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In vitro and *in vivo* toxicity of urban and rural particulate matter from California

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Abstract

Particulate matter (PM) varies in chemical composition and mass concentration based on location, source, and particle size. This study sought to evaluate the *in vitro* and *in vivo* toxicity of coarse ($PM_{10-2.5}$) and fine (PM_{25}) PM samples collected at 5 diverse sites within California. Coarse and fine PM samples were collected simultaneously at 2 rural and 3 urban sites within California during the summer. A human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) was exposed to PM suspensions (50 μg/mL) and analyzed for reactive oxygen species (ROS) after 5 hours of treatment. In addition, FVB/N mice were exposed by oropharyngeal aspiration to 50 μg PM, and lavage fluid was collected 24 hrs post-exposure and analyzed for total protein and %PMNs. Correlations between trace metal concentrations, endotoxin, and biological endpoints were calculated, and the effect of particle size range, locale (urban vs. rural), and location was determined. Absolute principal factor analysis was used to identify pollution sources of PM from elemental tracers of those sources. Ambient PM elicited an ROS and pro-inflammatory-related response in the cell and mouse models, respectively. These responses were dependent on particle size, locale, and location. Trace elements associated with soil and traffic markers were most strongly linked to the adverse effects *in vitro* and *in vivo*. Particle size, location, source, and composition of PM collected at 5 locations in California affected the ROS response in human pulmonary endothelial cells and the inflammatory response in mice.

Keywords

Aspiration exposure; *in vitro* exposure; particulate matter; reactive oxygen species; inflammation

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Competing interests

The authors declare that they have no competing interests.

1. Introduction

Ambient particulate matter (PM) has been associated with excess cardiovascular mortality (Dockery et al. 1993), lung cancer (Pope et al. 2002), and cardiac arrhythmias (Peters et al. 2000). Such effects vary in strength with sampling location, season, and size fraction (Zanobetti and Schwartz 2009). Due to the public health effects associated with PM exposure, PM is regulated by the U.S. Environmental Protection Agency (EPA) and the World Health Organization (WHO) based on total mass concentration and particle size range, regardless of PM composition.

The particle size distribution of PM in the ambient atmosphere is related to the sources from which they are generated and governs their deposition into the respiratory system. Coarse PM ($PM_{10-2.5}$, i.e. particles with aerodynamic diameter between 2.5 and 10 μm) tends to originate from resuspended dust, coal/oil fly ash, and mechanical shearing. Bioaerosols, such as endotoxin, are also elevated in this particle size fraction (Monn and Becker 1999). When inhaled, coarse particles deposit in the upper respiratory tract and conductive airways of the lungs. Fine PM ($PM_{2.5}$, i.e. particles with aerodynamic diameter less than 2.5 μm) in contrast, originates from fuel combustion, biomass burning, and secondary particles. These particles can deposit within the gas exchange region of the lungs (Valavanidis et al. 2008). Importantly, the chemical composition and concentration of PM has been found to vary (Brunekreef and Forsberg 2005) and is dependent upon the PM source, sampling location, time of year, and size fraction (Cassee et al. 2013).

In the past it has been assumed that, due to their inability to penetrate into the gas exchange region of the lung, coarse PM elicited only limited adverse health effects in humans. This paradigm does not account for observations of health associations that have been related to coarse PM exposure, including pro-inflammatory effects in the lungs (Wegesser and Last 2008), cardiovascular mortality (Malig and Ostro 2009), and decreased heart rate variability (Lipsett et al. 2006). On the other hand, some studies have not been able to make a clear association between coarse particles and adverse health effects (Peng et al. 2008) and currently, while overall PM_{10} is regulated by the EPA and WHO, coarse $PM_{10-2.5}$ is not.

The aim of this study was to evaluate the relative *in vitro* and *in vivo* toxicity of 2 different PM size fractions (coarse and fine) at rural and urban sites in California. In addition, PM that has an aerodynamic diameter greater than 10 μm (defined as super-coarse PM, $PM_{>10}$), a poorly studied size fraction of ambient particles that does not generally penetrate into the thorax, was also evaluated for 1 rural and 1 urban sampling location. Furthermore, we sought to determine whether our *in vitro* findings were consistent with *in vivo* effects by exposing mice to the same PM size-fractionated samples. Trace element concentrations were evaluated for correlations with toxicity-related endpoints and used in factor analyses to assess the sources contributing to the PM chemical composition and the association between sources and adverse effects.

2. Materials and methods

2.1. PM collection

2.1.1. Sampling—Ambient coarse and fine PM samples were simultaneously collected every 48 hours (weekdays) or 72 hours (weekends) for 1 month at 5 locations in California during the summer of 2009. Three urban site locations (Davis, Clovis, Bakersfield) and 2 rural site locations (Trinidad, Tranquility) were selected for this work (more detailed site descriptions can be found in the Supplemental Material). Between 11 and 15 samples/location were collected for each size fraction, for a total of 133 fine and coarse PM samples available for subsequent biological and chemical analyses. Field blank samples were collected at each location.

The PM samples were collected simultaneously with high volume cascade impactors (ChemVol model 2400, BGI, Inc., Waltham, MA). All collection substrates were stored at −20 °C after collection. Prior to, and following each collection, the PUF substrates were weighed using standard operating procedures in an environmentally controlled room. The tare and gross weights were measured on a microbalance (XS105 DualRange, Mettler-Toledo, Columbus, OH) to determine the amount of PM collected and collection efficiency. Detailed PM collection methods and techniques involved in the sterilization of the impactors and collection substrates can be found in the Supplemental Material.

2.1.2. Recovery of PM extracts and characterization—Fine, coarse, and super-coarse PM (n = 154 PM samples total) were extracted with 70% ethanol and Milli-Q water as described previously (Mirowsky et al. 2013). The extracted PM components, made up of soluble and insoluble material, were transferred to labeled, pre-weighed, sterile, polypropylene containers. These containers were placed in a −20°C freezer overnight and transferred to a −70°C freezer until they were lyophilized and resuspended in water. Aliquots were stored in sterile cryovials at −70°C.

Filter extracts of soluble and insoluble material were microwave digested and analyzed by inductively coupled plasma mass spectroscopy (ICP-MS) for trace element analysis as previously described (Mirowsky et al. 2013) with detailed methodology presented in the Supplemental Material. Briefly, the samples were digested with a 2-step microwave program using a MDS-200 Microwave System (CEM Corp., Worcestershire, UK). Samples were analyzed on an Axiom single-collector magnetic sector ICP-MS (VG Elemental, Winsford, UKProcedural blanks and NIST 1648 reference samples were incorporated into every digestion procedure. Appropriate procedural blank subtractions were made as previously described (Kinney and Thurston 1993).

2.1.3. Endotoxin concentration—Endotoxin concentrations were determined by a kinetic Limulus Amebocyte Lysate (LAL) assay (Lonza, Walkersville, MD) for a subset of samples (n = 57 samples), as previously described (Mirowsky et al. 2013). This subset of samples was determined by selecting the 2 highest and 2 lowest coarse PM responders from the *in vitro* ROS experiments. The corresponding fine and super-coarse PM samples for those locations were also analyzed for endotoxin.

2.2. In vitro exposure

2.2.1. Cell culture—A human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) was provided by Drs. James Kirkpatrick and Vera Krump-Konvalinkova at Johannes Gutenberg University (Mainz, Germany). Cells were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide and grown in Endothelial Growth Medium (EGM-2) containing 1% penicillin/streptomycin (Gibco, Grand Island, NY) and supplemented with an EGM-2 BulletKit and 5% fetal bovine serum (Lonza).

2.2.2. ROS assay—The oxidative potential of all the PM samples and field blanks (n = 154 samples) was determined using a 2′,7′-dichlorofluorescein diacetate (DCFH-DA) assay. Cells were seeded at 5,000 cells/well in black 96-well plates and grown to confluence (~2 days). 100 μL of 10 μM DCFH-DA (Invitrogen, Carlsbad, CA) in phosphate buffered saline (PBS) was added to each well and incubated for 30 minutes at 37°C. The dye-loaded cells were washed twice with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) media (Gibco), and 100 μL fresh DMEM/F12 media was added. 25 μL of 250 μg/mL PM samples, sonicated for 20 minutes and containing both insoluble and soluble components, were added to wells (in triplicate) for a final concentration of 50 μg/mL PM. Fluorescence measurements were taken at 0 hr and 5 hr using an automated plate reader (Spectra Max M2e, Molecular Devices, Sunnyvale, CA) at excitation/emission wavelengths of 485/535 nm. Fluorescence at 0 hr was used as baseline, and ROS production was calculated as the increase in fluorescence intensity over time. Positive controls (metal-rich fireworks PM) and negative controls (water) were included in each plate. To compare the toxicity of super-coarse PM to the other size fractions, additional plates of cells were treated with representative samples of PM collected on the same day. The concentration of PM chosen for this study was based on previous work in this laboratory in which 50 μg/mL PM was the highest non-toxic dose determined using a lactate dehydrogenase assay (Lippmann et al. 2013).

2.3. Animals

All fine and coarse PM samples were analyzed for lung inflammation and injury in mice. Additional mice were treated with samples of super-coarse PM collected on the top stage of the ChemVol sampler at a rural (Tranquility) and urban site (Bakersfield).

2.3.1. Animal care—Eight to ten week old male and female FVB/N mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred at New York University (NYU). Mice were housed in polycarbonate cages with corn-cob bedding in temperature (20–23°C) and humidity controlled HEPA-filtered rooms with a 12 hour light/dark cycle. Animals were provided standard chow and water *ad libitum*. All animal procedures and handling were performed under the NIH and Animal Welfare Act guidelines for the ethical treatment of animals, and under a protocol approved by the NYU School of Medicine Institutional Animal Care and Use Committee.

2.3.2. Oropharyngeal aspiration and bronchoalveolar lavage—An oropharyngeal aspiration technique (Rao et al. 2003) was used to disperse the insoluble and soluble PM into the lungs of mice (n = 3 per group). Groups either contained 2 females and 1 male, or 2

males and 1 female. Previous work at NYU has shown that both genders of FVB/N respond similarly to aspirated particles (unpaired t-test of %PMNs comparing males and females; p = 0.35). Briefly, mice were anesthetized with isofluorane until breathing slowed to approximately 1 breath/second and placed on a 45° angle board. 50 μL of sterile water (negative control) or 50 μL of 1 mg/mL PM from collection sites or field blanks suspended in sterile water was placed at the base of the tongue just before a deep inhalation, for a total dose of 50 μg/mouse. Animal weight was not correlated with % PMNs measured (Spearman Correlation Coefficent between % PMNs and weight = 0.05, p = 0.33).

Twenty-four hours after aspiration animals were sedated with an intraperitoneal (ip) injection of Ketamine at a concentration of 0.1 mg/g of body weight and euthanized 0.26 mg/g of ip sodium pentobarbital. The lungs were lavaged twice using 1.2 mL PBS and cell counts, cell differentials, and total protein (BCA Protein Assay Kit, Thermo Fisher, Waltham, MA) were measured in lavage fluid. Cell counts were performed on the recovered lavage using a standard hemocytometer and differential cell slides were made using a cytospin and stained.

2.4. Statistical analyses

Prism 5.0 (GraphPad, San Diego, CA) was used for analysis of the *in vitro* and *in vivo* response data and correlations, which are reported as means ± standard error (SE), unless otherwise noted. Data were tested for normality using the Kolmogorov-Smirnov test and then analyzed using Student's unpaired t-tests. For experiments using more than 2 groups for analysis, one-factor analysis of variance (ANOVA), followed by Tukey's Multiple Comparison Test, was used. Correlations were performed between trace element and endotoxin concentrations and *in vitro* and *in vivo* responses using Pearson's Correlation Coefficient. Statistical significance was set for $p \leq 0.05$. For the ROS response, the mean water vehicle blank was subtracted from the change in fluorescence intensity on each plate. For the %PMNs and protein, responses of control animals given a water aspiration were subtracted from the responses of the exposed mice. When analyzing by locale, values obtained from the urban (Davis, Bakersfield, Clovis) and rural locations (Trinidad, Tranquility) were averaged prior to analysis.

Principal factor analysis of the trace element data with Varimax rotation was used to identify pollution sources of coarse and fine particles. This analysis was based on the correlation matrix of trace element concentrations obtained using ICP-MS, and the loadings of (i.e. correlations with) each element concentration on common factor (pollution sources) and corresponding factor scores were calculated. For this model, 15 trace metals and 128 samples were included. Super-coarse PM samples were excluded from this analysis as they were only collected at two locations (Tranquility and Bakersfield), and ten coarse and fine PM samples did not have detectable concentrations and were thus excluded from this analysis. The variables included concentrations of: S, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Sb, and Pb. SAS (version 9.3; SAS Institute, Cary, NC) was used for factor analysis.

3. Results

3.1. PM characterization

Among the California locations, the mass concentration of $PM_{2.5}$ was greatest at Bakersfield and Clovis, while the ocean-side Trinidad site had the lowest concentration of $PM_{2.5}$ (Table 1). Trinidad had the greatest concentration for $PM_{10\text{-}2.5}$, though elevated levels of $PM_{10\text{-}2.5}$ were also observed at Bakersfield and Clovis, relative to other sites. Tranquility had similar mass concentrations for the $PM_{2.5}$ and $PM_{10\text{-}2.5}$ fractions. The super-coarse PM fraction was greater at Bakersfield compared to Tranquility, although there was great variability in day-to-day mass concentrations. By mass, the extraction efficiency of these samples was 70 ± 5% (mean ± standard error) for fine PM, 78 ± 1% for $PM_{10\text{-}2.5}$, and 80 ± 10% for super-coarse PM.

3.2. Trace metal analysis

Individual trace metal concentrations were assessed for each sample and grouped by location and particle size fraction (Supplemental Material). Among the locations, ocean-side Trinidad displayed unique trace element patterns compared to the other four locations. For example, Fe and Ti concentrations were much lower and Mg concentrations much higher in both the $PM_{2.5}$ and $PM_{10\text{-}2.5}$ fractions in Trinidad compared to the other locations. Additionally, concentrations of Cu were much lower in the rural locations (Trinidad and Tranquility) compared to the urban locations. Lastly, endotoxin was elevated in the urban locations compared to the rural locations, with the Davis $PM_{10\text{-}2.5}$ sample having the highest concentration.

Correlations were calculated between individual trace element and endotoxin concentrations and the *in vitro* and *in vivo* endpoints (Table 2). S and As were negatively and significantly correlated with *in vitro* ROS ($R = -0.27$, $p < 0.01$ and $R = -0.30$, $p < 0.001$, respectively) and *in vivo* %PMNs ($R = -0.51$, $p < 0.001$ and $R = -0.27$, $p < 0.01$, respectively). Positive correlations were observed with Sb, Sn, Cu, Mn, Ti, P, and Fe for *in vitro* ROS and *in vivo* %PMNs and protein measurements. Endotoxin was positively correlated with *in vitro* ROS and *in vivo* %PMNs and protein, but the correlation was only significant ($p < 0.001$) for ROS.

3.3. Source identification

The factor analysis loadings of each identified source are shown in Table 3. Five possible source categories were identified, based on the highest tracer loadings on each, reliability (i.e., % measured above detection), and interpretability as source tracers. The source categories identified in this work included soil dust, traffic, coal combustion, industrial-related sources, and biomass burning. The 5 factors account for 78.5% of the total variance of the data set, indicating that the variability of the element concentrations can be satisfactorily explained by the 5 factors.

The first factor loading, soil dust, was most associated with Ca, Ti, V, Mn, and Fe. V, which was also highly detected, may be associated with the soil component (instead of as a separate source component) because of re-entrainment of previously emitted and deposited

residual oil burning PM, as V has been reported in California soils (Deverel and Millard 1988). Because the second source was highly correlated with Cu and Sb, known tracers of brake wear (Thorpe and Harrison 2008), this factor was assigned as traffic. Consistent with this interpretation the factor scores for this source were significantly higher in the urban, compared to rural, locations. The third factor, identified as being associated with coal combustion (Thurston et al. 2011), had the highest loadings for As and Se. The fourth factor, rich in Cr, Ni, and Pb, presents as a mixture of industrial sources, including oil-fired power plants, steam boilers, and a steel mill (Yang et al. 2013). The last factor, heavily loaded with K, was assigned as biomass burning (Thurston et al. 2011).

Elements from soil and traffic were significantly associated with increases in ROS (Table 2). Elements related to coal combustion, industry, and biomass burning had no significant effect on ROS (Table 2). The same trend of associations was observed when correlations were calculated for %PMNs although endotoxin was not significantly correlated with %PMNs.

3.4. In vitro toxicity

The ROS response was examined to determine if PM-stimulated oxidative stress was significantly related to sampling location, particle size, and/or locale. When the influence of the particle size was assessed at each location (Figure 1A), significant size-dependent differences in ROS production were observed at the 3 urban locations; no significant differences in ROS were observed amongst the 2 rural locations. Therefore, it was unsurprising that the combined urban locations also exhibited a significant difference between size ranges (Figure 1B). For all the sampling locations, the $PM_{10\text{-}2.5}$ size fraction elicited a greater response compared to $PM_{2.5}$.

3.5. Animal toxicity

A mouse model was used to assess the effect of coarse and fine PM on %PMNs and total protein in lavage fluid following oropharyngeal aspiration. Differences between the coarse and fine PM size fractions for %PMNs were significant between all locations except Trinidad (Figure 2A). Similar to the ROS response, coarse PM was more potent than fine PM. Significant differences were seen for both the rural and urban locales (Figure 2B); the coarse PM size fraction elicited the greatest response in both the rural and urban locations. When the protein measurements were compared based on the particle size range with location (Figure 2C), Davis was the only location where a significant particle size range effect on %PMNs was observed (i.e. coarse PM had significantly greater effect than fine PM). When the locations were divided into urban or rural locales, no significant differences were observed (Figure 2D).

3.6. Super-coarse vs. coarse vs. fine PM

Super-coarse PM was collected simultaneously with fine and coarse PM at the Bakersfield and Tranquility sites. The relative effect of super-coarse PM on *in vitro* ROS production differed between sites (Figure 3). For the Bakersfield samples, super-coarse PM was less potent than the coarse PM and more potent than the fine PM collected with no significant differences (Figure 3A). A significant size-dependent effect was seen with the Tranquility samples, where the super-coarse PM was significantly elevated compared to the coarse and

fine PM size fractions (Figure 3A). When %PMNs were compared across particle size fractions, super-coarse PM elicited a greater response than coarse PM; fine PM elicited the least potent response (Figure 3B). This size-dependent effect was observed for both locations at which super-coarse PM was sampled. No size-dependent effects were observed for lavage protein (Figure 3C).

4. Discussion

This study compared the relative *in vitro* ROS and *in vivo* responses to 2 different PM size ranges (coarse and fine PM) collected at 2 rural and 3 urban sites in California, and super-coarse PM collected at 1 rural and 1 urban location. All particulate matter samples were simultaneously collected using high-volume cascade impactors, and characterized by mass, trace element, and endotoxin concentrations. Correlations between biological responses and individual trace element/endotoxin concentrations were evaluated. Lastly, significant pollution sources for each size fraction and location were assessed using factor analysis. The size-fractionated California ambient PM elicited an ROS response in a cell model as well as an inflammatory response in a mouse model. Overall, these responses were largely dependent on particle size range, location, and locale (urban vs. rural). These data demonstrate that the source and composition of ambient PM can influence *in vitro* ROS and *in vivo* inflammatory responses, suggesting that the use of PM mass concentration as the primary metric for the selection of health policies for ambient PM can be enhanced by also considering PM composition and sources.

Oxidative stress has been implicated as a possible mechanism to explain PM-related health effects, either directly by the innate oxidative potential of ambient particles or from a cellular response to the particles (Ghio et al. 2012; Mazzoli-Rocha et al. 2010). Oxidative stress occurs when there is an imbalance between antioxidants and oxidants. PM from various locations has been shown to induce ROS both in human lung epithelial cells (Shi et al. 2006) and in cell-free models (Ntziachristos et al. 2007). In previously published work from our laboratory, PM from various locations in the New York City metropolitan area was assessed for their ROS and pro-inflammatory potential (Mirowsky et al. 2013). In contrast to the current study, $PM_{2.5}$ was found to elicit a greater *in vitro* ROS response compared to $PM_{10\text{-}2.5}$. Additionally, in the current study endotoxin was associated with the ROS response whereas in the NYC samples endotoxin was correlated only with %PMNs. These study discrepancies could be attributed to differing components and sources of PM, further suggesting that PM composition rather than size fraction may be a better predictor of adverse responses. Further, in our previous work the correlations between trace elemental concentrations and ROS response were similar in magnitude to that presented in the current manuscript and it appeared that stronger correlations were seen with %PMNs. Similar correlation coefficients were seen in a previous study (Liu et al. 2014).

In studies assessing the toxicity of endotoxin, conflicting results have been obtained. For example, previous studies have found associations between endotoxin and inflammatory responses in cell and animals models (Alfaro-Moreno et al. 2007; Steenhof et al. 2011). Contrasting results from a study done in California, however, concluded that endotoxin was not related to the pro-inflammatory responses to coarse PM (Wegesser and Last 2008).

Similarly, we did not find associations between endotoxin and pro-inflammatory responses in our mouse model but did observe a positive association between endotoxin and ROS response in our cell model.

Limitations of the present study include a lack of organic and ionic composition data for the PM samples. Previous studies have shown associations between volatile organic constituents, organic carbon, and nitrates with inflammatory responses (Plummer et al. 2012; Wang et al. 2012; Wegesser and Last 2008). In the current work, the aqueous extraction and lyophilization methods would not be optimal for the collection of non-polar components, such as elemental carbon, or semi-volatile and volatile components, and therefore was not explored. It is also possible that we were unable to account for all sources in our model. For example, we were unable to measure the contribution of sea salt to our responses as Na and Cl could not be measured with ICP-MS; however, a previous review has speculated that exposure to sea salt at ambient concentrations would not present a health risk (Cassee et al. 2013). Thus, a lack of identification of all PM components may limit the comprehensiveness of our factor source conclusions. The *in vivo* portion of this study was somewhat limited by the number of animals used per group (n = 3). This low n per group was utilized in an effort to reduce the number of animals used in the study while surveying the *in vivo* effects of over 100 PM samples.

5. Conclusion

This study has shown that PM size range, location, and locale (urban vs. rural) across 5 sites in California influence the ROS response in human pulmonary endothelial cells, as well as am inflammatory response in a mouse model. $PM_{10\text{-}2.5}$ elicited the greatest ROS response in the cell model, and this was particularly evident in the urban locations. $PM_{10\text{-}2.5}$ also elicited the greatest increase in %PMNs, regardless of location or locale, with the exception of the rural ocean-side location. In addition, super-coarse PM (i.e., $PM_{>10}$ μm) produced as great, or more, of an inflammatory response in the mouse lung, thus suggesting that additional research should address the toxicity of this particle size that will deposit largely in the upper respiratory tract. Five factors (soil dust, traffic, coal combustion, industrial-related sources, and biomass burning) were identified based upon the composition of the collected PM, and within these factors, ROS and %PMN responses were positively associated with soil and traffic. This work supports that of previous studies in demonstrating that understanding the role of PM composition in adverse health effects, in addition to PM mass concentration, is necessary for a full evaluation of the toxic responses to PM exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- The toxicity of particulate matter was assessed at 5 California locations.
- Urban locations and coarse particles elicited the greatest biological effects.
- Soil and traffic markers were associated with the measured biological effects.
- Particle sources might be more influential than size when determining toxicity.

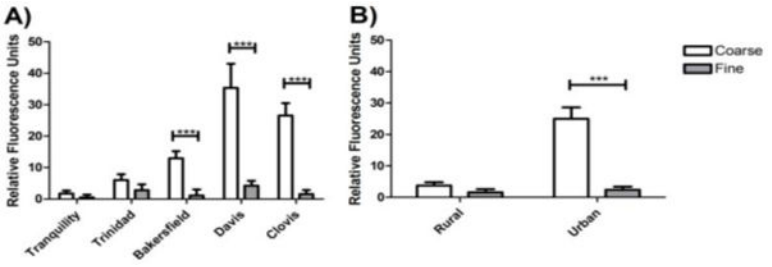

Fig. 1.

Comparison of the effect of coarse and fine PM on ROS activity in endothelial cells based on (A) location and (B) locale. Data represent the mean relative fluorescence units minus the mean vehicle water blank ± SE (in triplicate). P-values were calculated using an unpaired Student's t-test between coarse and fine PM for sampling sites and locales. ***p-value < 0.001.

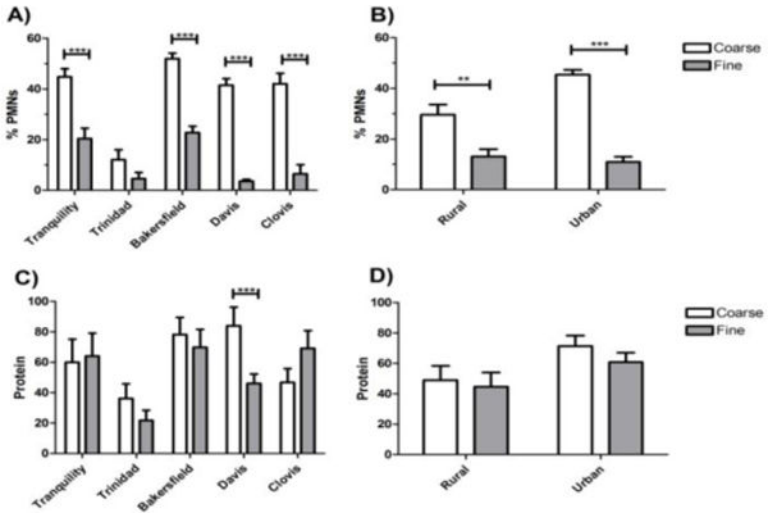

Fig. 2.

Comparison of the effect of coarse and fine PM on %PMNs and protein in the lavage fluid from FVB/N mice. (A) % PMNs separated by location; (B) %PMNs separated by locale; (C) protein separated by location; (D) protein separated by locale. Data represent the mean %PMNs or protein minus the mean vehicle water blank ± SE (n = 3 mice per group). P-values were calculated using an unpaired Student's t-test between coarse and fine PM for sampling sites and locales. **p-value < 0.01, ***p-value < 0.001

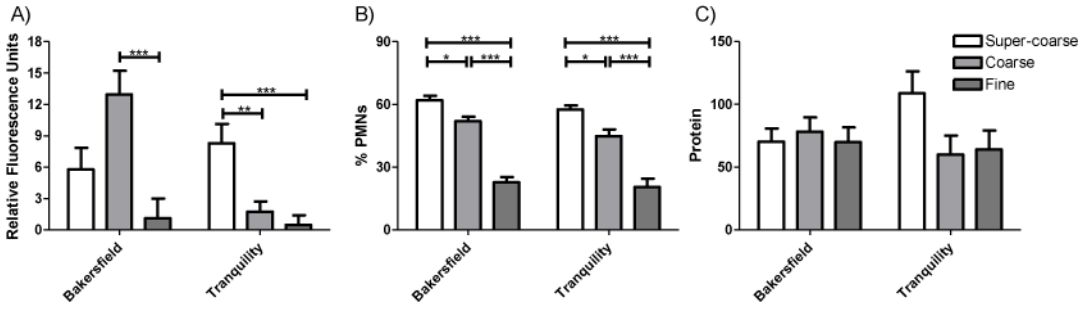

Fig. 3.

The effect of super-coarse, coarse, and fine PM on the *in vitro* and *in vivo* responses. (A) *In vitro* ROS responses, with the mean vehicle water blank subtracted, were measured in cultured HPMEC-ST1.6R endothelial cells (in triplicate). *In vivo* (B) %PMNs and (C) total protein were obtained from FVB/N mice (mean water vehicle blank values subtracted, n = 3 mice per group). Data represent the mean ± SE. P-values were calculated using 1-factor ANOVA followed by Tukey's Multiple Comparison Test. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.005

Table 1

Mass concentration for PM stratified by location and size fraction. All data are expressed as mean ± SD. Sample size = 11–15 samples/location.

	Super-coarse $PM_{>10}$ ($\mu g/m^3$)	**Coarse $PM_{10-2.5}$ ($\mu g/m^3$)**	**Fine $PM_{2.5}$ ($\mu g/m^3$)**
Tranquility	20.1 ± 37.6	15.0 ± 14.6	15.2 ± 9.9
Bakersfield	37.2 ± 23.6	37.0 ± 17.6	31.2 ± 16.4
Davis	NM[a]	15.5 ± 3.7	11.5 ± 5.6
Clovis	NM	23.7 ± 0.1	29.1 ± 11.8
Trinidad	NM	71.4 ± 18.8	5.7 ± 2.8

[a] NM = not measured

Table 2

Pearson correlation coefficients between trace element and endotoxin concentrations with both *in vitro* and *in vivo* responses. ROS responses were measured in endothelial cells (in triplicate). %PMNs and total protein were obtained in mice (n = 3). Elements are ranked, from largest to smallest, based on overall concentration, followed by endotoxin.

	***in vitro* ROS**	***in vivo* %PMNs**	***in vivo* protein**
S	−0.27**	−0.51***	−0.19*
Ca	0.27**	0.66***	0.10
Fe	0.41***	0.80***	0.20*
K	−0.04	−0.01	−0.03
Mg	0.14	0.07	−0.15
P	0.29**	0.72***	0.36***
Zn	0.06	−0.04	0.05
Ti	0.33***	0.76***	0.22*
Mn	0.44***	0.77***	0.26**
Cu	0.44***	0.60***	0.21*
Sr	0.25**	0.54***	0.06
Sn	0.27**	0.37***	0.21*
Pb	0.03	−0.13	−0.12
Cr	0.07	0.13	0.01
Ni	0.14	0.19*	−0.01
Sb	0.42***	0.42***	0.21*
V	0.33***	0.65***	0.12
Se	−0.14	−0.10	0.03
As	−0.30***	−0.27**	0.16
La	0.30***	0.73***	0.14
Cd	−0.01	−0.09	−0.08
Co	0.47***	0.75***	0.15
Ag	−0.06	0.16	−0.03
Endotoxin	0.54***	0.27	0.13

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

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Table 3

Varimax rotated factor loadings for the 5-factor solution obtained from pooled coarse and fine PM. Key trace elements with factor loadings larger than 0.50 are in bold.

	Soil Dust	Traffic	Coal	Industrial	Biomass burning
S	−0.33	−0.22	0.52	0.04	0.50
K	0.10	−0.04	−0.11	−0.05	**0.88**
Ca	**0.86**	0.11	−0.23	0.00	0.23
Ti	**0.87**	0.34	−0.16	0.08	−0.05
V	**0.89**	0.12	0.05	0.25	0.05
Cr	0.41	−0.03	−0.08	**0.66**	−0.15
Mn	**0.85**	0.29	−0.20	0.22	−0.07
Fe	**0.87**	0.34	−0.18	0.16	−0.10
Ni	0.28	0.17	−0.17	**0.86**	−0.04
Cu	0.50	**0.80**	−0.17	0.04	−0.09
Zn	0.10	0.49	0.40	0.14	0.50
As	−0.39	0.10	**0.72**	0.01	0.06
Se	−0.01	−0.12	**0.89**	−0.08	−0.12
Sb	0.30	**0.89**	−0.01	0.11	−0.02
Pb	−0.15	0.05	0.30	**0.52**	0.22
% variance[a]	42.0	13.8	8.5	8.0	6.2
Cumulative % variance[b]	42.0	55.8	64.3	72.3	78.5

[a] % variance contains the percent of total variance accounted for by each factor.

[b] Cumulative % variance contains the cumulative percentage of variance accounted for by the current and all preceding factors.