Lung Toxicity of Ambient Particulate Matter from Southeastern U.S. Sites with Different Contributing Sources: Relationships between Composition and Effects

JeanClare Seagrave,1 Jacob D. McDonald,1 Edward Bedrick,2 Eric S. Edgerton,3 Andrew P. Gigliotti,1 John J. Jansen,4 Lin Ke,5 Luke P. Naehler,6 Steven K. Seilkop,7 Mei Zheng,5 and Joe L. Mauderly1

1Lovelace Respiratory Research Institute, Albuquerque, New Mexico, USA; 2University of New Mexico, Albuquerque, New Mexico, USA; 3Atmospheric Research and Analysis Inc., Cary, North Carolina, USA; 4Southern Company, Birmingham, Alabama, USA; 5Georgia Institute of Technology, Atlanta, Georgia, USA; 6University of Georgia, Athens, Georgia, USA; 7SKS Consulting Services, Siler City, North Carolina, USA

BACKGROUND: Exposure to air pollution and, more specifically, particulate matter (PM) is associated with adverse health effects. However, the specific PM characteristics responsible for biological effects have not been defined.

OBJECTIVES: In this project we examined the composition, sources, and relative toxicity of samples of PM with aerodynamic diameter ≤ 2.5 µm (PM2.5) collected from sites within the Southeastern Aerosol Research and Characterization (SEARCH) air monitoring network during two seasons. These sites represent four areas with differing sources of PM2.5, including local urban versus regional sources, urban areas with different contributions of transportation and industrial sources, and a site influenced by Gulf of Mexico weather patterns.

METHODS: We collected samples from each site during the winter and summer of 2004 for toxicity testing and for chemical analysis and chemical mass balance–based source apportionment. We also collected PM2.5 downwind of a series of prescribed forest burns. We assessed the toxicity of the samples by instillation into rat lungs and assessed general toxicity, acute cytotoxicity, and inflammation. Statistical dose–response modeling techniques were used to rank the relative toxicity and compare the seasonal differences at each site. Projection-to-latent-surfaces (PLS) techniques examined the relationships among sources, chemical composition, and toxicologic end points.

RESULTS AND CONCLUSIONS: Urban sites with high contributions from vehicles and industry were most toxic.

KEY WORDS: chemical mass balance, intratracheal instillation, in vivo, lung, particulate matter, PM2.5, projection to latent surfaces, source apportionment. Environ Health Perspect 114:1387–1393 (2006). doi:10.1289/ehp.9234 available via http://dx.doi.org/ [Online 12 June 2006]

Concern over possible health effects of environmental particulate matter ≤ 2.5 µm (PM2.5) [U.S. Environmental Protection Agency (EPA) 2004] has stimulated numerous studies of its chemical/physical properties, the sources that contribute the most hazardous components, and biological mechanisms for the adverse effects. Although epidemiologic studies indicate that significant effects are often associated with PM2.5 exposure, the magnitude of the effect varies with location. In vitro studies have shown correlations between effects of PM and the contributing sources or composition (Alfaro-Moreno et al. 2002; Aust et al. 2002; Becker et al. 1996; Don Porto et al. 2001; Hatch et al. 1985; Huang et al. 2002; Imrich et al. 2000; Karlsson et al. 2005; Long et al. 2001; Maciejczyk and Chen 2005; Schins et al. 2004). Furthermore, in vivo studies have shown that effects of inhaled concentrated ambient particles (CAPS) vary with the daily CAPS composition (Clarke et al. 2000; Ghio and Huang 2004; Gurgueira et al. 2002; Saldiva et al. 2002; Schins et al. 2004), but such studies are limited to variation in composition and effect at single sites as a function of time. Other studies have examined the in vitro (Li et al. 2003) or in vivo (Dick et al. 2003) effects as functions of particle size. A well-known series of experiments compared the effects of materials collected from the Utah Valley during periods of operation or closure of a local steel mill (reviewed by Ghio 2004). Becker et al. (2005) examined the in vitro effects of PM from a single site as a function of season, and an epidemiologic study examined seasonal differences across 100 U.S. cities (Peng et al. 2005). However, few studies have directly compared the effects of ambient respirable PM from different locations in vivo (Gavett et al. 2003; Hatch et al. 1985). Such studies are critical to rational regulation of PM based on source/composition/toxicity relationships rather than size alone.

In the present study we used intratracheal instillation to compare toxicity of PM2.5 collected during summer or winter from four sites with different contributing sources. This technique, although a nonphysiologic method of administration, is useful for comparative studies in which the nature of collected samples precludes inhalation exposures (McDonald et al. 2004; Seagrave et al. 2002). We did not include in vitro analyses because we observed poor correlations with in vivo results for a series of engine exhaust samples (Seagrave et al. 2003).

The selected sites within the Southeastern Aerosol Research and Characterization (SEARCH) network represented a range of urban to rural areas with different contributing PM sources (Hansen et al. 2003). We collected PM2.5 during two seasons and performed source apportionment for these samples using the chemical mass balance (CMB) receptor model (Zheng et al. 2002). In addition to the SEARCH sites, we evaluated the toxicity of a sample collected downwind from a series of prescribed forest burns (smoke). Assessment of toxicity/site/composition relationships included relative toxicity rankings by site and projection-to-latent-surfaces (PLS) analysis (McDonald et al. 2004).

Materials and Methods

Site description. The selected sites represented a range of urban to rural areas in the southeastern United States with different contributing PM sources as previously described by Hansen et al. (2003). Briefly, the Birmingham, Alabama (BHM), site was an undeveloped building lot in an urban area, 3 km north of the downtown area (courthouse), within a few kilometers of heavy transportation and industry, including a coke production facility. The Jefferson Street, Atlanta, Georgia (JST), site was also an urban site located 4.2 km northwest of downtown Atlanta, amid parking lots, city streets, warehouses, and storage and within 250 m of a bus maintenance facility. The Pensacola, Florida (PNS), site was mixed urban and residential, near an elementary school, and 4.7 km from the Gulf of Mexico.

Address correspondence to J. Seagrave, Lovelace Respiratory Research Institute, 2425 Ridecrest SE, Albuquerque, NM 87108 USA. Telephone: (505) 348-9499. Fax: (505) 348-8567. E-mail: jseagrave@LRRI.org

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whereas the Centreville, Alabama (CTR), site was rural and forested, proximal to the Talladega National Forest.

Sample collection and processing. We collected ambient PM$_{2.5}$ for toxicity testing on Teflon filters using high-volume samplers and extracted them by sonication, first with a 9:1 acetone:dichloromethane mixture and then with purified water. Both fractions were concentrated and combined to produce a 1:1 (vol/vol) acetone:aqueous mixture, thus reconstituting the atmospheric ratio of constituents. An extract control sample from unexposed filters was processed identically. Additional details of these methods are presented in Supplemental Material (available online at http://www.ehponline.org/docs/2006/9234/suppl.pdf).

Chemical characterization of atmospheres and extracted samples. We collected parallel air samples to determine average atmospheric concentrations for each site/season and to estimate mass and species available for extraction on the filters for the toxicity testing. Briefly, we measured PM$_{2.5}$ mass gravimetrically, trace elements by X-ray fluorescence (Hansen et al. 2003), sulfate and nitrate by ion chromatography, and ammonium by automated colorimetry. Organic carbon (OC) and elemental carbon (EC) were analyzed by thermal-optical reflectance at Desert Research Institute (Reno, Nevada) (Chow et al. 2001). Organic compounds were analyzed by gas chromatography/mass spectrometry (GC/MS) (Zheng et al. 2002). The Supplemental Material (available online at http://www.ehponline.org/docs/2006/9234/suppl.pdf) provides additional information on these methods.

The extracts generated for toxicity testing were also analyzed for selected constituents shown in previous studies to discriminate among sources.

Source apportionment. We performed source apportionment based on the atmospheric chemistry using a CMB method previously described by Zheng et al. (2002). Briefly, chemical profiles of well-defined aerosol source emissions were defined by separate analyses. The chemical composition of the sample was then determined, and equations corresponding to linear combinations of the source profiles were solved using an effective variance-weighted least-squares analysis technique (Watson et al. 1984, 2001). The sources considered important for these sites included emissions from diesel and gasoline engines, wood combustion, paved road dust, meat cooking, vegetative detritus, natural gas combustion, and emissions from coke facilities (Zheng et al. 2006). Source profiles for wood combustion and paved road dust were modified as appropriate for the local composition of these sources (Zheng et al. 2002). Time-resolved and spatially resolved analyses of the sources of PM$_{2.5}$ at the SEARCH sites are published separately (Zheng et al. 2006).

Measurement of in vivo toxicity. Animals. Charles River Laboratories (Wilmington, MA) supplied the 8 x 1-week-old male F344/Crl BR rats, which were quarantined for 3 weeks and confirmed free of common pathogens by serology. The rats, housed two per cage under a controlled light/dark cycle, temperature, and relative humidity conditions, had ad libitum access to food (Harlan Teklad Lab Blox; Harlan Teklad, Madison, WI) and water. The Institutional Animal Care and Use Committee approved all animal work, assuring humane use with regard for alleviation of suffering.

Reagents and supplies. All chemicals were obtained from Sigma Chemical Company (St. Louis, MO) unless otherwise specified. Acetone (optima grade) and dichloromethane (HPLC/GC-MS grade) were obtained from Fisher Scientific (Fairlawn, NJ).

Sample preparation. We prepared PM$_{2.5}$ suspensions and the extract control for instillation as previously described (Seagrave et al. 2002) as suspensions in vehicle (0.9% NaCl/1% acetone/0.01% Tween-80), with dilutions in the same vehicle. To confirm similar responsiveness among the different experimental series, we used National Institute of Standards and Technology (NIST; Gaithersburg, MD) standard reference material 2975 (forklift diesel soot) suspended in vehicle.

Intratracheal instillation. We instilled anesthetized rats (5% halothane in oxygen with nitrous oxide) with a sample or control material in 0.5 mL via a trans-oral cannula and returned them to their cages after recovery from anesthesia.

Each experimental series consisted of two samples at three doses (0.75, 1.5, and 3 mg/rat), the extract control, and the NIST diesel soot positive control, with five rats per dose. Because a significant fraction of each sample is soluble material, these doses would not be expected to cause overload phenomena (Oberdorster 1995). In addition, one series also included a group of uninstilled control rats.

Euthanasia and processing. We killed the rats with Euthasol (Virbac Labs, Fr. Worth, TX) 24 hr after instillation [the time of the maximal inflammatory and cytotoxic effects (Seagrave et al. 2002)] and recorded their body weights. Processing, lavage of the right lung lobes, and fixation were as previously described (Seagrave et al. 2002).

We evaluated total lavage cells using a hemacytometer and differential cell counts on Wright-Giemsa–stained cyt centrifuge preparations (Seagrave et al. 2002). We analyzed cell-free lavage fluid for lactate dehydrogenase (LDH) (Gay et al. 1968), total protein (Watanabe et al. 1986), and alkaline phosphatase (APase) using a Hitachi 911 (Roche Diagnostics, Basel, Switzerland) autoanalyzer.

A board-certified veterinary pathologist (A.P.G.) graded the lung histopathology. In accordance with guidelines of the Society of Toxicologic Pathologists (Crisman et al. 2004), we did not attempt a “blinded” evaluation. Furthermore, foreign matter was obvious in the lungs of treated animals. Responses were graded using a scale from 0 (normal) to 5 (extreme pathology: severe and widespread presence of a particular response/diagnosis). Each rat received scores summarizing responses in cytotoxic or inflammatory categories and a total score as previously described (Seagrave et al. 2002).

Statistical analysis of toxicity data. We graphed the dose–response relationship for each sample. Responses to the extract control were similar for the series of experiments done for the winter and smoke samples, but these were slightly different from the responses to the extract control prepared in the experimental series to test the summer samples. Baseline values for the two series were therefore considered separately. As previously described, we fit an exponential function to the toxicity data and used the exponent of the equation (“potency factor”) to compare the toxicity of the samples (Seagrave et al. 2002). Using the entire dose–response curve provides substantially more statistical power to discriminate among samples than do individual dose-to-dose comparisons.

We evaluated differences among samples for each end point using p-values from pairwise F-tests, adjusted for multiple comparisons using the modified Bonferroni procedure of Hochberg (1988), with p = 0.05 as the criterion for statistical significance.

PLS analysis. We used SIMCA (version 8; Umetrics Inc., Kinnelon, NJ) to perform a PLS analysis on the SEARCH site samples with the mass fractions of chemical classes as predictors and the toxicologic potency factors as responses. Because detailed organic specification was not performed on the smoke sample, this sample was not included in the analysis. Table 1 shows the simplified organic composition classes used as predictors. OC was also considered as a separate predictor element, along with EC, ammonium, NO$_3^-$, SO$_4^{2-}$, arsenic, bromine, copper, manganese, lead, selenium, titanium dioxide, zinc, and a composite of metal oxides collectively referred to as major metal oxides (MMOs). Data were centered and scaled to unit variance before analysis. A second iteration of the analysis used the CMB-attributed sources as predictors. In the PLS analysis, the fraction of the total variation ($R^2$) in the toxicologic responses and chemical constituent predictors was assessed for each component. A cross-validated cumulative prediction accuracy measure ($Q^2$) was used to select the optimal number of components for the final models. Loading plots visually display...
the relationship between the predictors and responses as functions of the PLS components with the highest predictive capacity.

Results

Atmospheric chemistry. Analysis of the atmospheric chemistry showed both season- and site-related differences (Figure 1A,B). SO$_4^{2-}$, aluminum oxide (Al$_2$O$_3$), and silicon dioxide (SiO$_2$) were higher at all sites during the summer, whereas OC, NO$_3^-$, and potassium oxide (K$_2$O) were higher in winter. BHM-winter, BHM-summer, and JST-winter had the highest EC and ferric oxide (Fe$_2$O$_3$) levels. BHM-summer also had the highest levels of MMOs. The smoke sample contained predominantly OC; the only significant MMO in this sample was K$_2$O.

Figure 1C shows the major classes of organic compounds as a percentage of the total mass. The organic mass (OM) fraction was higher in all sites in the winter. PNS-winter exhibited the highest fraction of many organic-compound classes, including alkane and aromatic diacids, branched alkanes, carboxylic acids, cholesterol, levoglucosan (LG), nonanal, and resin acids. However, BHM-winter had the highest levels of polycyclic aromatic hydrocarbons (PAHs), followed by BHM-summer, JST-winter, and PNS-winter. Cholesterol was highest at the PNS and JST sites, whereas hopanes and steranes were highest in JST-winter and BHM-winter, followed by PNS-winter and BHM-summer. The pattern for branched and straight alkanes was similar: highest in BHM-winter followed by the JST- and PNS-winter. CTR was noteworthy in having the lowest levels of n- and branched alkanes, hopanes and steranes, alkane and aromatic diacids, and PAHs in both seasons, but in the summer it had the highest resin acids.

Source apportionment. Figure 2 shows results of the CMB analysis for the SEARCH sites. As expected, wood smoke and secondary NO$_3^-$ contributed more mass to the winter samples. In contrast, summer samples contained more secondary SO$_4^{2-}$. Diesel exhaust was a minor component of the CTR and PNS samples (both seasons) but contributed substantially to the mass in the urban/industrial sites, especially BHM-winter. Gasoline emissions were also quite high in BHM-winter and JST-winter. Meat cooking contributed more to the mass in the winter, except for BHM, whereas road dust was significant only in the summer. Unidentified OM (other OM), which includes secondary organic aerosol, was substantial in all sites in both seasons but was generally greater in summer.

Sample chemistry. Mass recovered in the extracts for toxicity testing averaged 60% of the total mass estimated from the filter loading of parallel filters collected for the chemical

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Table 1. Chemical classes and key sources.

| Compound            | Source                                      |
|---------------------|---------------------------------------------|
| Organic             |                                             |
| n-Alkanes           | Vegetative detritus, vehicles (diesel)       |
| Branched alkanes    | Vegetative detritus, motor vehicles         |
| Alkane diacids      | Secondary organic aerosol                   |
| Aromatic diacids    | Secondary organic aerosol                   |
| Benzo(a)anthracene-7-one | Coke, other combustion                |
| Carboxylic acids    | Combustion sources, vegetative detritus, microbes |
| Cholesterol         | Meat cooking                                |
| Hopanes and steranes| Vehicle emissions, lube oil                 |
| LG                  | Wood combustion                             |
| Nonanal             | Meat cooking                                |
| PAHs                | Combustion (wood, coke, motor vehicles)     |
| Resin acids         | Wood combustion                             |
| DC                  | Combustion (wood, meat, motor vehicles)     |
| Inorganic           | Agriculture/livestock and gasoline exhaust  |
| Ammonium            |                                             |
| EC                  | Diesel, other combustion                    |
| MMOs and other metals| Resuspended (road dust)                    |
| Manganese           | Motor vehicles and road dust                |
| NO$_3^-$            | Combustion (wood, meat, motor vehicles, coal)|
| Lead                | Motor vehicles and road dust                |
| SO$_4^{2-}$         | Combustion (coal, motor vehicles, others)   |
| Zinc                | Motor vehicles and road dust                |

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Figure 1. Atmospheric chemistry varies as a function of site and season. (A) Major classes of components (OC is not corrected for total OM). (B) Metals and MMOs. (C) Identified organic classes.
analyses with a somewhat lower recovery from the smoke sample. The organic solvent extracted a larger fraction of the collected mass for the winter and smoke samples, whereas the aqueous extract contained more of the mass from the summer samples (Figure 3A).

NO$_3^-$ was not detected in any of the winter extracts, possibly due to losses during storage between sample extraction and analysis (Schaap et al. 2006). Examples of the recovered mass relative to the predicted mass for selected inorganic and organic analytes are presented in Figure 3B and C. Not surprisingly, the largest discrepancies were observed in analytes with the lowest starting masses (e.g., LG in the summer samples). Recovery of MMOs was around 50% for all samples. Recoveries > 100% were occasionally observed, possibly due to methodologic differences. However, the range of recoveries was rarely > 2-fold, whereas the range in actual mass among the different samples was much greater, and thus the rank order of the samples was usually preserved through the extraction process.

Toxicity. Figures 4 and 5 show the potency factors for the inflammatory (including lung weight:body weight ratio) and cytotoxic parameters, respectively. Among the samples collected in the winter, JST-winter caused significantly more toxicity (LDH, APase, total protein, and histopathologic cytotoxicity and increases in lung weight) than the other winter samples. BHM-winter was the second most potent for these indicators except histopathology and was significantly more potent than PNS-winter or CTR-winter for increases in APase and total protein. JST-winter also most potently induced inflammation. It was significantly more potent than CTR-winter, PNS-winter, and smoke for total cells, neutrophils, macrophages, and lung weight:body weight ratio and significantly greater than smoke for lymphocytes and histopathologic indication of inflammation. Although JST-winter was not significantly different from BHM-winter, BHM-winter was significantly more potent than the other samples for total cells and significantly more potent than smoke for neutrophils, macrophages, and histopathologic inflammation. PNS-winter caused a statistically significantly negative potency for macrophages. The smoke sample had a similar effect that did not reach statistical significance.

There were smaller differences among the summer samples. Among the toxicity indicators, only APase demonstrated significant differences among the samples: JST-summer and PNS-summer suppressed this enzyme activity. All summer samples significantly increased neutrophils, with BHM-summer being significantly more potent than CTR-summer and PNS-summer. BHM-summer also significantly increased macrophages, although the response was not significantly different from the other summer samples.

Interestingly, JST-summer was significantly less potent than JST-winter for all end points. The potency of BHM-summer was also less than BHM-winter for most end points, but only the effect on protein reached statistical significance. In contrast, CTR-summer was more potent than CTR-winter.
for lymphocytes. Similarly, BHM- and PNS-summer increased lymphocytes more than the corresponding winter samples, although the differences were not statistically significant. The only significant difference between BHM-winter and BHM-summer was the greater suppression of APase by the summer sample.

**Discussion**

This study showed that the biological effects of intratracheal instillation of equivalent masses of PM$_{2.5}$ differ as a function of site and season, thus implicating specific constituents and/or sources in its effects. Although this is intuitively reasonable and is supported by other experimental evidence, current air quality regulations are based only on mass in specific size fractions. Identification of the most potent constituents should lead to more targeted regulation to protect populations at risk.

Intratracheal instillation of collected and extracted samples has limitations, including the high doses usually used. Furthermore, the non-physiologic route of administration results in deposition of all particle sizes with the same spatial distribution, which may be nonuniform and different from that achieved by inhalation. However, this method is very useful for preliminary screening studies for direct comparisons of multiple materials (Costa et al. 2006; Driscoll et al. 2000; Seagrave and McDonald 2004; Warheit et al. 2005). Another limitation is that recovery of the mass from filters used to collect the PM$_{2.5}$ is rarely 100% efficient. Therefore, if the extraction is selective, leaving behind more or less toxic constituents should lead to more targeted regulations are based only on mass in specific size fractions. Experimental evidence, current air quality regulations are based only on mass in specific size fractions. Identification of the most potent constituents should lead to more targeted regulation to protect populations at risk. Environmental Health Perspectives • VOLUME 114 • NUMBER 9 • September 2006
constituents, the toxicity results may underestimate or overestimate (respectively) the toxicity of the original material. It is therefore important to optimize the extraction and, where possible, compare the composition of the extracted material with the original filter samples. Extraction of these ambient samples included a secondary aqueous extraction not included in the previous studies of engine emission samples (Seagrave et al. 2002, 2005a). The aqueous extract contained substantial additional mass, particularly for the summer samples, most likely due to increased NH$_4$SO$_4$ in these samples. However, interpretation must be tempered by the fact that 100% recovery was not achieved.

Wood smoke can be a significant contributor to ambient PM$_{2.5}$ mass, especially in the winter. Previous studies have indicated potential health effects of relatively high concentrations of smoke (Barrett et al. 2006; Boman et al. 2003; Burchiel et al. 2005; Park et al. 2004; Seagrave et al. 2005b; Tesfagzi et al. 2002, 2005; Zelikoff et al. 2002). However, we observed little toxicity from the smoke sample, which consisted of relatively fresh smoke from prescribed forest burns (primarily smoke from forest understory: live or dead branches, stumps, leaves, pine needles, shrubs, and grass). In contrast, the wood smoke in the SEARCH site samples was most likely from aged fireplace and woodstove emissions. Given the lack of effect of the smoke sample, in combination with the fact that neither the chemicals associated with wood smoke nor the wood smoke source from the CMB apportionment correlated with the toxicity in the PLS analyses, it seems unlikely that wood smoke PM$_{2.5}$ contributed significantly to the toxicologic responses.

The winter samples from the two more urban/industrial sites produced the greatest responses, with JST-winter being significantly more potent than BHM-winter for several of the cytotoxicity responses. BHM-summer and BHM-winter were similar in potency, but JST-summer was significantly less potent than JST-winter for most end points. The ambient composition for the sites from which the most potent samples were collected includes higher levels of EC, $n$-alkanes, hopanes and steranes, and NO$_3$. However, NO$_3$ was not detectable in the winter extracts, so it is unlikely that NO$_3$ could have contributed to the toxicity. PAHs were also higher in both BHM samples, but JST-winter and PNS-winter were similar for this class of chemicals.

Source apportionment suggested that the three most potent samples include more PM$_{2.5}$ from diesel and gasoline exhaust. The impact of these emissions is supported by the PLS analysis.

A limitation to these PLS analyses is the poor prediction capacity of $Q^2$, which reflects the sensitivity of the analysis to inclusion of individual samples and the large number of chemical constituent predictor variables relative to the small number of samples (eight). In addition, poor prediction capacity could also indicate that the most toxic constituents were not measured or that variation in extraction efficiency interfered with the composition/toxicity correlation. Although PLS analysis using the attributed sources introduces an additional level of uncertainty, the results of this analysis generally support the analysis using the primary chemical composition.

In summary, this study supports the concept that PM$_{2.5}$ composition affects its toxicity. Specifically, the most toxic samples were from the sites during seasons with the largest contributions of diesel and gasoline emissions, whereas wood burning was only weakly correlated with toxicity end points. The PLS analysis also indicated that SO$_4^{2-}$, secondary organic aerosols, meat cooking, and vegetative detritus were not correlated with the biological responses.

**Figure 7.** PLS analysis based on source apportionment. (A) Loading plot showing relationships between predictors (sources) and responses (toxicity end points) based on a two-component model. Observed versus predicted responses for (B) total cells and (C) lavage LDH. Abbreviations: S, summer; W, winter.

**References**

Alfaro-Moreno E, Martinez L, Garcia-Cueilar C, Bonner JC, Murray JC, Rosas I, et al. 2002. Biologic effects induced in vitro by PM$_{2.5}$ from three different zones of Mexico City. Environ Health Perspect 110:715–720.

Aust AE, Ball JC, Hu AA, Lighty JS, Smith KR, Straccia AM, et al. 2002. Particle characteristics responsible for effects on human lung epithelial cells. Res Respir Health Eff Inst 110:1–65.

Barrett ED, Henson RD, Selikoff SK, McDonald JD, Reed MD. 2006. Effects of hardwood smoke exposure on allergic airway inflammation in mice. Inhal Toxicol 18:33–43.

Becker S, Dailey LA, Soukup JM, Grambow SC, Devlin RB, Huang YC. 2005. Seasonal variations in air pollution particle-induced inflammatory mediator release and oxidative stress. Environ Health Perspect 113:1032–1038.

Becker S, Soukup JM, Dilmur MR, Devlin RB. 1996. Stimulation of human and rat alveolar macrophages by urban air particulates: effects on oxidant radical generation and cytokine production. Toxicol Appl Pharmacol 141:637–648.

Boman EC, Forsberg AB, Jarvholm BG. 2003. Adverse health effects from ambient air pollution in relation to residential wood combustion in modern society. Scand J Work Environ Health 29:251–260.

Burchiel SW, Lauer FT, Dunaway SL, Zsivadi J, McDonald JD, Reed MD. 2005. Hardwood smoke alters murine splenic T cell responses to mitogens following a 6-month whole body inhalation exposure. Toxicol Appl Pharmacol 202:229–236.

Chow JC, Watson JD, Crow D, Lowenthal DH, Merrifield T, 2001. Comparison of IMPROVE and NIOSH carbon measurements. Aerosol Sci Technol 34:23–34.

Clarke RW, Coull B, Reinsch U, Catalano P, Killingsworth DR, Koutrakis P, et al. 2000. Inhaled concentrated ambient particles are associated with hematologic and bronchoalveolar lavage changes in canines. Environ Health Perspect 108:1179–1187.

Costa DL, Lehmann JR, Winsett D, Richards J, Ledbetter AD, Dreher KL. 2006. Comparative pulmonary toxicological assessment of oil combustion particles following inhalation or instillation exposure. Toxicol Sci 91:237–246; doi:10.1093/toxsci/kfj123 [Online 31 January 2006].

Crissman JW, Goodman DG, Hildebrandt PK, Maronpot RR, Prater DA, Riley JH, et al. 2004. Best practices guideline: toxicologic histopathology. Toxicol Pathol 32:126–131.

Dick DA, Singh P, Daniels M, Evansky P, Becker S, Dilmur MJ. 2003. Murine pulmonary inflammatory responses following instillation of size-fractionated ambient particulate matter. J Toxicol Environ Health A 66:2193–2207.
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Dan Porto CA, Hoet PH, Verschaeve L, Schoeters G, Nemery B. 2001. Genotoxic effects of carbon black particles, diesel exhaust particles, and urban air particulates and their extracts on a human alveolar epithelial cell line (A549) and a human monocytic cell line (THP-1). Environ Mol Mutagen 37:155–163.

Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, et al. 2000. Intratracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. Toxicol Sci 55:24–35.

Gavett SH, Haykal-Coates N, Copeland LB, Heinrich J, Gilmour M. 2003. Metal composition of ambient PM$_2.5$ influences severity of allergic airways disease in mice. Environ Health Perspect 111:1471–1477.

Gay RJ, McComb RB, Bowers GN Jr. 1968. Optimum reaction conditions for human lactate dehydrogenase isoenzymes as they affect total lactate dehydrogenase activity. Clin Chem 14:740–753.

Ghio AJ. 2004. Biological effects of Utah Valley ambient air particles in humans: a review. J Aerosol Med 17:157–164.

Ghio AJ, Huang YC. 2004. Exposure to concentrated ambient particles (CAPs): a review. Inhal Toxicol 16:53–59.

Gurgueira SA, Lawrence J, Coull B, Murthy GG, Gonzalez-Flecha B, Lawrence J, Coull BA, Stearns RC, Lawrence J, Coull BA, Stearns RC. 2005. Health effects of subchronic exposure to low levels of wood smoke in rats. Toxicol Sci 85:505–513.

Hansen DA, Edgerton ES, Hartsell BE, Jansen JJ, Kadasamy N, Gilmour M, Heinrich J, Gilmour M. 2003. Metal composition of ambient PM$_2.5$ influences severity of allergic airways disease in mice. Environ Health Perspect 111:1471–1477.

Huang SL, Cheng WL, Lee CT, Huang HC, Chan CC. 2002. Ultrafine particle pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect 111:455–460.

Long CM, Suh HH, Kobak L, Catalano PJ, Ning YH, Kouttrakis P. 2001. A pilot investigation of the relative toxicity of indoor and outdoor fine particles: in vitro effects of endotoxin and other particulate properties. Environ Health Perspect 109:1019–1026.

Maciejczyk P, Chen L. 2005. VIII. Source-related daily variations in in vitro responses to CAPs. Inhal Toxicol 17:243–253.

McDonald JD, Ede I, Seagrave J, Zielinska B, Whitney K, Lawson DR, et al. 2004. Relationship between composition and toxicity of motor vehicle emission samples. Environ Health Perspect 112:1527–1538.

Oberdorster G. 1995. Lung particle overload: implications for occupational exposures to particles. Regul Toxicol Pharmacol 21:123–135.

Park MS, Cancio LC, Jordan BS, Brinkley WW, Rivera VR, Dubick MA. 2004. Assessment of oxidative stress in lungs from sheep after inhalation of wood smoke. Toxicology 195:97–112.

Peng RD, Dominici F, Pastor-Barriuso R, Zeger SL, Samet JM. 2005. Seasonal analyses of air pollution and mortality in 100 US cities. Am J Epidemiol 161:585–594.

Saldive PH, Clarke RW, Coull BA, Stearns RC, Lawrence J, Murthy GG, et al. 2002. Lung inflammation induced by concentrated ambient air particles is related to particle composition. Am J Respir Crit Care Med 165:1610–1617.

Schaap M, Spindler G, Schulz M, Acker K, Maenhaut W, Berner A, et al. 2006. Artefacts in the sampling of nitrate studied in the “INTERCOMP” campaigns of EUROTRAC-AEROSOL. Atmos Environ 40:6487–6496.

Schins RP, Lightbody JH, Birm PJ, Shi, Donaldson K, Stone V. 2004. Inflammatory effects of coarse and fine particulate matter in relation to chemical and biological constituents. Toxicol Appl Pharmacol 195:1–11.

Seagrave J, Gigliotti A, McDonald JD, Seilkop SK, Whitney K, Zielinska B, et al. 2005a. Composition, toxicity, and mutagenicity of particulate and semivolatile emissions from heavy-duty compressed natural gas-powered vehicles. Toxicol Sci 87:232–241.

Seagrave JC, McDonald JD. 2004. Respiratory toxicity testing: alternatives to inhalation exposure. In: Effects of Air Contaminants on the Respiratory Tract: Interpretations from Molecular to Meta-analysis (Heinrich U, ed). Stuttgart:Franhofer/IRG Verlag, 209–217.

Seagrave JC, McDonald JD, Gigliotti AP, Nikula KJ, Seilkop SK, Gurewich M, et al. 2002. Mutagenicity and in vivo toxicity of combined particulate and semivolatile organic fractions of gasoline and diesel engine emissions. Toxicol Sci 70:212–226.

Seagrave J, McDonald JD, Reed MD, Seilkop SK, Mauderly JL. 2005b. Responses to subchronic inhalation of low concentrations of diesel exhaust and hardwood smoke measured in rat bronchoalveolar lavage fluid. Inhal Toxicol 17:657–670.

Seagrave JC, Seilkop SK, Mauderly JL. 2003. In vitro relative toxicity screening of combined particulate and semivolatile organic fractions of gasoline and diesel engine emissions. J Toxicol Environ Health 66:1113–1122.

Tesfaigzi Y, McDonald JD, Reed MD, Singh SP, De Sanctis GT, Eynott PR, et al. 2005. Low-level subchronic exposure to wood smoke exacerbates inflammatory responses in allergic rats. Toxicol Sci 88:505–513.

Tesfaigzi Y, Singh SP, Foster JE, Kubatsko J, Barr LP, Fine PM, et al. 2002. Health effects of subchronic exposure to low levels of wood smoke in rats. Toxicol Sci 65:115–125.

U.S. EPA. 2004. The Particle Pollution Report: Current Understanding of Air Quality and Emissions through 2003. Research Triangle Park, NC:U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards. Available: http://www.epa.gov/airtrends/pm.html [accessed 25 February 2006].

Warheit DB, Brock WJ, Lee KP, Webb TR, Reed KL. 2005. Comparative pulmonary toxicity inhalation and instillation studies with different TiO$_2$ particle formulations: impact of surface treatments on particle toxicity. Toxicol Sci 88:514–524.

Watanabe N, Kami S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K, et al. 1986. Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin Chem 32:1551–1554.

Watson JG, Chow JC, Fujita EM. 2001. Review of volatile organic compound source apportionment by chemical mass balance. Atmos Environ 35:1927–1934.

Watson JG, Cooper JA, Huntzicker J. 1984. The effective variance weighting for least squares calculations applied to the mass balance receptor model. Atmos Environ 18:1347–1355.

Zelikoff JT, Chen LC, Cohen MD, Schlesinger RB. 2002. The toxicology of inhaled woodsmoke. J Toxicol Environ Health B Crit Rev 5:269–283.

Zheng M, Cass GR, Schauer JJ, Edgerton ES. 2002. Source apportionment of PM$_2.5$ in the southeastern United States using solvent-extractable organic compounds as tracers. Environ Sci Technol 36:2381–2387.

Zheng M, Ke L, Edgerton ES, Schauer JJ, Dong M, Russell AG. 2006. Spatial distribution of carbonaceous aerosol in the southeastern United States using molecular markers and carbon isotope data. J Geophys Res 111:D10S06; doi:10.1029/ 2005JD006777 [Online 31 May 2006].