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Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/es506048k • Publication Date (Web): 24 Feb 2015

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Abstract

Near-road exposures to air pollutants have been associated with decreased lung function and other adverse health effects in susceptible populations. This study was designed to investigate whether different types of near-road particulate matter (PM) contribute to exacerbation of allergic asthma. Samples of upwind and downwind coarse, fine, and ultrafine PM were collected using a wind direction-actuated ChemVol sampler at a single site 100 meters from Interstate-96 in Detroit, MI during winter 2010/2011. Upwind PM was enriched in crustal and wood combustion sources while downwind PM was dominated by traffic sources. Control and ovalbumin (OVA)-sensitized BALB/cJ mice were exposed via oropharyngeal (OP) aspiration to 20 or 100 μg of each PM sample two hours prior to OP challenge with OVA. In OVA-allergic mice, 100 μg downwind coarse PM caused greater increases than downwind fine/ultrafine PM in bronchoalveolar lavage neutrophils, eosinophils, and lactate dehydrogenase. Upwind fine PM (100 μg) produced greater increases in neutrophils and eosinophils compared to other upwind size fractions. Cytokine (IL-5) levels in BAL fluid also increased markedly following 100 μg downwind coarse and downwind ultrafine PM exposures. These findings indicate coarse PM downwind and fine PM upwind of an interstate highway promote inflammation in allergic mice.

Introduction

The prevalence of asthma and allergic diseases has increased worldwide¹ in recent years. Near-road exposure to air pollutants is a potentially important contributing factor to these conditions. Several epidemiological and observational studies have shown that traffic-related air pollutants result in adverse effects on respiratory health including decreased lung function, increased allergic responses, and higher rates of hospitalization²⁻⁴. Near-road traffic-related air pollutants also produce more severe respiratory outcomes in susceptible populations⁵⁻⁷. Children living in close proximity (within 50 m) to roadways with high levels of air pollution have increased risk of allergy, bronchitis, and reduced lung growth⁸⁻¹⁰. These studies demonstrate a need for improved understanding of near-road environments and associated health effects¹¹.

Specific characteristics of particulate matter (PM) in near-road environments may contribute in different ways to produce adverse respiratory effects. Individuals with existing asthma walking along a heavy diesel traffic street with high levels of PM for two hours had decreased lung function and increased inflammatory biomarkers compared with walking in a traffic-free park⁷. In mice, studies showed that near-road PM induces pulmonary inflammation and allergic responses which differ by particle size^{5,12}. However, there is still uncertainty about the impact of specific PM composition and meteorological factors associated with near-road exposures on the exacerbation of asthma in susceptible individuals.

Variation in PM-associated toxicity has been attributed to differences in geographical location, seasonal influences, and size components¹³⁻¹⁵. Coarse PM (2.5-10 μm) is a combination of crustal material and particles from sources such as brake and tire wear and resuspended road dust¹⁶ while fine PM (0.1-2.5 μm) and ultrafine PM (<0.1 μm) typically originate from combustion processes¹⁷. Seasonal and wind conditions may further impact how different PM

types are dispersed and transported. These differences lead to highly complex PM mixtures within near-road environments that may cause specific health effects such as exacerbation of asthma.

The Near Road Exposures and Effects of Urban Air Pollutants Study (NEXUS) was designed to investigate the effects of traffic-related air pollutant exposure on respiratory outcomes for asthmatic children in Detroit, MI¹⁸. NEXUS used predictive modeling tools to assess the impacts of near road air pollutants on children's health, contrasting different traffic exposures and adverse respiratory outcomes¹⁹. The present investigation was designed to complement the NEXUS epidemiological study. Field campaigns were conducted using a wind direction-actuated ChemVol sampler located near Interstate-96 (I-96). Size-fractionated PM (coarse, fine, and ultrafine) was collected from a single site separating downwind and upwind sources during the winter season, and administered to allergic and control mice to evaluate exacerbation of allergic airway responses. The allergic airways disease model was generated by sensitization and challenge with ovalbumin (OVA) antigen, which results in several features of human allergic asthma, including enhanced airway responsiveness, increased bronchoalveolar lavage (BAL) eosinophils, and allergy-related cytokines²⁰. We hypothesized that equal mass of downwind (highway-enriched) wintertime PM samples would produce greater effects on immune sensitization, pulmonary responsiveness, and inflammatory responses than urban-source enriched upwind samples in a mouse model of allergic airways disease.

87 **Materials & Methods**

88 *PM Collection, Extraction and Recovery*

89 The sampling site was located 100 m north of I-96 in Detroit, Michigan, in Eliza Howell
90 Park (42°23'12.46"N; 83°16'13.73"W) (Figure S1A). Integrated samples were collected during
91 four separate winter 2010/2011 sampling periods (12/10/2010-12/15/2010, 1/27/2011-2/1/2011,
92 3/4/2011-3/9/2011, and 3/28/2011-3/29/2011) in three sizes (coarse, fine, and ultrafine) using
93 two ChemVol High Volume Cascade Impactors (Model 2400, BGI Inc., Waltham, MA).

94 This EPA ChemVol system was re-engineered for improved field collection of size-
95 fractionated ambient particulates. The 3-phase Gast regeneration blower was housed in a
96 converted utility trailer with a 50amp-220V shore plug and power inverter. The blower was
97 operated by a computer-controlled system programmed to maintain volumetric flow at 900 L
98 min⁻¹ (±5%) by adjusting for filter loading and for changes in atmospheric conditions. Wind
99 direction-actuated sampling was achieved by using two collocated ChemVol impactor heads. An
100 ultrasonic anemometer (Model 85000, R. M. Young Co., Traverse City, MI) measured wind
101 direction and speed which signaled two solenoid valves to either sample onto the “upwind” or
102 “downwind” impactor head. The system was programmed such that the downwind impactor
103 head would sample when the wind direction was from 80° to 280° indicating when the site was
104 impacted by I-96 mobile source emissions. The downwind impactor head also sampled during
105 no/low wind speed conditions (< 0.5 m/sec) when meteorological stagnation was hypothesized to
106 result in the local buildup of mobile source emissions from the roadway. The upwind samples
107 (281° to 79°) were intended to represent a regional background sample primarily from the
108 northerly direction.

Detailed methodology for PM extraction and recovery are described in the Supporting Information (SI).

Elemental Analyses of PM

Following extraction, PM aliquots were prepared for elemental analysis by two-stage acid digestion. Ion chromatography, and high resolution inductively-coupled mass spectrometry (HR-ICP-MS) for elemental analysis, were conducted as previously described²¹.

Exposure to PM and Ovalbumin.

All experimental protocols using laboratory animals were approved by the U.S. EPA NHEERL Institutional Animal Care and Use Committee. Young adult (7 weeks old, 18–21 g) female BALB/cJ mice were obtained from Jackson Laboratories (Bar Harbor, ME) and housed 4/cage in an AAALAC-accredited barrier-isolated animal facility. Food and water were provided *ad libitum*. Animals were held for a minimum of five days before treatment. To examine PM effects on OVA allergen responses, mice were grouped into three cohorts (72/size fraction) and exposures were conducted as follows. Allergic groups were sensitized with 20 µg OVA administered intraperitoneally (i.p.) in 0.2 mL aluminum hydroxide adjuvant (Alhydrogel; Accurate, Westbury, NY), while non-allergic groups received i.p. adjuvant only. Fourteen days later, mice were exposed on an equal mass basis to 20 or 100 µg of either upwind or downwind PM filter extract in 50 µL sterile saline, or an equivalent volume of control (non-exposed) filter extract ($n=7-8$ /group) by oropharyngeal aspiration under isoflurane anesthesia. Two hours later, all mice were challenged by aspiration of 20 µg OVA, followed by assessment of immediate airway responses (IAR) to OVA (0h post exposure). Responsiveness to methacholine (MCh) aerosol and bronchoalveolar lavage fluid (BALF) parameters were measured 48 hours after OVA challenge. Mice were euthanized and necropsied one hour after MCh challenge (see below).

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133 ***Respiratory Responses to OVA Antigen and MCh.***

134 Whole body plethysmography (Buxco Electronics, Wilmington, NC) was used to
135 measure IAR to antigen exposure and airway responsiveness to MCh aerosol in unanesthetized,
136 unrestrained mice²². IAR were determined by the change in an index of airflow obstruction
137 (enhanced pause; Penh) measured immediately before and after OVA challenge. Penh is derived
138 from timing of expiration and pressure changes associated with respiration in the
139 plethysmograph chamber^{22,23}. Baseline values were determined over a 20 min period. Mice were
140 removed and challenged with OVA antigen by aspiration as described above and returned to the
141 chambers within 5-10 min of challenge. Recording was resumed and measurements were
142 averaged over a one hour period. Two days later, baseline values were recorded for 10 min,
143 followed by aerosolization of saline and then increasing concentrations of methacholine (6.25, 12
144 and 25 mg/mL).

145 ***BALF Collection and Analysis.***

146 Anesthesia was induced with a combination of pentobarbital and phenytoin (120 and 15
147 mg/kg), and then mice were exsanguinated via cardiac puncture. The left lung was clamped and
148 the right lobes lavaged *in situ* with three aliquots of Hanks' balanced salt solution (0.6 mL each).
149 The BALF was centrifuged at 300 x g for 10 min. An aliquot was centrifuged at 250 rpm for 10
150 min (Shandon 3 Cytospin, Shandon, Pittsburg, PA) to prepare cell differential slides. Total cell
151 counts were determined using a Z1 Coulter counter (Coulter Inc., Miami, FL). BAL cell slides
152 were stained with Wright-Giemsa using an automated slide stainer (Hematek 2000; Miles Inc.,
153 Elkhart, IN). Cells were enumerated (300 cells/sample) to determine differential cell counts.
154 BALF supernatant was assessed for the following: lactate dehydrogenase (LDH), protein

(Thermo Fisher Diagnostics, Rockford, IL), albumin (DiaSorin, Stillwater, MN), and *N*-acetyl- β -D-glucosaminidase (NAG, Roche Diagnostics, Indianapolis, IN). Assays were carried out using a Konelab 30 clinical chemistry analyzer (Thermo Chemical Lab Systems, Espoo, Finland).

Serum Antibody levels.

Serum concentrations of OVA-specific IgG₁ and IgE were determined using an enzyme-linked immunosorbent assay (ELISA) kit from Cayman Chemical (Ann Arbor, MI) per manufacturer's instructions.

Cytokine Measurements.

Murine interleukin (IL)-5, IL-13, interferon gamma (IFN- γ) and macrophage inflammatory protein (MIP-2) were quantified in BALF using ELISAs per the manufacturer's recommendations (Millipore, Billerica, MA, USA). The lower limit of quantification for each cytokine were as follows: IFN- γ – 1.0 pg/mL; IL-5 – 1.0 pg/mL; IL-13 – 25.0 pg/mL; MIP-2 – 125.0 pg/mL.

Endotoxin Measurements

Each PM suspension containing 0.5% methanol and saline was vortexed and sonicated for 15 min. The suspension was diluted in endotoxin free water (1 mg/mL) followed by centrifugation at 2100 rpm for 15 min. Endotoxin concentration measurements were performed on the supernatants using the Limulus amoebocyte lysate assay (QCL-1000; Lonza, Walkersville, MD) according to the manufacturer's instructions.

Lung Histopathology

Left lung lobes were collected and inflated at 20 cm water pressure with 10% neutral buffered formalin. Fixed tissues were paraffin-embedded, sectioned at 5 μ m, and stained with hematoxylin and eosin for histopathologic evaluation. One transverse section of the left lung lobe

was made for each animal and evaluated by light microscopy by a board-certified pathologist using established pathologic criteria²⁴. Differences in lesion incidence between groups were evaluated using Fisher's Exact Test.

Statistical Analyses

Graphpad Prism v4.0 software was used to perform statistical analyses. Values were expressed as means $\pm$ standard error of the mean. Statistical analyses between groups were conducted using a one way analysis of variance with Bonferroni post-hoc test. The result was considered significant when $p < 0.05$.

Results

ChemVol Characterization

During the four sampling periods, the wind direction was nearly evenly split between upwind (50.7%) and downwind (49.3%). Figure S1B indicates the winds were widely distributed across all directions, but there was a strong northwesterly influence on wind speed and frequency. Meteorological conditions were categorized as stagnant 14% of the time, (< 0.5 m/sec) which activated downwind sampling.

ChemVol mass collection performance was evaluated against a Federal Equivalency Method (FEM) for fine PM concentration. A collocated Thermo Scientific Model 2025 Partisol Sequential Dichotomous Sampler (Waltham, MA) collected 24-hour integrated fine and coarse PM samples. The 5-day average fine PM concentrations from the FEM sampler were compared against the sum of the ChemVol fine and ultrafine fractions. A linear regression analysis ($n=10$) found good precision between the samplers with a coefficient of determination of 0.80. The slope of the regression was 1.25 ($p=0.0005$), and the higher Partisol concentrations suggest the ChemVol sampler was underestimating fine PM mass by 25%. Linear regression analysis

showed the ChemVol sampler coarse fraction mass was 12% higher than the FEM sampler ($m=0.88$, $p=0.0019$, $r^2=0.72$), which may indicate the mass median aerodynamic diameter (MMAD) cut point of the ChemVol and FEM sampler are different. Combined coarse, fine, and ultrafine masses were, on average, 11% underestimated by the ChemVol compared to the FEM sampler ($m=1.11$, $p=0.0008$, $r^2=0.77$).

PM Mass and Composition

PM concentrations and collected masses for the three size fractions and two wind directions along with recovery fractions and chemical analyses are shown in Table 1. Greater ambient concentration and total collected PM mass (>2-fold) were collected from the downwind direction of the I-96 freeway compared with the upwind direction. Water soluble extraction recovery ranged from 72-87% of collected mass, and was slightly higher (82-87%) for the downwind coarse and fine PM fractions when compared to the corresponding upwind samples (72-78%). Major ion analysis revealed the coarse PM fractions from either direction had high concentrations of sodium (Na^+), and chloride (Cl^-), indicative of road salt and crustal sources, when compared to other size fractions, while nitrate (NO_3^-), and sulfate (SO_4^{2-}) levels were higher in the fine and ultrafine fractions (Table 1). Organic carbon (OC) in the coarse fraction was double in the upwind direction compared to the downwind direction, but were similar in both wind directions in the fine and ultrafine fractions. Elemental carbon (EC) was only detected in the fine and ultrafine fractions and was much lower than OC levels. The PM collected along I-96 contained negligible amounts of measurable endotoxin (<1 EU/g).

Figure 1A illustrates the total mass balance percentages of the major classes of chemical components in each fraction of upwind and downwind PM, incorporating data from all assays. The coarse fractions from both sampling directions possessed a similar chemical composition.

High levels of Cl^- (~23% downwind, ~15% upwind) were observed in coarse fractions in comparison to other size fractions. The proportions of other major chemical classes were comparable in both directions among coarse or fine PM fractions, except for the OC mentioned in the previous paragraph. However, SO_4^- content was higher in the upwind fractions. Figure 1B illustrates the concentrations of metal components, analyzed by HR-ICP-MS, associated with specific sources in PM samples. Metals indicative of brake and tire wear, including barium (Ba), copper (Cu), and lead (Pb) were highest in the coarse fractions, especially downwind, and were slightly lower in the fine fractions. Manganese (Mn), indicative of industrial emissions, was highest in both the coarse and fine upwind fractions. Vanadium (V) and nickel (Ni) are associated with burning of residual oil, and Ni was highest in the ultrafine fractions, especially downwind.

HR-ICP-MS findings were consistent with those of ion chromatography; all samples had high levels of sulfur (11-25% of total mass), while Na^+ (12-16%), and calcium (Ca^{2+}) (5-6%) were elevated in the coarse fractions of both wind directions (Table 1). Downwind coarse PM contained the following concentrations of elements that were at least 30% higher than the collected upwind PM fraction: Na^+ (16% of total), and Ba, cadmium (Cd), and selenium (Se) (each <0.1%). The upwind coarse fraction was higher in silicon (Si), magnesium (Mg), potassium (K), aluminum (Al), and phosphorus (P), several of which are indicative of crustal soil resuspension (ranging from 0.2-2.4% of total). Within the fine PM fractions, downwind PM had higher ratios ($\geq 30\%$) of Pb, molybdenum (Mo), and Se (each <0.1%). Upwind fine PM had elevated ratios of Na (2.8%), Ca (0.9%), and Mg (0.4%). Downwind ultrafine PM had elevated levels of Mg (0.6%), Al (0.5%), and Ni; (0.1%). Upwind ultrafine PM had elevated ratios of Si (0.4%), iron (Fe, 0.1%), zinc (Zn) and P (each 0.2%).

247

248 *Respiratory responses*

249 There were no significant changes in immediate responses to OVA challenge as
250 determined by WBP in non-allergic or allergic mice exposed to control filter extract or PM
251 (Table S1). Two days after OVA challenge, WBP testing indicated increasing concentrations of
252 MCh aerosol produced marginal but statistically insignificant increases in PenH values among all
253 exposure groups, and there were no significant differences among groups at the same MCh
254 concentration (Table S2).

255 *BALF Cells, Biochemical Indices and Cytokines*

256 Following 100 µg coarse particle exposure, BALF macrophages significantly increased in
257 the allergic groups when compared to both non-allergic and allergic control filter groups (Figure
258 2A). Further, exposure of allergic mice to 100 µg downwind coarse PM produced a significant
259 increase in both neutrophils and eosinophils compared to both control filter groups (Figure 2B,
260 2C). Eosinophils in the 100 µg downwind coarse group were significantly greater than in the 100
261 µg upwind allergic treatment group (Figure 2C) and also increased 4-fold after exposure of
262 allergic mice to 100 µg upwind fine PM (Figure 2F). A significant increase in neutrophils was
263 observed in the 100 µg upwind fine allergic group compared to both control filter groups (Figure
264 2E). In contrast, no significant changes in BALF cell counts were observed following exposure
265 to ultrafine PM (Figure 2G-2I). Lymphocytes did not differ among any of the treatment groups
266 (data not shown).

267 Exposure of allergic mice to 100 µg coarse downwind PM induced a significant increase
268 in IL-5 BALF levels (Figure 3A). Further, these increases were noted following exposure to 100
269 µg fine and ultrafine upwind or downwind PM (Figure 3B, 3C). Levels of IFN-γ, IL-13, and
270 MIP-2 were near or below the limit of quantitation (data not shown).

Biochemical indicators of acute lung injury were also examined in BALF two days after aspiration of PM samples (Table S3). Downwind coarse PM (100 μg) increased all of the endpoints ~2-fold relative to control filter; the differences were statistically significant for LDH and NAG. Downwind fine PM (100 μg) increased these endpoints to a lesser degree in allergic mice, and only NAG was significantly increased. Non-allergic mice that received downwind ultrafine PM (100 μg) experienced a significant 2-fold increase in total protein. Overall, the downwind PM treatment produced more notable changes in acute lung injury endpoints than upwind PM exposure.

OVA-specific IgG₁ and IgE Responses.

Ovalbumin sensitization increased both serum IgG₁ and IgE antibody responses in comparison to the unsensitized groups (Figure S2). Exposure to either dose of upwind or downwind coarse, fine, and ultrafine PM did not significantly alter IgG₁ and IgE serum levels in comparison to control OVA-allergic mice.

Histopathological Changes in the Lung

Independent of PM treatment, OVA sensitization and challenge resulted in an increased incidence of mixed cell inflammation in perivascular, peribronchiolar, and alveolar regions (Figure S3). Inflammatory cells included eosinophils, neutrophils, lymphocytes, macrophages, and rare multinucleated giant cells (Table S4). Other allergic changes included an increased incidence of mucus within bronchiolar airways and multifocal alveolar histiocytosis (Table S4). Near-road PM effects included intracytoplasmic brown particles within alveolar macrophages and multifocal alveolar histiocytosis, which were increased across all PM sizes compared to respective control groups (independent of dose or allergic status). Pigmented particles varied from scattered granules 1-2 μm in diameter to larger aggregates up to ~10 μm in diameter.

Across all PM sizes and doses, no significant differences in incidence were observed between upwind and downwind PM groups. The only difference among PM size groups was alveolar macrophage pigment, which had a greater incidence in coarse and fine PM groups compared to ultrafine PM groups. The incidence of intracytoplasmic pigment and alveolar histiocytosis was also higher overall for the 100 μg PM dose compared to the 20 μg PM dose across groups. Among 100 μg non-allergic treatment groups, the incidence of mixed cell inflammation was greatest for ultrafine PM, intermediate for coarse PM, and lowest for fine PM.

Discussion

This study was conducted to investigate acute respiratory effects, specifically the exacerbation of allergic asthma of winter time Detroit PM collected along I-96 using a ChemVol sampler in an allergic airways disease mouse model. Our results indicate the following: (i) the chemical composition of upwind PM contained higher mass fractions of species indicative of crustal elements (coarse Si and Ca), wood combustion (fine K) and regional transport of air pollution (fine SO_4^{2-}), whereas downwind PM was higher in species consistent with roadway impacts such as road salt (coarse Na, Cl), brake wear (coarse Ba, Cu), and diesel fuel combustion (fine EC); (ii) 100 μg downwind coarse PM significantly increased neutrophils and eosinophils in OVA-allergic groups while the same dose of upwind fine PM significantly increased neutrophils; and (iii) IL-5 levels were increased to a greater degree by exposure to 100 μg downwind coarse PM than by exposure to the same dose of upwind coarse PM. Collectively, these findings show that on an equivalent mass basis, downwind coarse PM elicited a greater inflammatory response in allergic mice than upwind coarse PM, while conversely upwind fine PM elicited a greater neutrophilic inflammatory response compared to downwind fine PM.

Sulfate concentrations were higher in fine and ultrafine PM relative to coarse PM, and were slightly higher upwind of the freeway. Epidemiological studies and regional profile data indicate that nitrate concentrations are considerably higher and sulfate concentrations are lower during winter with the dominating contribution derived from upwind sources in the eastern US including Detroit²⁵. Despite the elevated levels of sulfates and nitrates in samples tested here, their biological reactivity was comparatively lower than that of samples taken from other locations in the US²⁶. On a mass fraction basis, downwind coarse and fine PM samples were dominated by elemental tracers suggesting brake wear (Ba, Mg, Cu, and Pb), road salt, and industrial emissions such as iron/steel production/mixed industry (Cd, Fe, Mn, Mo and Zn) in the Detroit area²⁷. Conversely, upwind PM composition suggests entrainment of soil (Sr, Si, Ti, Sn, Ca²⁺ and Al), wood smoke (K) and residual oil combustion (Cr, Ni, and V)²⁸. Ultrafine PM analyses indicated tailpipe combustion emissions and burning motor oil (OC, EC), and industrial/residual oil combustion (Ni) as contributing sources. The high levels of Na⁺ and Cl⁻ suggest general urban road salt aerosolization in both wind directions during the winter months while elevated K levels were suggestive of wood smoke²⁹. The I-96 sampling site is considered a low diesel site with high vehicle traffic consistent with measured OC/EC ratios¹⁸. In the collected PM samples, very little EC was found, consistent with the dominant presence of gasoline powered vehicles on this stretch of I-96, whereas higher levels are associated with the use of diesel powered vehicles^{30,31}. The negligible (<1 EU/g) amounts of endotoxin suggest it was not an important contributing factor in the inflammatory response. Overall, the composition profiles of PM samples used in this toxicology study were similar to the composition of urban PM in a previous study of PM from six major US cities²⁹.

PM-associated water-soluble metals are known for contributing to adverse health effects including pulmonary injury in animal models^{32,33}. In this study, downwind coarse PM contained higher levels of Cu and Ba. Soluble copper-rich PM collected from Utah Valley was associated with epithelial toxicity and stress responses³⁴. Intratracheal instillation of barium sulfate nanoparticles significantly increased neutrophils³⁵. These findings suggest that the observed toxicity of the roadway-enriched coarse PM fraction may be partly attributed to copper and barium. Upwind fine PM contained the highest levels of Mn. Manganese is considered a general industrial emission in Detroit which contributes to pronounced lung inflammation³⁶. Ultrafine PM contained significant amounts of Ni in comparison to other size fractions, and caused significant increases in BALF protein levels (Table S3). In rats exposed to residual oil fly ash containing high levels of Ni, BALF protein levels were also significantly increased^{28,37}. Water-insoluble copper nanoparticles also promote eosinophil recruitment which coincides with our finding that downwind coarse PM produces eosinophilia³⁸. Altogether, these reports suggest that the concentrations of PM-associated metals may have contributed to acute lung injury and cellular inflammation.

The allergic airways disease model was generated by sensitization and challenge with ovalbumin antigen and previously used within our laboratory²⁰. This model exhibits features of human allergic asthma, including enhanced airway responsiveness, increased BALF eosinophils, and allergy-related cytokines. Within the allergic mice, increases in serum IgG₁, eosinophils and IL-5 levels were demonstrated confirming successful immune sensitization. Increased serum IgG₁ and IL-5 cytokine levels observed after downwind coarse PM exposure is consistent with prior reports where significant increases in these endpoints were observed in mice following exposure to PM collected 50 m downwind of a high traffic road¹². IL-5 acts as a

chemoattractant, and activator for eosinophils and likely contributed to the increased eosinophil numbers seen herein^{39,40}. Conversely, IgE does not participate in eosinophil activation⁴¹; eosinophil numbers would not necessarily be expected to parallel serum IgE antibody levels. This serves as a partial explanation for the divergence of responses among these endpoints in the allergic mice.

Both upwind and downwind PM had significant effects on allergic inflammation and lung injury. Downwind PM samples increased cell number in a dose dependent manner in OVA-sensitized mice, supporting the idea that exposure to traffic-related emissions may induce adverse respiratory outcomes⁵. The increases in BALF macrophage number, alveolar histiocytosis, and macrophage pigment following both upwind and downwind coarse PM at the higher dose are consistent with particle phagocytosis and stimulation of macrophage recruitment to the sites of PM deposition. These effects are similar to prior studies in which ambient urban PM increased macrophages 2-3 days after PM exposure⁴². Downwind coarse PM also produced higher numbers of BALF neutrophils and eosinophils in the context of allergen challenge similar to previous reports indicating that coarse PM exposure has pro-inflammatory effects⁴³⁻⁴⁵.

An increase in lung inflammation was observed in allergic mice, including increased mixed cell infiltrates, mucus, eosinophils, and macrophages. Increased airway mucus is considered one of the hallmark observations in asthmatics, and rodents with allergy-induced airway hyperresponsiveness also exhibit this feature⁴⁶. Increased mucus in OVA-allergic mice may potentially be mediated by increases in IL-5 and eosinophilia, as suggested by previous research⁴⁷. Upwind and downwind PM may potentiate components of allergic inflammation, evidenced here by increased histiocytic inflammation in both non-allergic and allergic mice.

Repeated or chronic exposure to the different PM samples is needed to determine whether more extensive pathological effects would be observed.

None of the PM samples affected ventilatory responses immediately after allergen challenge. Likewise, the MCh challenge produced no significant differences among treatment groups. These findings are consistent with certain reported studies examining concentrated ambient PM of similar sizes in humans, which suggests that coarse, fine and ultrafine PM do not produce acute effects on pulmonary function^{48, 49}. In contrast, other studies showed exposure to coarse PM collected from near roadway sites can lead to adverse physiologic effects *in vivo*^{50, 51} and influence pulmonary responses^{5, 45, 52}.

It is important to note the limitations within this study. Collection of samples during winter results in lower amounts of semi-volatile organic aerosols and photochemically transformed secondary organic aerosols. Notably, distance of the sampler from the roadway plays a significant role in the severity of responses. Samples collected < 50 m downwind from a major roadway produced alterations in pulmonary function whereas samples collected 150 m downwind did not produce statistically significant changes^{5, 12}. In contrast, the sampler in this study was approximately 100 m from the roadway which may have attenuated any respiratory responses. The proximity of the sampler to secondary or trunk roads may have potentially impacted our results. It is important to note however, downwind sampling resulted in almost double the mass of upwind sampling over the same time period. Although mice were dosed on an equal mass basis, human exposures to downwind PM would have been greater, and it is plausible that for this air shed, the dose of particles is more important than the chemical composition. Further, significant effects were demonstrated only at the higher of the two particle doses, and application of double the downwind sample would be predicted to result in

substantially higher effects than the high dose upwind sample. Future studies might consider conducting exposures that take into account mass concentrations at various locations to better model actual human exposure scenarios across a near road gradient.

Substantial evidence has been found showing an elevated risk of asthma in susceptible individuals near roadways. Our study indicates that short-term exposure of allergic mice to downwind coarse PM produced significant increases in airway eosinophils, neutrophils, pro-allergic cytokines, and cell injury markers in BALF. Pulmonary inflammation was more prevalent following instillation of the downwind coarse fraction and declined as particle size decreased, similar to previous studies, while the upwind fine fraction also had significant adverse effects. PM chemical analysis revealed the chemical composition of upwind PM was higher in species indicative of crustal (coarse Si and Ca), wood combustion (fine K) and regional transport of air pollution (fine SO_4^{2-}), whereas downwind PM was higher in species consistent with roadway impacts such as road salt (coarse Na, Cl), brake wear (coarse Ba, Cu), and diesel fuel combustion (fine EC). Collectively, these findings show that downwind coarse PM (associated with traffic pollution) elicited a greater inflammatory response in allergic mice than upwind coarse PM, while upwind fine PM (associated with regional scale air pollution) elicited a greater neutrophilic inflammatory response when compared to downwind fine PM. Future studies are needed to better characterize and assess near-road pollutant gradients in different locations and their potential health effects.

Acknowledgements

The authors thank Debora Andrews, Elizabeth Boykin, Lisa Copeland, Mary Daniels, Eugene Gibbs, Richard Jaskot, Kasey Kovalcik, Jim Lehmann, and Judy Richards for technical assistance, and Drs. Janet Burke and Marsha Ward for manuscript review. This project was supported by the EPA-UNC training agreement, Curriculum in Toxicology, University of North Carolina at Chapel Hill (CR83515201-0).

Supporting Information

PM extraction and recovery methods, data of IAR (Table S1), MCh challenge (Table S2), BAL biochemistry (Table S3), study site (Figure S1), OVA serum immunoglobulins (Figure S2), and histopathology (Table S4, Figure S3) are included. This information is available free of charge via the internet at <http://pubs.acs.org/>.

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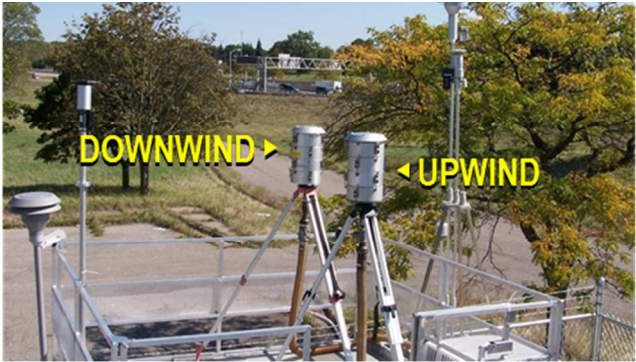
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TOC/Abstract graphic

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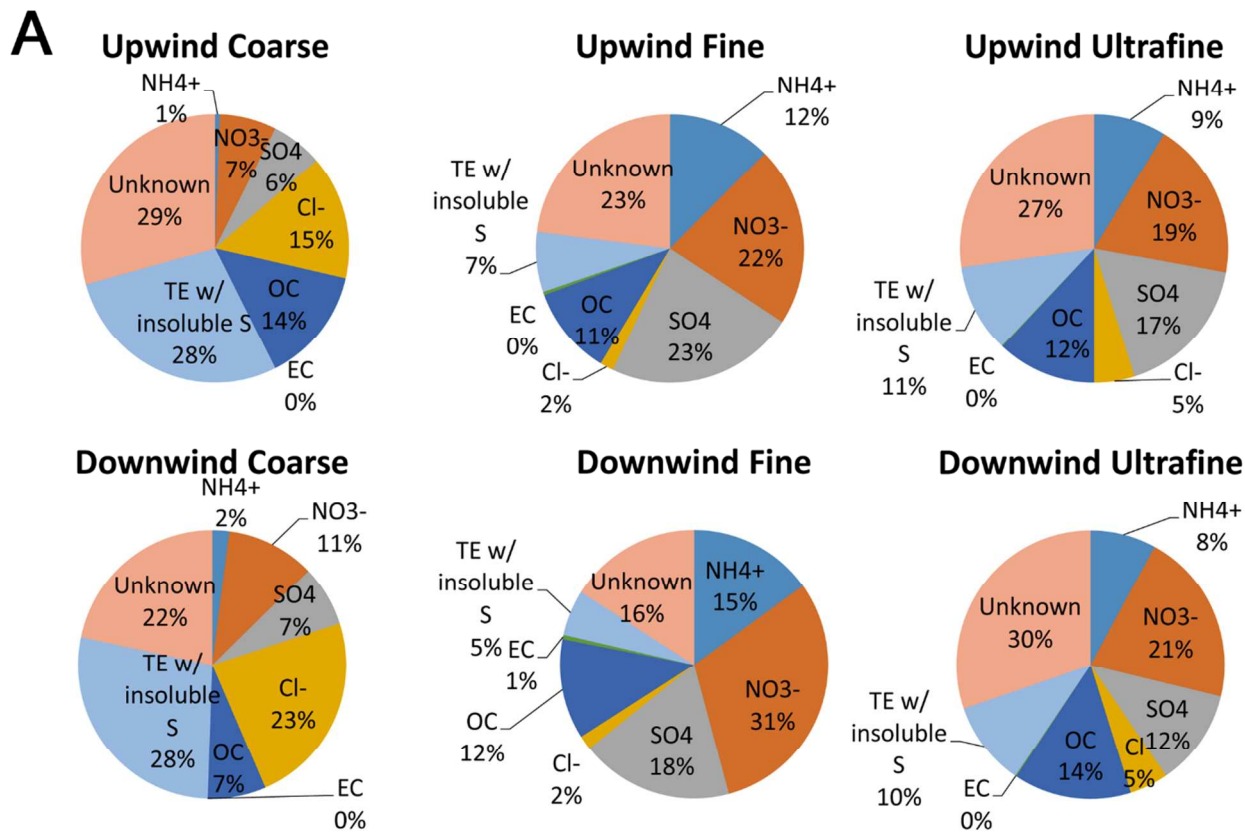
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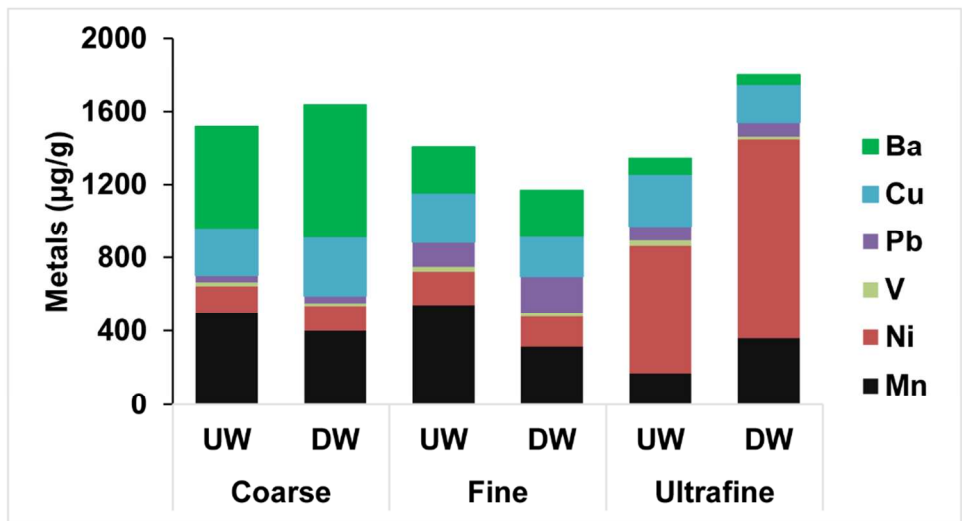
Table 1. Ambient PM concentrations, collected mass, extraction recovery, ion, elemental (by HR-ICP-MS) and carbon species analysis, and endotoxin levels in different PM size fractions collected upwind and downwind at the sampling site near I-96 in Detroit, MI. Mass concentration ($\mu\text{g}/\text{m}^3$) = (Mass (mg))/(flow rate * sampling time (total mins)). Bold font indicates greater value in upwind or downwind direction.

	Coarse Upwind	Coarse Downwind	Fine Upwind	Fine Downwind	Ultrafine Upwind	Ultrafine Downwind
Collected mass (mg)	47.46	80.55	34.05	72.03	31.42	82.68
Mass concentration ($\mu\text{g}/\text{m}^3$)	19	34	13	28	12	30
Recovery (%)	72	82	78	87	77	74
Ionic components ($\mu\text{g}/\text{g}$)						
NO_3^-	68409	107118	216874	307320	190987	208258
SO_4^{2-}	62545	72557	228026	184367	172501	117226
Cl^-	149108	234800	16493	17085	48772	46536
NH_4^+	5881	20599	125108	150867	87677	79420
Na^+	91405	129851	27789	14115	48356	36359
Ca^{2+}	38969	36955	8255	4762	9065	8499
Mg^{2+}	6358	6083	3572	2110	1308	5825
K^+	1994	1388	4055	3495	4428	3771
Elemental constituents ($\mu\text{g}/\text{g}$)						
S (calculated as SO_4^{2-})	113014	123931	249074	209750	229280	182676
Na	121951	163049	27545	14440	53057	44872
Ca	58310	50820	9350	5631	12431	11733
SiO_2	24427	16988	6853	6708	3926	2059
Fe	11665	11528	5001	5327	1015	486
Mg	11536	7205	3796	2349	2758	5762
K	4209	2828	4855	4087	5537	5300
Al	5904	3600	2764	2916	2919	5400
Zn	1497	1342	2262	2482	2430	1828
P	1582	991	934	649	1881	1211
Ni	145	135	183	162	694	1090
Mn	498	400	540	313	170	361
Ba	552	721	251	248	84	48
Cu	262	321	271	221	283	205
Sr	333	294	71	47	66	1
Ti	276	242	104	86	44	20
Pb	39	41	135	202	82	78
Sn	49	48	87	63	8	4
Sb	42	50	47	46	19	16
Cr	50	45	36	37	28	19
Mo	25	27	17	23	17	21
V	22	17	27	21	28	15
As	6	7	21	21	22	16
Se	1	4	21	37	9	16
Cd	2	3	10	3	10	10
Carbon Species						
Total Carbon (%)	14	7	11.1	12.6	12.1	14.3
Organic ($\mu\text{g}/\text{g}$)	139821	70264	107180	120826	119953	142049
Elemental ($\mu\text{g}/\text{g}$)	0	0	3980	5257	1159	1396
Endotoxin (EU/g)	0.84	0.49	0.63	0.64	0.23	0.23

602



B



603

604 **Figure 1. (A)** Mass balances (%) of major classes of chemical components in each size fraction of
605 Upwind and Downwind PM in the saline suspension used for oropharyngeal aspiration on mice. TE
606 w/insoluble S indicates trace elements with insoluble sulfur (calculated as ICPMS SO_4 - IC SO_4). **(B)**
607 Concentrations ($\mu\text{g/g}$) of metals associated with specific sources in each size fraction (UW: Upwind, DW:
608 Downwind).

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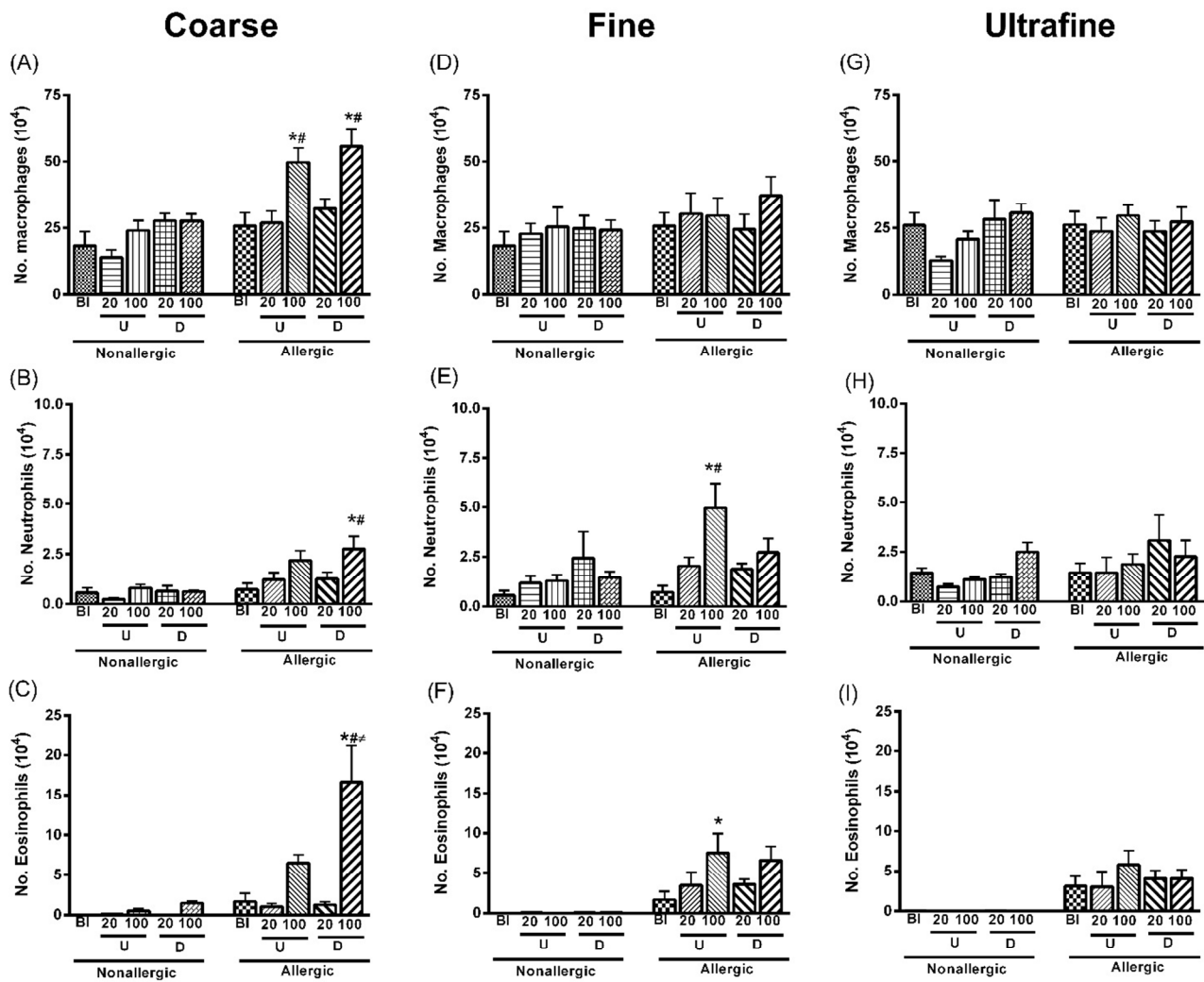
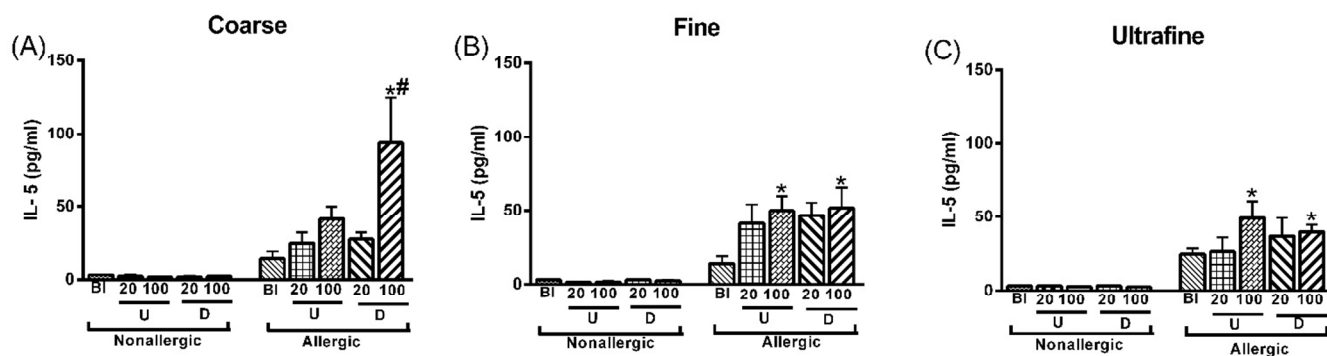


Figure 2. Numbers of BAL alveolar macrophages, neutrophils, and eosinophils recovered 2 days after OVA challenge in non-allergic and allergic mice exposed to 20 or 100 µg Upwind (U) or Downwind (D) coarse (panels A-C), fine (panels D-F) and ultrafine (panels G-I) PM at the challenge phase. Values are means $\pm$ SEM of 6-8 observations. *p< 0.05 versus control non-allergic mice. # p<0.05 versus control allergic mice. ≠ p<0.05 versus upwind allergic mice.



615
 616 **Figure 3.** BAL supernatant IL-5 levels in non-allergic and allergic mice exposed to 20 or 100 µg Upwind
 617 (U) or Downwind (D) Coarse (A), Fine (B) and Ultrafine (C) PM. Values are means $\pm$ SEM of 6-8
 618 observations. * $p < 0.05$ versus control non-allergic mice. # $p < 0.05$ versus control allergic mice.