (EAVES). This sampler operates in a tissue culture incubator maintained at 37 °C, as described previously. [25] Prior testing of the direct exposure EAVES sampler has demonstrated that in an hour long exposure: (1) no significant cytotoxicity or inflammatory mediator production occurs from cellular exposure to the electrical field; (2) no cellular response was observed due to the low ozone concentration produced by the corona wire; (3) no cellular response was observed due to the electrical charges added to the PM; (4) no secondary organic aerosol formation was observed from very-reactive VOCs flowing through the sampler; (5) no cellular response was observed following exposure to toxic gases without PM present; and (6) no response was observed when mixtures of toxic gases and PM pass through the sampler without the deposition field on, while a significant response was observed from the same mixture when deposition voltage field is applied.[25] Thus, the EAVES sampler can expose cells to PM while maintaining equilibria with the gaseous components of the atmosphere being sampled with no observable response caused by the operation of the device.

In this study, the EAVES sampler was operated in exactly the same manner (with no disruption of the equilibria between the gas and particle phase) as described by Ebersviller *et al.*.[33, 34] The duration of each exposure was ca. one hour.

Analysis of mRNA levels

Total RNA was isolated from the cells using Trizol (Invitrogen, Carlsbad, CA) nine hours post-exposure and stored at −20 °C until analysis, per the manufacturer's instructions. Evaluation of inflammatory response was measured with real-time, reverse-transcriptase polymerase chain reaction (RT-PCR) for expression of Cyclooxygenase 2 (COX-2) and Interleukin 8 (IL-8) as described previously.[50] COX-2 and IL-8 mRNA levels were normalized against a housekeeping gene (β-actin mRNA) and reported as "fold-increase" over control. Each sample was tested for mRNA expression with three replicates.

Statistical Analysis

All data are presented as the mean ± standard error from the mean and expressed as fold increase over the same measurements performed on a set of control cells maintained in the incubator throughout the exposure period. Data sets were analyze and compared using unpaired Student-t test with Welch's correction. Differences were considered significant when p was less than 0.05.

Results

Chemical Characterization of Chamber Contents

As the chamber contents were irradiated by sunlight most of the primary hydrocarbons oxidized, thereby producing carbonyl-containing secondary organics. These types of atmospheric reactions have been extensively studied and described in the literature.[28, 51, 52] Figure 2 contains two chromatograms showing chamber hydrocarbon concentrations. The top chromatogram shows VOCs detected after injection (but prior to sunrise) from the mixture of emissions from the 1980 Mercedes vehicle and 2.0 ppmC of SynUrb54. The

bottom chromatogram shows VOCs detected after sunset following day-long irradiation. The speciation data show that initially injected hydrocarbons have mostly been consumed by the end of the day, resulting in a lower number of peaks, and a smaller magnitude for the peaks that remain (2b). The numbers labeling the peaks correspond to the species listed in Table S1.

Table S1 summarizes the concentrations for each compound identified in the test atmosphere before sunrise and after sunset. This analysis is included to demonstrate that photochemical-transformation of the test atmosphere occurred. Some of the major compounds initially present included: isopentane, benzene, toluene, m-xylene, and 1,2,4-tri-methyl-benzene. By the end of the day, isopentane and m-xylene had been completely consumed, while the concentrations of benzene, toluene, and 1,2,4-tri-methyl-benzene decreased by 77.7, 70.1, and 95.0%.

Figure 3 contains a selective-ion chromatogram of the derivatized carbonyl samples taken in the dark (after day-long irradiation) from the 1980 Mercedes/SynUrb54 mixture. As stated above, the samples were taken with a mister apparatus and no attempt was made to separate the gases and PM prior to collection in the derivatization solution. Therefore, the chromatograms represent a mixture of the gaseous and PM-bound carbonyls that were present in the chamber. The numbers above the peaks in Figure 3 are identified by compound name in Table S2. Compounds that were present in the photochemically oxidized test atmosphere are formaldehyde, acetaldehyde, methyl ethyl ketone, 2-pentanone, 2-hexanone, glyoxal, and methylglyoxal.

We also determined the chemical composition of the resuspended PM material to help understand any observed differences in response between the exposure methods. Chromatograms in Figure 4 represent the same type of selective-ion-filtered chromatograms of derivatized carbonyls present in Figure 3, and demonstrate that there were almost no carbonyls detected in the PM samples. The peaks that did appear included: formaldehyde, the unreacted PFBHA reagent, and the internal standard (fluorobenzaldehyde), all of which were measured in the water and media blanks. Thus, the resuspension liquid did not contain any carbonyls from the filter-sampled PM.

Biological Effects of Exposures

Figures 5 and 6 represent COX-2 and IL-8 expression induced by the two exposure methods (resuspension and via direct deposition). Figure 5 shows the response to exposures conducted using the 1980 Mercedes with SynUrb54, and Figure 6 shows the results from the 2006 Volkswagen with SynUrb54.

Figure 5 shows that direct exposure of cells to the test atmosphere using direct deposition did induce a significant increase in both COX-2 and IL-8 expression. No response was observed following exposure to the resuspended PM at the same exposure concentration. A similar pattern was observed for the tests using the 2006 Volkswagen with SynUrb54 (shown in Figure 6). While exposure using direct deposition induced significant increases in gene expression a small decrease for COX-2 was observed from cells exposed to the same amount of PM by the resuspension method (2.6 $\mu g/cm^2$).

To facilitate comparison to resuspension exposure levels that are often used in the literature, we performed a resuspension exposure 16 times higher than those shown in Figures 5 and 6. Comparisons of the responses from cells exposed to 2.6 and to 42.5 $\mu g/cm^2$ of resuspended PM from the Mercedes with SynUrb54 are illustrated in Figure 7. The COX-2 expression was not increased over the control for either resuspension exposure. For IL-8 expression,

however, there was a two-fold increase in response compared to the incubator control at the 42.5 μg/cm^2 resuspension exposure level.

Discussion

The gas and particle test atmospheres created in this study were developed to model important chemical features needed to elicit toxicant-induced biological effects. For our study to successfully mimic the variety of gases and PM present in the ambient environment, we needed to create complex, urban-like mixtures of gases and PM that contained a large number of chemical species. Prior work with SynUrb54-only systems clearly shows that urban-like, gas-only atmospheres can create a large variety of gas-phase toxicants.[28, 33] For the purpose of the comparisons we are making here, it is sufficient to observe that: 1) chemical analyses of chamber contents show that many of the injected VOCs reacted and that a large variety of secondary and higher generation oxygenated organics (carbonyls and multi-functional carbonyls) were formed; 2) direct sampling of the PM from the chamber on to the cells led to exposure outcomes that showed significant response thereby demonstrating that the PM had toxic properties; and 3) therefore, it is reasonable to expect a positive response from other sampling methods and subsequent exposures to same type of cells..

Results by Ebersviller, et.al.[33, 34], using the same outdoor chamber and the EAVES sampler (in combination with a second gas-only exposure system), clearly demonstrated the transfer of gas-phase-produced toxicity to a non-toxic PM. The direct sampling and exposures that occurs in the EAVES sampler maintains the equilibria between the gases and particles in the exposure system, thereby preventing the loss of volatile species from the particle phase during the sub-second time it takes to put them on the cell surface. Thus, if toxic VOCs were partitioned to the PM, direct deposition sampling would deliver this PM carrying such toxic species to the cell surface, while these volatile species are likely lost in PM extracted with a filter and suspended in a liquid media.

To challenge the *in vitro* methods using emissions from two test vehicles provided a mechanism by which we could generate distinct test atmospheres, with different gas and PM compositions. The results indicated that, for the direct deposition exposures, both vehicles induced a 2-fold expression of COX-2, and the Mercedes produced twice the expression of IL-8 than the Volkswagen. This shows that the direct deposition sampling system can see a difference in toxicity from different vehicle emissions and that emissions derived from a vehicle with fewer emission controls in place produces more toxicity.

We have shown the photochemically reacted test atmospheres to be highly complex mixtures of multiple generations of daughter products that represent an approximation of the complexity of an ambient urban environment. Further, these *in situ* generated test atmospheres contain unidentified carbonyls and other oxygenates (e.g., epoxides), often at low-levels that are not commercially available for any other type of testing. Thus, this test environment allows us to make a more “holistic” assessment of the effects of the urban gaseous and PM environment.

The EAVES sampler does not modify the chemical composition of the sample prior to the delivery of PM to the cellular surface, as documented by de Bruijne née Lichtveld, *et al.*.[25] Therefore, all soluble and insoluble compounds surrounding the carbon core of the DE particle are retained and available to elicit a response from exposed cells. Likewise, PM size distributions are not modified.

Filter collection and extraction techniques are popular because of their ease of use and relatively low cost. Our data indicate that extensive sample handling and modification of the

PM during the resuspension method (illustrated in Figure 1). Furthermore, when we compare the composition of the carbonyls present during the direct deposition exposures to those measured in the resuspension media, it is apparent that the composition has been modified (Figures 3 and 4). Not only has the carbonyl composition been modified in the resuspension media, the carbonyl-containing species have completely disappeared. As reported by Cooney *et al.*, particles can be seen to agglomerate when resuspended in liquid.[53] Using SEM, they showed the mode particle diameter of the resuspended DE increased relative to non-suspended DE. In addition, particles in liquid settle very slowly at these particle diameters, with only the largest particles likely to reach the cellular surface. This means that during a resuspension exposure not all the particles will deposit onto the cellular surface to cause a response prior to RNA collection, making it difficult to accurately determine the amount of PM delivered to the cells.

Direct sampling deposition and exposure elicited a significant response from both test atmospheres. Based on previous experiments using photochemically-reacted SynUrb54 we expected to observe an increase in biological response relative to controls.[28, 33] All exposures using resuspended PM obtained during the corresponding direct exposure, however, failed to induce a positive response from cells at exposure concentrations equal to the direct method for either of the biological endpoints assayed. Although there was a small, but significant, decrease for COX-2 observed from cells exposed by the resuspension method it is likely due to a slight increase of cytotoxicity making the mRNA less stable.

For the resuspended PM, there was a small but significant response observed when 16-times the amount of PM mass was used for exposure. The observed response, however, was still only about 50% of that induced from the EAVES-exposed cells at 2.6 $\mu g/cm^2$. As described above, when using the resuspended technique we observed that the final material used in the resuspended exposure was missing toxicants from the test atmosphere (Figure 4). The many carbonyls present in the direct exposure (Figure 3), and the lack of any in the resuspended material (Figure 4), strongly suggests that the response from the larger applied resuspended-PM mass is not likely to be caused by the same toxicant as the direct exposure. Rather, any observed response maybe the result of inherent, nonvolatile, non-soluble components in the treated PM (e.g., metals) that persisted through the extensive modification during filter collection and resuspension stages.[33, 40] Furthermore, differences in biological effect between the ‘dirty’ and ‘clean’ vehicles could be detected by the direct deposition exposures, but not by the resuspended exposures. These results taken together are biological evidence that the resuspended PM has lost an important source of toxicity.

The goal of this study was to determine if the separation of phases and post-treatment of filter-collected PM from a reactive gas-and-PM airstream would significantly modify the toxicity of the collected PM compared to that detected by direct exposure of the same PM. Chemical measurements during the photochemical aging process assured that primary hydrocarbons were consumed and that a large variety of gas-phase carbonyls and other oxidized products were produced, many of which are likely to partition to the PM.[33, 54] The same analysis of resuspended PM, however, showed that no carbonyls or other oxidized organics, other than those in the water and media blanks, were present. This is chemical evidence that filter-collected and resuspended PM has lost an important source of cellular toxicity. As stated above, neither chemical composition nor PM size distribution was modified prior to exposure by the EAVES sampler. Also, by exposing at an air-liquid interface the EAVES sampler delivers all PM, regardless of size, to the cellular surface. Moreover, with the EAVES sampler, we are able to reliably estimate the amount of PM that actually reached the cellular surface by using collection efficiencies measured during initial development of the method.[25]

Extrapolating our test conditions to the ambient environment, we conclude that it is highly likely that the separation of phases and post-collection treatment of filter-collected PM significantly modifies the toxicity of the resuspended PM. These modifications may result in a distorted view of the potential health effects compared to those elicited by direct sampling and exposure to the PM. While researchers may be attracted to the potential for high-throughput studies with resuspension techniques, we have demonstrated that sample modifications by the loss of toxicity are likely to bias the results. We recommend that resuspension techniques, therefore, be used with caution, and suggest that results obtained may represent only a lower bound on PM toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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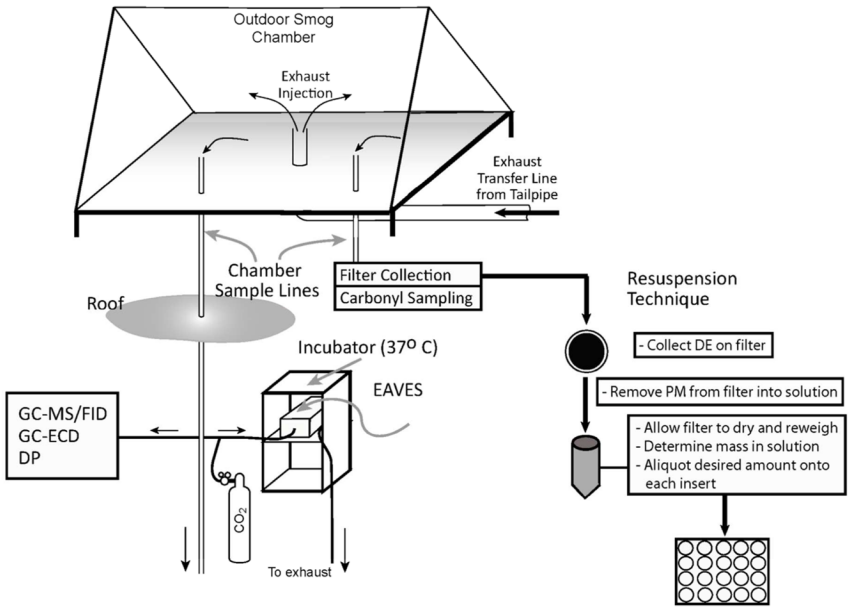

Figure 1.

Schematic diagram of the outdoor smog chamber, the laboratory sampling systems, and the biological exposure system (EAVES). Filters were collected directly under the chamber floor and processed post-collection for the exposures to resuspended PM. The diesel exhaust sample injection lines into the chamber are also depicted.

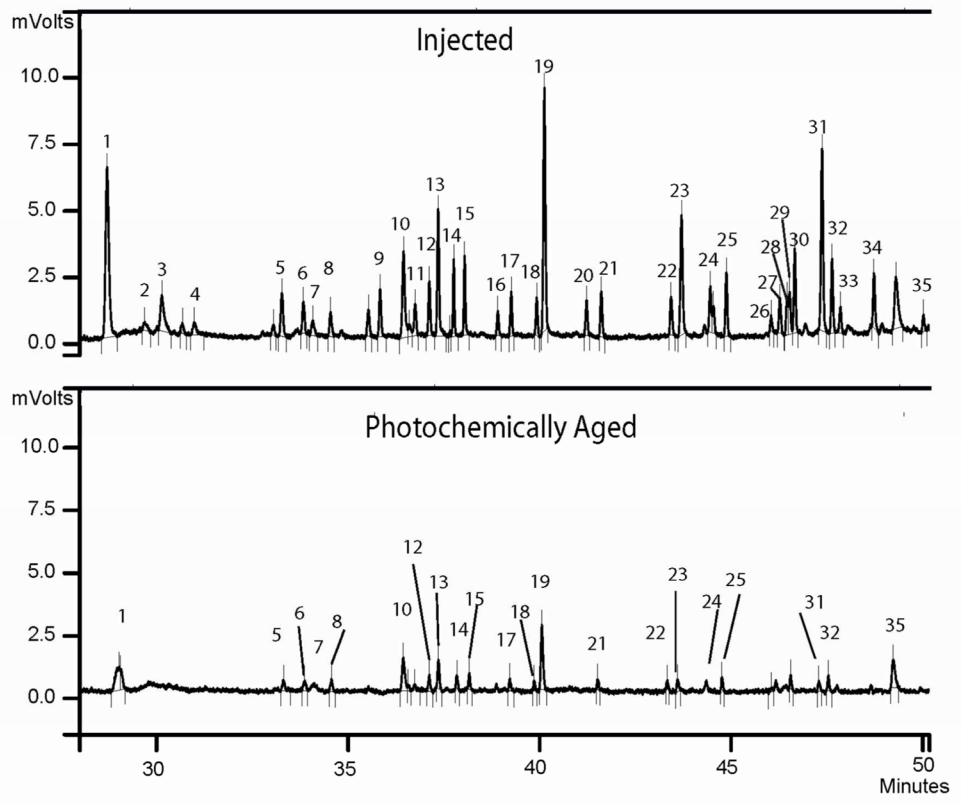

Figure 2.

Gas chromatogram of VOC species in outdoor chamber. Top: after injection of exhaust from 1980 Mercedes (50 seconds) and 2.0 ppmC injection of SynUrb54 mixture, but before sunrise. Bottom: in dark after daylong sunlit reaction. The peak numbers correspond to the species listed in Table S1. Major compounds present in the chromatogram labeled injected were: 1 – iso-pentane, 10 – benzene, 19 – toluene, 23 – m-xylene, and 31 – 1,2,4-tri-methyl-benzene. After photochemical aging the species either completely reacted away or had significantly decreased.

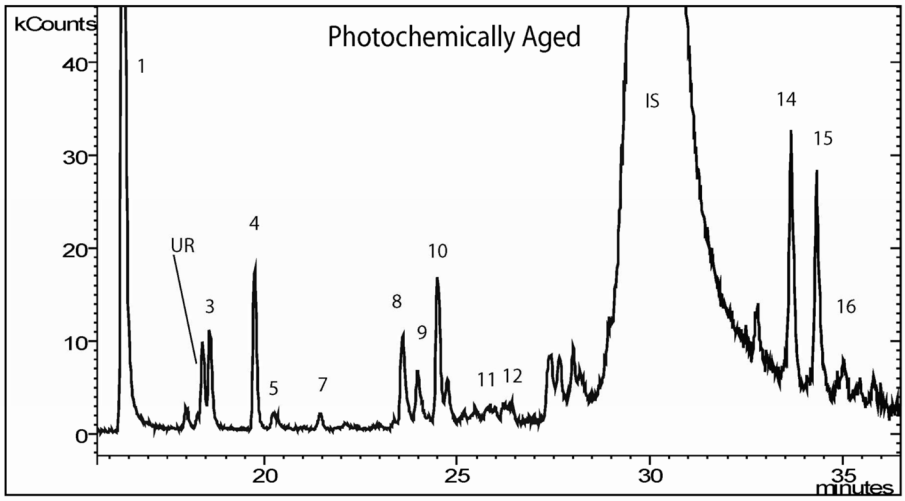

Figure 3.

Selective-ion chromatogram for the injections from the 1980 Mercedes and 2.0 ppmC of SynUrb54 in the chamber. of carbonyls from mister samples. Samples were collected after daylong irradiation. The peak numbers correspond to the species identified in Table S2. UR – Unreacted PFBHA solution, IS – Internal Standard, and some of the major peaks are 1 – formaldehyde, 3 – acetaldehyde, 6 – methyl ethyl ketone, 8– 2-pentanone, 10 – 2-hexanone, 14 – glyoxal, and 15 – methylglyoxal.

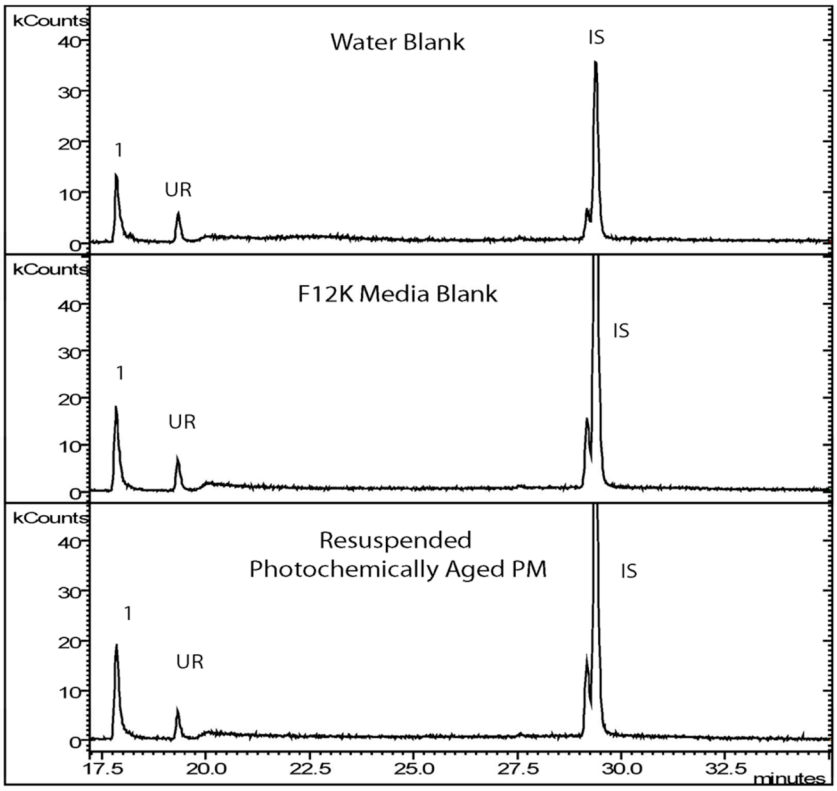

Figure 4.

Selective-ion chromatogram of carbonyls from a water blank, a F12K media blank, and a filter sample taken after day-long irradiation of the injections from the 1980 Mercedes and 2.0 ppmC of SynUrb54 in the chamber. The peak numbers over the peaks correspond to the identities in Table S2. UR – Unreacted PFBHA reagent, IS – Internal Standard.

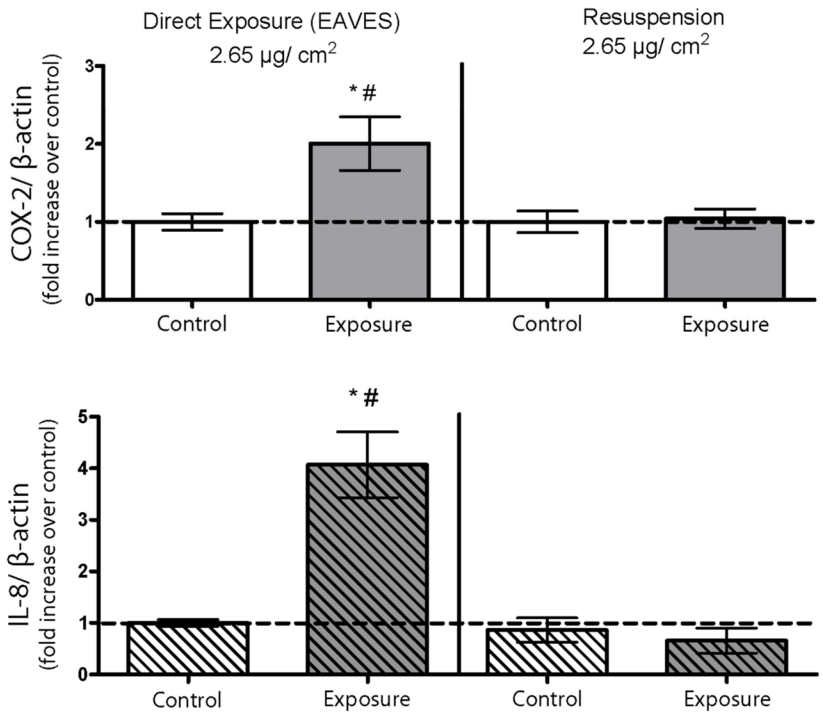

Figure 5.

COX-2 and IL-8 m-RNA expression induced by exposure to directly deposited PM from the aged exposure in the 1980 Mercedes with SynUrb54 experiment and induced by resuspension exposures. The symbols * indicates statistically different from non-exposed incubator control; # indicates statistically significant difference compared to resuspension exposures. The error bars represent the mean ± standard error from the mean.

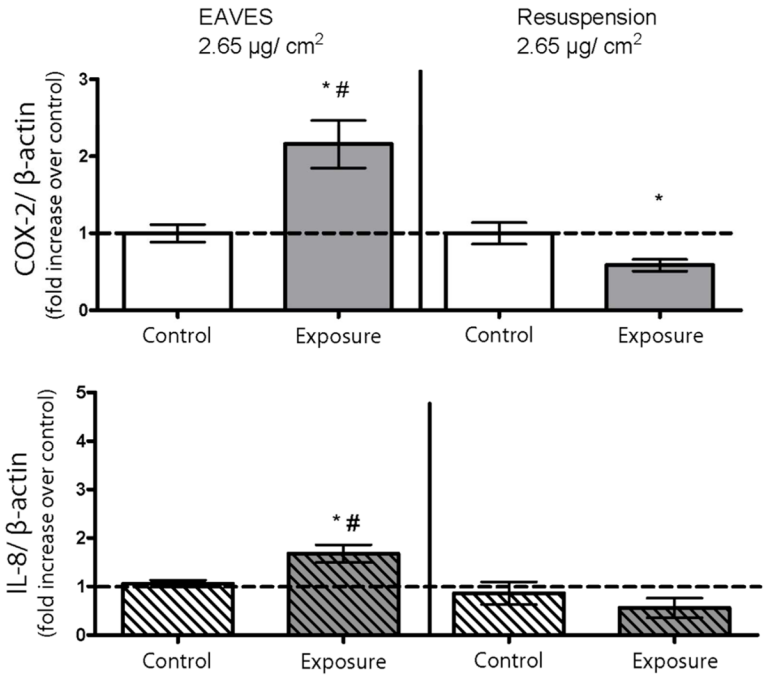

Figure 6.

COX-2 and IL-8 m-RNA expression induced by exposure to directly deposited PM from the aged exposure in the 2006 Volkswagen with SynUrb54 experiment and induced by resuspension exposures. The symbols * indicates statistically different from non-exposed incubator control; # indicates statistically significant difference compared to resuspension exposures. The error bars represent the mean ± standard error from the mean.

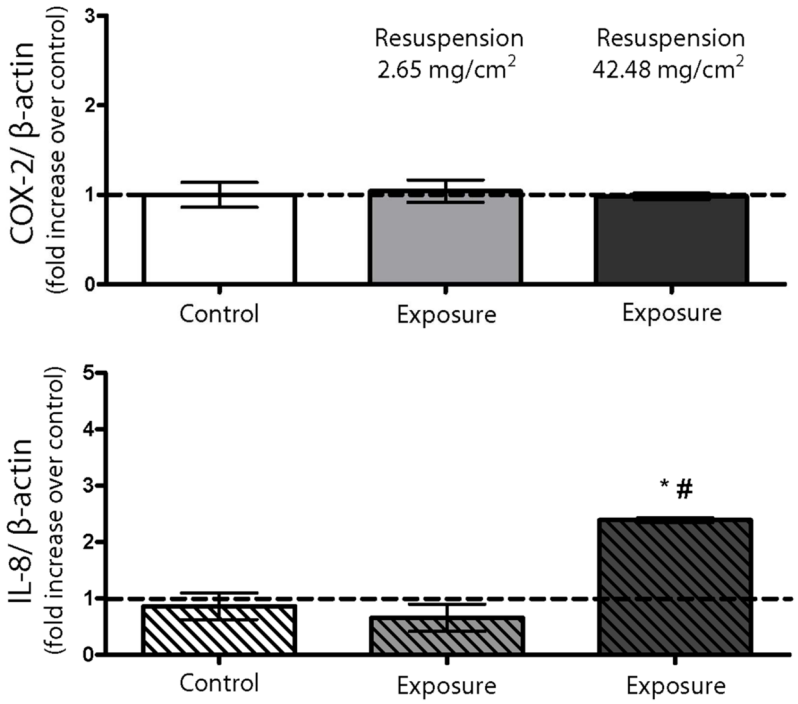

Figure 7.

COX-2 and IL-8 m-RNA expression induced PM from the AGED Exposure in the 1980 Mercedes with SynUrb54 experiment from resuspension exposures at concentrations of 2.65μg/cm^2 and 42.48μg/cm^2. The symbols * indicates statistically different from non-exposed incubator control; # indicates statistically significant difference compared to resuspension exposures. The error bars represent the mean ± standard error from the mean.