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Effects of Metals within Ambient Air Particulate Matter (PM) on Human Health

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We review literature providing insights on health-related effects caused by inhalation of ambient air particulate matter (PM) containing metals, emphasizing effects associated with in vivo exposures at or near contemporary atmospheric concentrations. Inhalation of much higher concentrations, and high-level exposures via intratracheal (IT) instillation that inform mechanistic processes, are also reviewed. The most informative studies of effects at realistic exposure levels, in terms of identifying influential individual PM components or source-related mixtures, have been based on (1) human and laboratory animal exposures to concentrated ambient particles (CAPs), and (2) human population studies for which both health-related effects were observed and PM composition data were available for multipollutant regression analyses or source apportionment. Such studies have implicated residual oil fly ash (ROFA) as the most toxic source-related mixture, and Ni and V, which are characteristic tracers of ROFA, as particularly influential components in terms of acute cardiac function changes and excess short-term mortality. There is evidence that other metals within ambient air PM, such as Pb and Zn, also affect human health. Most evidence now available is based on the use of ambient air PM components concentration data, rather than actual exposures, to determine significant associations and/or effects coefficients. Therefore, considerable uncertainties about causality are associated with exposure misclassification and measurement errors. As more PM speciation data and more refined modeling techniques become available, and as more CAPs studies involving PM component analyses are performed, the roles of specific metals and other components within PM will become clearer.

Metals that are components of ambient air particulate matter (PM), and especially some of those that are within the fine PM (FPM) fraction, have been cited as PM components that are most likely to be toxic. The focus has often been on transition metals such as iron (Fe), vanadium (V), nickel (Ni), chromium (Cr), copper (Cu), and zinc (Zn) on the basis of their ability to generate reactive oxygen species (ROS) in biological tissues. Most of the evidence pointing to the biological effects of metals has come from studies involving exposures to laboratory animals in vivo, or to cells in vitro. We know of no studies involving exposures to laboratory animals in vivo, or to cells in vitro to pure metals and their compounds at doses with environmental relevance, that have been positive. On the other hand, some studies using high FPM dose exposures to source-related mixtures containing multiple metals, such as residual oil fly ash (ROFA), coal fly ash (CFA), and concentrated ambient particles (CAPs) have produced effects that appear to be related to their relatively low metals contents. Brief reviews of such studies are presented in the third, fourth, and fifth sections of this article. However, it has been difficult to determine the roles played by the individual metals in the effects observed. Also, most of the ROFA, CFA, and CAPs studies have used relatively high doses, and the relevance of the effects observed to human ambient air exposures at much lower FPM mass levels is therefore uncertain. While effects found in high-dose laboratory exposures have occasionally been suggested to also occur with exposures to near ambient concentrations (e.g., inflammatory indicators in the CAPs study of Maciejczyk & Chen, 2005), more often those effects have not been found at exposures near ambient concentrations (e.g., no abnormal levels of cytokines in human volunteers in the CAPs exposure study of Ghio et al., 2000).

Studies of the effects of relatively low concentrations of airborne metals in humans have also been limited to complex mixtures containing metals. These include those short-term exposures to (1) CAPs in healthy human volunteers and (2) those time-series studies of large human populations where

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data from simultaneous PM compositional analyses were also available. Due to the limitations of statistical power in such studies, the epidemiological analyses have focused more on the identifying the contributions to the effects of factors or source-related mixtures than of individual metals or other components within the mixtures. Clinical laboratory studies in humans have involved instillation of particle suspensions into human lungs and subsequent analyses of bronchoalveolar lavage fluid (BALF) samples for particle retention and biomarkers of effects.

It is important to recognize that ambient air FPM is a chemically nonspecific pollutant, and may originate from, or be derived from, various emission source types. Thus, FPM toxicity may well vary, depending on its source and chemical composition. If the FPM toxicity could be associated with specific source signatures, then health effects research could be better focused on specific FPM components that come from those sources, and specific biological mechanisms could be postulated for further consideration by toxicological studies. FPM health effects research is therefore now being increasingly focused on source apportionment of FPM using chemical speciation data, and this review of the metals literature is limited to those studies that used PM compositional data to identify associations of exposures to source categories or to individual metal components that have been associated with health-related effects.

This review article consists of: (1) an Introduction; and sections discussing peer-reviewed papers on (2) concentrations and sources of trace metal components of ambient air PM; (3) clinical studies in humans; (4) epidemiologic studies in human populations; (5) animal toxicology studies; (6) in vitro studies and mechanisms of metal induced biological effects; and (7) discussion and conclusions.

CONCENTRATIONS AND SOURCES OF TRACE METAL COMPONENTS OF AMBIENT AIR PM

According to the most recent PM Criteria Document (PMCD) (U.S. EPA, 2004), the median $PM_{2.5}$ concentration nationwide was about 13 $\mu g/m^3$. Annual mean $PM_{2.5}$ concentrations were above 17 $\mu g/m^3$ at 5% of the sites, mainly in California and in the southeastern United States. The 98th percentile 24-h average concentrations were below 47 $\mu g/m^3$ at 95% of the sites sampled. Most of the sites with higher levels were in California.

The U.S. Environmental Protection Agency (EPA), working with state and local air quality agencies, began implementing an ambient air-monitoring network in 1999/2000 to provide a consistent data set for the characterization and evaluation of trends in $PM_{2.5}$ chemical species. The speciation and trends network (STN) was designed to include about 54 core trends sites across the United States, and to provide a stable ongoing national perspective. In 1999, an initial 13 sites were operated as a model for the deployment of the more comprehensive network consisting of the 54 core trends sites and roughly 200 additional locally relevant sites (PMCD, U.S. EPA, 2004). Some sites are operated every third day, and others every sixth day, which greatly limits their usefulness for time-series studies of daily mortality and morbidity.

Data from the initial 13 STN sites were presented in the Third External Review Draft of the PMCD, released in 2001 for public review and comment by its Clean Air Scientific Advisory Committee (CASAC). These data were used to evaluate the suitability of various aerosol samplers being considered for use in the network system used to obtain $PM_{2.5}$ composition data. Three types of colocated aerosol sampling devices were operated from February through July 2000. A complete description of the data, techniques used to analyze the filters, and the results of the evaluation of the performance of the sampling devices can be found in Coutant and Stelzer (2001), and the analyses of data are described by Coutant et al. (2001).

Table 1 shows the mean concentration of $PM_{2.5}$as well as the concentration of each chemical species at each of the 13 sites. The measurement methods, number of samples (n), the AIRS site code, the mean, minimum, and maximum component concentrations, and minimum detection limits for each component were described in detail in the PMCD (U.S. EPA, 2004). The concentrations of anions and cations in Table 1 (ammonium, nitrate, sodium, potassium, and sulfate) were determined by ion chromatography; carbonaceous species were determined by the thermal optical transmittance method (NIOSH method); and trace elements (Al through Zr) were determined by x-ray fluorescence (XRF) spectrometry (PMCD, U.S. EPA, 2004).

The concentrations of many of these elements were beneath minimum detection limits (MDLs) and are not shown in Table 1. The usual practice of denoting table entry values below MDL by (—) is followed here. Missing data for $PM_{2.5}$ are also indicated by (—). Environmental concentration data below the MDL are often represented by 0.5 × MDL for various purposes such as risk assessments and for mass closure. Unfortunately, speciation data are not available for the $PM_{10-2.5}$ size fraction. Some research studies have analyzed metals concentrations by inductively coupled plasma–mass spectrometry (ICP-MS). ICP-MS methods have lower MDLs than do XRF.

Sulfur (S) was the major element analyzed in the $PM_{2.5}$ size fraction at all AIRS sites, and is highly correlated with $PM_{2.5}$; ranging from as low as 8% of the $PM_{2.5}$mass in Sacramento, CA, to 10% in Houston, TX. In general, the sulfur (or sulfate) is higher in the eastern part of the US than in the west.

The concentrations of the crustal elements Al, Si, K, Ca, and Fe were highest at Phoenix, AZ. A number of trace elements (e.g., As, Cr, Cu, Fe, Mn, Ni, Pb, Se, Ti, V, and Zn) were detectable in the $PM_{2.5}$ data sets, and the concentrations of many of these elements were much greater than the uncertainty in their determination. Sb, Ru, and Mo were not detectable at many sites. The concentrations of all of the other trace elements were below 15 ng/m^3, with the exceptions of Fe (below 240 ng/m^3) and Zn (below 35 ng/m^3). Among the 13 sites, St. Louis had the highest concentrations of Cu (18 ng/m^3), Fe (240 ng/m^3), Pb (14 ng/m^3), Mn (14 ng/m^3), and Zn (33 ng/m^3). In general, Ni and V were higher in the eastern United States (Burlington, VT;

TABLE 1
Mean concentrations of $PM_{2.5}$ and chemical species of AIRS sites

AIRS Site	Burlington, VT	Philadelphia, PA	Atlanta, GA	Detroit, MI	Chicago, IL	ST. Louis, MO	Houston, TX	Minneapolis, MN	Boulder, CO	Phoenix, AZ	SEATTLE, WA	Sacramento, CA	Riverside-Rubidojx, CA	Mean	SD	Percent of $PM_{2.5}$
$PM_{2.5}$ (FRM Mass)	10	14.2	—	16.6	15.7	15.6	12.4	10.3	9.5	9.9	8.2	9.4	28.6	13.4	5.6	
$PM_{2.5}$ (Reconst-ructed Mass)	10.9	16	16.3	18	15	16.4	11	12.4	11.3	12	8	15	30.5	14.8	5.5	
Sulfate (Calculated)	2.6	4.3	4.8	4.4	4.3	4.3	3.4	2.3	1.5	1.2	1.4	1.2	3.6	3.023	1.376	20.4%
Sulphate (by I.C.)	2.8	4.4	4.8	4.6	4.2	4.3	3.5	2.4	1.6	1.3	1.3	1.3	3.7	3.092	1.371	20.9%
Ammonium by (by I.C.)	1.1	2	1.3	7.2	1.9	1.9	1.2	1.2	0.79	0.5	0.47	0.74	4.8	1.546	1.134	10.4%
Sodium Ion (by I.C.)	0.22	0.21	0.27	0.27	0.052	0.19	0.22	0.27	0.24	0.28	0.16	0.38	0.49	0.250	0.104	1.7%
Potassium (by .C.O.)	0.035	0.042	0.044	0.061	0.066	0.069	0.051	0.059	0.032	0.062	0.045	0.077	0.075	0.055	0.015	0.4%
Nitrate	1.3	2.1	0.7	3.1	2	2.3	0.68	2.3	1.5	1.1	0.67	2.3	12.3	2.488	3.045	16.8%
Volatile Nitrate	—	0.94	—	—	0.71	—	0.37	—	—	—	0.25	—	—	0.543	0.278	3.7%
Nonvolatile Nitrate	—	0.61	—	—	1.3	—	0.32	—	—	—	0.41	—	—	0.660	0.444	4.5%
Elemental Carbon	0.26	0.66	0.9	0.68	0.61	0.72	0.3	0.39	1	0.75	0.6	0.66	1.2	0.672	0.265	4.5%
Organic Carbon	2.5	3.1	4.3	3.2	2.8	3.5	2.2	2.6	3.2	4	2.6	5.2	6.0	3.267	0.867	22.0%
Aluminum	0.02	0.019	0.028	0.025	0.03	0.044	0.092	0.028	0.092	0.12	0.015	0.04	0.057	0.047	0.034	0.32%
Arsenic	—	—	0.001	0.002	0.001	0.002	0.001	0.002	—	0.002	0.001	0.002	0.002	0.002	0.001	0.01%
Barium	—	—	—	—	—	0.024	—	—	—	—	—	—	—	0.024	0.000	0.16%
Bromine	0.002	0.004	0.003	0.003	0.003	0.004	0.003	0.002	0.002	0.003	0.002	0.002	0.006	0.003	0.001	0.02%
Cadmium	—	—	—	—	—	—	—	—	—	—	—	—	—	0.000	0.000	0.00%
Calcium	0.035	0.037	0.037	0.069	0.058	0.13	0.055	0.071	0.12	0.15	0.029	0.043	0.17	0.077	0.048	0.52%
Chlorine	—	0.011	0.003	0.017	0.017	0.027	0.05	0.008	0.019	0.047	0.055	0.052	0.072	0.032	0.023	0.21%
Chromium	0.002	0.002	—	0.002	0.001	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.002	0.001	0.01%
Copper	0.002	0.004	0.002	0.006	0.004	0.014	0.003	0.003	0.004	0.006	0.003	0.006	0.006	0.005	0.004	0.03%
Iron	0.041	0.084	0.084	0.12	0.091	0.24	0.073	0.065	0.13	0.17	0.053	0.079	0.17	0.108	0.057	0.73%
Lead	—	0.005	0.003	0.006	0.006	0.014	0.002	0.005	0.005	—	0.004	—	0.006	0.006	0.003	0.04%
Magnesium	—	—	0.008	0.02	0.017	0.011	0.02	0.018	0.02	0.025	0.014	0.021	0.032	0.019	0.007	0.13%
Manganese	0.002	0.002	0.002	0.004	0.003	0.014	0.002	0.002	0.003	0.004	0.003	0.002	0.004	0.004	0.003	0.02%
Malvbdenum	—	—	—	—	—	—	0.002	—	—	—	0.002	—	—	0.002	0.000	0.01%
Nickel	0.002	0.006	—	0.002	0.001	0.002	0.002	0.002	0.001	0.003	0.002	0.01	0.002	0.003	0.003	0.02%
Phosphorous	—	—	—	—	0.002	0.004	0.002	—	0.006	0.006	—	0.006	0.007	0.005	0.002	0.03%
Potassium	0.041	0.053	0.06	0.078	0.085	0.099	0.072	0.068	0.063	0.11	0.05*S*	0.1	0.11	0.076	0.023	0.52%
Rubidium	—	—	0.005	—	—	—	—	—	—	—	—	—	—	0.005	0.000	0.03%
Setenlwn	—	0.002	0.001	0.002	0.001	0.001	0.001	—	—	—	0.001	—	—	0.001	0.000	0.01%
Silicon	0.069	0.086	0.11	0.11	0.11	0.17	0.23	0.12	0.28	0.36	0.049	0.12	0.2	0.158	0.093	1.06%
Sodium	0.1	0.087	0.056	0.096	0.048	0.045	0.17	0.093	0.078	0.1	0.15	0.19	0.2	0.109	0.052	0.73%
Strontium	—	—	0.001	—	0.001	0.001	0.001	—	—	0.003	0.001	—	—	0.001	0.001	0.01%
Sulfur	0.88	1.45	1.6	1.5	1.4	1.4	1.1	0.75	0.49	0.39	0.45	0.41	1.2	1.004	0.459	6.77%
Tin	—	—	—	—	—	0.008	—	—	—	—	0.007	—	0.014	0.010	0.004	0.07%
Titanium	0.004	0.006	0.006	0.007	0.004	0.008	0.007	0.006	0.01	0.013	0.003	—	0.012	0.007	0.003	0.05%
Vanadium	—	0.004	0.001	0.002	0.001	0.001	0.003	0.002	—	—	0.003	—	0.006	0.003	0.002	0.02%
Zinc	0.008	0.015	0.008	0.025	0.023	0.033	0.006	0.008	0.023	0.009	0.009	0.005	0.023	0.015	0.009	0.10%

All concentrations are mean at each site and are given as $\mu g/m^3$.
—concentration below minimal detection limit (MDL).

Philadelphia; Atlanta, GA). As, Cu, Pb, Mn, Ti, and Zn were higher in the Midwest (Detroit, MI, Chicago, Minneapolis, MN, St. Louis, MO, and Houston, TX). Fe and Ti were higher in the Western states (Phoenix, AZ, and Boulder, CO) as well as in Riverside, CA.

In summary, the STN data have provided new opportunities for $PM_{2.5}$ source identification and exploratory studies of the influence of $PM_{2.5}$ source classes and components on human health and welfare. Unfortunately, their limited scope in terms of spatial and temporal coverage, and lower detection limits, as well as their initial focus on the $PM_{2.5}$ size range, impose limits on their usefulness to health effects researchers.

REVIEW OF CLINICAL STUDIES IN HUMANS

Ghio et al. (2000) exposed 37 normal human volunteers by inhalation for 2 h to CAPs in Chapel Hill, NC, and reported that the exposures caused neutrophilic inflammation in the lungs and increased fibrinogen levels in the blood. Based on a further analysis of the concentrations of the soluble elemental components extracted from the air sampling filters used in that study, Huang et al. (2003) reanalyzed the data to determine the extent to which the nine most abundant CAPs components correlated with cellular and biochemical endpoints in the BALF, and in the peripheral blood samples that were collected 18 h after the CAPs exposures and stored. Of the soluble components extracted from the air sampling filters, Fe, As, Se, and sulfate were highly correlated with the FPM mass concentration, while Ni and Cu were least correlated. In terms of biological responses, an Fe/Se/sulfate factor was associated with increased BALF percentage of neutrophils, and a Cu/Zn/V factor with increased blood fibrinogen. The increase in plasma fibrinogen correlated with decreases in neutrophils and platelets, consistent with a state of systemic inflammation and increased platelet aggregability. However, Huang et al. did not analyze other types of emissions that could be correlated with these two factors, e.g., soluble organics. Salvi et al. (1999) found, in human volunteers, that diluted diesel exhaust also caused increases in neutrophils in airway lavage fluid, as well as in peripheral blood. Thus, it remains unclear what components of Chapel Hill air may be most associated with this measure of lung inflammation.

In another CAPs inhalation exposure study in human volunteers for which there were data on FPM composition, Urch et al. (2004) exposed 24 healthy adults in Toronto for 2 h to a mixture of FPM concentrated by a factor of 10 and ozone at 120 ppb. The CAPs averaged 148 $\mu g/m^3$. Using a measurement of brachial artery diameter (BAD), as measured by ultrasonography, as an index of cardiovascular response, there was an exposure related mean decrease in BAD of 0.09 mm. The linear regression analyses of change in BAD in relation to FPM components yielded p values of .04 for organic carbon (OC), .05 for EC, .06 for Cd, and .09 for K. The values were between 0.13 and 0.17 for Zn, Ca, and Ni, and values were even larger for all of the other measured components. The value for FPM as a whole was 0.40. Thus, for a study with a relatively small number of healthy subjects in a city with relatively low levels of FPM pollution, the results were inconclusive. However, they do suggest that more research is needed on the cardiovascular effects of FPM components in human subjects.

Lanki et al. (2006) studied the influence of ambient air FPM component exposures on exercise-induced ischemia in 45 elderly nonsmokers with stable coronary heart disease in Amsterdam (the Netherlands), Erfurt (Germany), and Helsinki (Finland). Two FPM source classes (traffic and long-range transport) were associated with ST-segment depression during submaximal exercise testing in a clinical laboratory. In a multipollutant model, with which the authors were able to separate effects of secondary sulfate from effects of vehicular emissions, only the traffic emissions were significantly associated with the effect. The authors also examined whether potentially toxic transition metals (Fe, Cu, Zn, and V) might be associated with ST-segment depression, given that both these, and OC, may have the capability to induce oxidative stress in the lung. However, when adjusted for ABS (absorbance, a measure of elemental carbon [EC] emissions from motor vehicles), none of the metals that were measured were associated with ST segment depression, while the ABS associations remained significant with only slight variation.

Chuang et al. (2007) studied a panel of 46 patients with coronary heart disease (CHD) in Taipei, Taiwan. Cardiac function was monitored for 1 wk using a continuous Holter monitor. They had hourly air quality measurements of PM10, FPM, and FPM components (sulfate, nitrate, OC, and EC), as well as the gaseous criteria pollutants. Heart rate variability (HRV) was reduced in proportion to elevations in FPM, sulfate, and OC, but not with any of the other measured pollutants in single pollutant models. In multipollutant models, only sulfate remained significant. While the concentrations of transition metals were not measured, they would be more closely correlated with sulfate than with total FPM mass or with OC, suggesting that they influenced the reductions in HRV.

The only other studies in human volunteers for which component analyses were available involved administration of PM by intratracheal instillation of particle suspensions.

On the basis that Fe is the most abundant of the transition metals in ambient air, Lay et al. (1998) and Ghio et al. (1998a) instilled ~5-mg doses containing both soluble and insoluble 2.6-μm Fe particle agglomerates suspended in saline into the lungs of volunteer subjects to investigate oxidative stress. BALF samples were collected from 1 to 91 days later. At 1 day, Lay et al. reported inflammatory responses, and Ghio et al. reported decreased transferrin concentrations and increased concentrations of ferritin and lactoferrin. By 4 days, iron homeostasis was normal. In a subsequent inhalation study in the same laboratory, Lay et al. (2001) determined that neither low or high solubility iron oxide particles (12.7 mg/m^3, MMAD = 1.5 μm, 30 min of exposure) had any effect on lung epithelial cell permeability or pulmonary function in human volunteers. They did not look for effects in other organ systems. In studies reported

in Schlesinger and Cassee (2003), Kleinman and coauthors "exposed normal and asthmatic adults [humans] for 2 h to 81 to 100 μm/m^3 ferric sulfate (1.3 μm MMAD) and found no significant exposure-related effects" on various pulmonary functional endpoints.

These kinds of tests have proved to be more informative when they were applied to real-world PM samples that were associated with adverse effects in human populations, such as those from the Utah Valley, where there was a 14-mo-long strike at a steel mill complex. There were significantly lower rates of mortality and hospital admissions during the strike than in the preceding and following years (Pope et al. (1989, 1991, 1992). Analyses of the PM collected on air sampling filters during those 3 years indicated that the concentrations of many airborne metal PM components were also significantly lower during the strike interval than in the preceding and following years (Frampton et al., 1999; Ghio & Devlin, 2001; Dye et al., 2001). Extracts of metals from sampling filters were used to test whether soluble components or ionizable metals, which accounted for 20% of the PM mass, could be responsible for the adverse health effects.

Human subjects were instilled with 500 μg (in 20-ml sterile saline) of Utah Valley dust extracts (UVD1, 2, and 3, which were collected during the 3 successive years) in the left segmental bronchus, and on the right side they were instilled with sterile saline as controls. Phagocytic cells were obtained from the segmental bronchi at 24 h postinstillation (Samet et al., 1997). Alveolar macrophages (AMs) from subjects instilled with UVD, which were obtained by BAL 24 h postinstillation, were incubated with fluoresceinated yeast (*Saccharomyces cerevisiae*) to assess their phagocytic ability. Although in the same proportion of AMs that were exposed to UVD phagocytized yeast, AMs exposed to UVD1 (which was collected while the local steel mill was open) took up significantly less PM than AMs that were exposed to the other extracts (UVD2 from when the steel mill was closed, and UVD3 from when the plant reopened). AMs exposed to UVD1 also exhibited a small decrease in oxidant activity (using dihydrorhodamine-123 [DHR]). AMs from healthy volunteers were incubated in vitro with the various UVD extracts to assess whether effects on human AMs could be observed that were similar to those seen following in vivo exposure. The percentage of AMs that engulfed yeast particles was significantly decreased by exposure to UVD1 at 100 μg/ml, but not at 25 μg/ml. However, the amount of PM engulfed was the same following exposure to all three UVD extracts. AMs also demonstrated increased oxidant stress after in vitro exposure to UVD1, and this effect was not abolished with pretreatment of the extract with the metal chelator deferoxamine (DEF). As with the AMs exposed to UVD in vivo, AM exposed to UVD in vitro had a decreased oxidant activity (DHR assay). UVD1 contained 61 times and 2 times the amount of Zn compared to UVD 2 and UVD3, respectively, whereas UVD3 contained 5 times more Fe than UVD1. Ni and V were present only in trace amounts, and did not differ from year to year (Frampton et al., 1999; Ghio & Devlin, 2001; Dye et al., 2001).

Another clinical study is that of Sorensen et al. (2003). This study found that personal exposure of healthy human volunteers to soluble V and Cr caused significant increases in oxidative stress and DNA damage (as measured by 8-oxodG concentrations in lymphocytes). Other soluble metals (Fe, Ni, Cu, and Pt) were not so associated.

REVIEW OF EPIDEMIOLOGIC STUDIES IN HUMAN POPULATIONS

Source Apportionment and PM Component Studies of Responses to Short-Term Exposures

The availability of data sets that include appropriate speciation data, and especially of metallic elements that serve as tracers for source apportionment of health effects, has been quite limited until recent years. The National Research Council (NRC 2001) acknowledged the value of receptor-oriented source apportionment models, but it also pointed out that "a number of approaches have been presented in the literature, but they are typically applied to only a single location or region." As also noted in NRC (2001), there has not been an extensive effort to test the effectiveness of these methods. To address this issue, Thurston et al. (2005) organized a workshop in 2003 to analyze two sets of $PM_{2.5}$ speciation data (Phoenix, AZ, and Washington, DC) by eight research groups applying a variety of multivariate source apportionment techniques, and then conducting mortality analyses (Thurston et al., 2005; Hopke et al., 2006; Ito et al., 2006; Mar et al., 2006). The intercomparison of source apportionment approaches found an acceptable degree of agreement of source-apportioned $PM_{2.5}$ for a variety of sources (Thurston et al., 2005; Hopke et al., 2006). While the mortality analyses of the source-apportioned $PM_{2.5}$ in the two cities found some common source types (i.e., sulfate and traffic) that were associated with mortality (Ito et al., 2006; Mar et al., 2006), some critical issues were identified for further investigation, including the consistency of lag structure of associations for a given source type and outcome, and the influence of possibly differential exposure error across source types. More analyses of speciation data using multiple cities with larger populations and a range of PM source types are needed. In particular, there is a need to have better data relating to local emissions, in order to produce more reliable epidemiological results (Ito et al., 2004).

The review of the literature on source apportionment and health-related effects that follows needs to be interpreted with caution insofar as the studies that are summarized here did not have the same kind of uniform framework as the workshop (Thurston et al., 2005; Hopke et al., 2006; Ito et al., 2006; Mar et al., 2006) comparison of results related to mortality in two specific cities. By contrast, the studies summarized next were done at various times in a variety of locations that varied in FPM compositions, different numbers of metals analyzed, and different lower limits of detection, and were addressing different hypotheses. Thus, their ability to clarify the possible roles of specific sources in causing health effects is quite limited.

They have provided little information that implicates specific metals, which serve as source tracers, in the associated health effects. Furthermore, they sometimes lacked specific information on which metals were reliably measured, or how they were used to identifying source-related mixtures. Thus, the reader will need to refer to the cited papers to examine the influence of these factors on the reliability of the reported results. Still, given these caveats, at this time, and at this stage of the development of the art and science of source apportionment for health effects studies, the studies summarized next can be informative.

A growing number of epidemiological studies have conducted analyses based on the elemental compositions of the PM (Özkaynak & Thurston, 1987; Özkaynak et al., 1996; Tsai et al., 2000; Laden et al., 2000 [reanalyzed by Schwartz, 2003, and by Grahame and Hidy 2004]; Mar et al., 2000 [reanalyzed by Mar et al., 2003]; Roemer et al., 2000; Burnett et al. 2000; Janssen et al. 2002; Penttinen et al., 2006; Ostro et al., 2007; Lipfert et al., 2006). As discussed in the section on toxicology, some recent toxicological studies have also used source apportionment techniques (Maciejczyk & Chen, 2005; Lippmann et al., 2005c) that provided suggestive evidence that PM from certain combustion sources (i.e., residual oil, secondary aerosols, and traffic), but not other sources (e.g., soil), were associated with biological responses, but the results to date have been too limited to be conclusive.

Thurston et al. (1984) and Ozkaynak and Thurston (1987) used compositional data from the U.S. Environmental Protection Agency (EPA) Inhalable Particle (IP) Network to develop source factors (indications of the sources, on the basis of their emission profiles) that most strongly influence the site-to-site and day-to-day variations in FPM mass across the nation. The elemental loadings on these factors suggested that five major source groups were most influential: wind-blown soil (Si, Fe); motor vehicle emissions (Pb, Br); residual oil combustion (Ni, V); iron/steel/metals industry emissions (Mn, Zn); and coal combustion (Se, S).

Some recent studies of acute health effects (Finkelstein et al., 2004, 2005; Gehring et al., 2006; Hoek et al., 2002; Nafstad et al., 2003, 2004; Vinzents et al., 2005 have used more refined exposure methodologies that gave more emphasis to local emissions than to central monitor stations, following the guidance of Ito et al. (2004), and they have indicated relatively larger and more statistically significant health risks for exposures to motor vehicle emissions. Those studies that examined this issue did not find centrally monitored fine PM or sulfate to be associated with the endpoints examined (Vinzents et al., 2005; Lipfert et al., 2006; Schwartz, 2003; Janssen et al., 2002; Grahame & Hidy, 2004). In addition, Maciejczyk and Chen (2005) found effects attributable to emissions from traffic, but not to emissions attributable to coal combustion.

An analysis of the short-term mortality effects of FPM air pollution sources has also indicated that the toxicity of FPM can vary depending upon its sources. In a study of the Harvard Six-City data set from the late 1970s and early 1980s, Laden et al. (2001) and Schwartz (2003) used the elemental composition of FPM to identify several distinct source-related fractions, and examined the association of these fractions with daily mortality in each of the Harvard six cities. Using specific rotation factor analysis for each city, they identified an Si factor (classified as soil and crustal material), a Pb factor (classified as motor vehicle exhaust), an Se factor (representing coal combustion), and up to two additional factors. They estimated city-specific associations of daily mortality with each source factor by Poisson regression, adjusting for time trends, weather, and the other source factors. The estimated mortality risks for each source type were mostly nonsignificant in each city, and only the "traffic"-related FPM was significantly associated with mortality in the combined estimates. They also noted that the mortality risk estimate for "residual oil"-related FPM was an order of magnitude larger than those for other source types, but this estimate had wider confidence bands because only two of the six cities identified a "residual oil" factor. In a further reanalysis of the Laden et al. (2000) study that considered the contribution of local Boston area residual oil combustion sources to the Se and S levels, Grahame and Hidy (2004) concluded that residual oil combustion effluents, which the U.S. EPA data show contain small amounts of Se, were most likely responsible for the fact that Se-related mortality and S-related mortality were much higher in Boston than in the other five cities, and were significant only in Boston, even though Se and S levels were low in comparison with other localities.

Tsai et al. (2000) regressed daily cardiopulmonary deaths in Camden, Newark, and Elizabeth, NJ, against PM source categories. Significant source factors for daily mortality were residual oil burning, and industrial processing of Zn and Cd.

For Phoenix, AZ, Mar et al. (2000, reanalyzed in 2003) found associations between daily cardiovascular mortality and source factors for vegetative burning, motor vehicle exhaust, resuspended road dust, and regional sulfate, in factor analysis that identified only five factors. The Mar et al. studies did not identify a smelter factor, as did other factor analyses of the time (Ramadan et al., 2000). Yet smelters and incinerators emit significant amounts of Cu, Zn, Pb, and As, among other metals (World Bank, 1998; Ayres & Simonis, 1994).

Burnett et al. (2000) studied the associations between 47 elements within both coarse thoracic PM and FPM, as well as pollutant gases, on daily mortality in eight Canadian cities. The strongest associations with mortality were for four specific components of FPM, i.e., sulfate, Fe, Ni, and Zn. Their total effect estimate was greater than that for FPM mass. There were also significant associations of 1-day lagged daily mortality with four specific coarse thoracic PM components, i.e., Sc, Mn, Ni, and Zn. Combining the coarse and fine concentrations for Ni (change $= 2.9$ ng/m^3), there was a 1.2% increase ($T = 2.9$), while for Zn (change $= 41$ ng/m^3) there was a 1.25 times increase ($T = 3.1$). In contrast, a larger study by many of the same authors (Burnett et al., 2004), using 19 yr of data from 12 Canadian cities, found that vehicles were the main emission source

category associated with daily mortality. These contrasting results demonstrate the need for more sophisticated methodologies, in particular with regard to accurate exposure information for variable local emissions (neither of the Burnett et al. studies had such information).

Roemer et al. (2000) examined the roles of FPM components on short-term health respiratory effects in humans in Europe, and reported significant associations with traffic markers. However, the findings were limited to associations of traffic markers with respiratory effects, and were not significant for other source-related categories or metal components,

Janssen et al. (2002) made estimates of source-specific contributions to PM_{10} on the basis of emissions data in 14 U.S. cities, and regressed them against hospital admissions data for those cities in terms of cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD), and pneumonia admissions. In order to get a better sense of exposure to source-related emissions, the authors utilized a measure of the prevalence of air conditioning in each locality, reasoning that in hotter climates with more air conditioning, people would spend more time in relatively tight microenvironments, and would therefore be less exposed to outdoor pollutants than people in cooler environments. They reported that there were significant associations of CVD admissions with four source categories, i.e., highway vehicles, highway diesels, oil combustion, and metals processing. They did not report on associations with the specific metals used as source tracers. The associations for COPD and pneumonia were not statistically significant.

Penttinen et al. (2006) studied a panel of 57 adult asthmatics in Helsinki, Finland, for 181 cool/cold season days while recording daily symptoms and pulmonary function. FPM and elemental components and criteria pollutants were measured every other day at a central site. The only significant associations were between FPM from local combustion source category and reductions in peak expiratory flow (PEF). None of the individual elements were significantly associated with reduced PEF, including Cu, Zn, Mn, and Fe, which were the source tracers for local combustion.

Ostro et al. (2007) examined the roles of FPM components on short-term mortality in humans in California, and reported associations of traffic markers, sulfate, Ca, Cl, Cu, Fe, Mn, Pb, Ti, V, and Zn in the cool season with all-cause and or cardiovascular short-term mortality, but no significant associations for Al, Br, or Ni.

Sinclair et al. (2004), Metzger et al. (2004), and Peel et al. (2005) described a study conducted in Atlanta in which a large variety of fine PM types were monitored, including the mass of all soluble metals. Metzger et al. (2004) found no association of the mass of all soluble metals with cardiovascular emergency department visits, and Peel et al. (2005) found no association of all soluble metals with respiratory emergency department visits. However, it should be noted that residual oil was not used for power generation anywhere near Atlanta, and also that Atlanta doesn't have ocean shipping, so it would be expected that levels of V, Ni, and ROFA would be very low in ambient air in this location.

TABLE 2
Source contributions to 1980 mortality in 36 U.S. MSAs (Ozkaynak & Thurston, 1984; Thurston & Ito, 2001)

Emission source class	Mean U.S. $PM_{2.5}$ impact	Percent mortality effect estimate
Soil	4.4 $\mu g/m^3$	0.4%
Auto emissions	2.9 $\mu g/m^3$	0.6%
Oil combustion	3.8 $\mu g/m^3$	0.6%
Metals (iron/steel)	1.1 $\mu g/m^3$	1.2%[a]
Coal burning	11.0 $\mu g/m^3$	7.3%[a]

[a] Signficant at $p < .01$.

Responses to Long-Term Source-Related and PM Component Exposures

Some past epidemiological studies suggest that sulfate-associated particles (i.e., fossil fuel combustion products) are among the most toxic in terms of effects on annual mortality rates (e.g., Thurston & Ozkaynak, 1987; Dockery et al., 1993; Pope et al., 1995; Lippmann & Thurston, 1996). As shown in Table 2, when the IP Network source factors were entered into a cross-sectional regression of 36 U.S. MSAs, the regressions of annual mortality rates on pollution and other mortality, cofactors (age, poverty, etc.) indicated that long-term exposures to the coal and metals/steel FPM components were more significantly associated with increased mortality than the other components identified in this early work (Ozkaynak & Thurston, 1987; Thurston & Ito, 2001). Soil particles were clearly shown to be nonsignificant contributors to annual mortality. Thus, these associations of mortality with source-specific FPM contributions, while rudimentary in terms of the mortality assessment method (cross-sectional), indicated that differing source categories may have differing impacts on mortality.

Lipfert et al. (2006) examined the influences of FPM sources on survival in a cohort of male U.S. military veterans using county-level data on FPM composition and vehicular traffic density for 1997–2002 and cohort member mortality for those living in those communities for 1997–2001. Using single-pollutant models, traffic density was the strongest predictor of mortality, but other components also appeared to be influential, including NO_2, nitrate, EC, Ni, and V. Sulfate was not significant in any of the models used in this study. In multipollutant models, the traffic density variable (which may or may not be related to metals content) was more robust than other emission categories, remaining significant when others ceased to be significant, with loss of significance occurring only when EC (another proxy for vehicular emissions) was also in the model. When traffic density was not in the models, V and Ni were often significant in multipollutant runs. Because these emissions are not nearly as widespread as

vehicular emissions, it is possible that their importance might have been understated in this study.

Pope et al. (2007) examined the influence of a copper smelter strike in four southwestern states on monthly mortality in those, adjacent, and other States. They estimated that the strike-related reduction in mortality in the four southwestern states was 2.5% (95 CI = 1.1–4.0%). There was also a 60% reduction in sulfate pollution in the four southwestern States during the strike. Since the major source of the measured sulfate was a Cu smelter, there must also have been a large reduction in transition metal concentrations during the strike.

Schober et al. (2006) studied the association between blood lead (Pb) and mortality in the third National Health and Nutrition Examination Survey (NHANES III), a national probability sample of the U.S. population. The data were analyzed from 1988 through 1994 for 9757 people over 40 years of age. Blood Pb was <5 μg/dl for 68% and between 5 and 9 μg/dl for 26% of them. How much of the blood Pb was from inhalation exposure was not known, nor whether it was due to exposures deposited in bones from exposures in past decades. Using blood Pb below 5 μg/dl as a baseline, there were significant increases in the relative risks (RRs) for total and cancer mortality in those with blood Pb between 5 and 10, and larger RRs for those with blood Pb $>$ 10 μg/dl. For cardiovascular mortality, there was a significant RR for blood Pb $>$ 10 μg/dl. This study confirmed and extended the analyses for NHANES II, which reported excess mortality for blood Pb $>$20 μg/dl (Jemal et al. 2002; Lustberg & Silbergeld, 2002).

Menke et al. (2006) found effects at relatively low Pb blood levels, with increased risks of MI and stroke mortality associated with blood lead levels $>$0.10 mmol/L ($>$2 mg/dL). After multivariate adjustment, in comparing the highest tertile of blood lead ($>$ 0.17 mmol/L [$>$3.62 mg/dl]) with the lowest level ($<$0.09 mmol/L [$<$1.94 mg/dl]), large and significant hazard ratios of 1.25 and 1.55 were found for all-cause and cardiovascular mortality.

Glenn et al. (2006) found associations between increases in blood and bone Pb levels with increased systolic blood pressure in young adults with occupational exposures to Pb, while Jain et al. (2007) found that a one standard deviation increase in blood or bone Pb levels in a cohort of middle-aged and elderly military veterans was associated with a significant increase in the rate of ischemic heart disease events.

Hong Kong Sulfur-in-Fuel Intervention Study

A mandated switch to fossil fuels with low S contents in Hong Kong took place as of July 1, 1990. Hedley et al. (2002) compared monthly mortality and treatment for bronchial hyperreactivity for the population for the 5 yr preceding the intervention, and for the 5 yr following it. They showed that the switch to low-S fuels caused a drop in SO_2 of about 50% in ambient air, but no intervention-associated changes in the concentrations of other airborne criteria pollutants. The drop in SO_2 was associated with prompt and persistent reductions in daily mortality of 2.2% overall, with about 2% for cardiac mortality, and about 4.5% for respiratory mortality, but the authors speculated that the health-related changes could have been due to other, unmeasured pollutants whose concentrations changed in parallel with those of SO_2. In a subsequent poster presentation, Hedley et al. (2004) documented large reductions in the concentrations of Ni and V, but not in other metals, in Hong Kong after July 1, 1990, as a result of the mandated switch to fossil fuels with low S contents in power plants. Taken together, these data can be interpreted as being consistent with significant effects of ROFA or its Ni and/or V components on daily mortality and bronchial hyperreactivity.

Time-Series Coefficients of Daily Mortality in the NMMAPS Study and Average Transition Metals Concentrations in Those Cities

The results obtained by Hedley et al. (2002, 2004), cited earlier, led Ito (as reported in Lippmann et al., 2006) to examine the possible role of PM components (i.e., transition metals, ions, and crustal soil tracers) on the city-to-city variation of PM_{10} mortality risk estimates. For this analysis, Ito analyzed the association between FPM components from the U.S. EPA FPM speciation network and the NMMAPS PM_{10} daily mortality risk estimates. The speciation data were for the years 2000–2003. The NMMAPS PM_{10} mortality risk estimates (updated estimates using GLM) for the 90 largest U.S. metropolitan statistical areas (MSAs) (for the time-series analysis that was conducted for 1987–1994) were obtained from the Johns Hopkins School of Public Health's Internet-based Health and Air Pollution Surveillance System (IHAPSS) web site. Although there were more than 40 FPM component species, the analysis focused on the 16 key components that were most closely associated with major source categories, i.e., Al, As, Cr, Cu, elemental carbon (EC), Fe, Mn, Ni, NO_3^-, organic carbon (OC), Pb, Se, Si, SO_4^{2-}, V, and Zn. An annual average for each FPM monitor was computed, and then averaged across available monitors for each MSA. The resulting MSA-averaged FPM component values were then matched with mortality in the 60 NMMAPS MSAs that had FPM speciation data. The PM_{10} mortality risk estimates (expressed as percent excess deaths per 10-μg/m^3 increase in PM_{10}) were then regressed on each of the FPM components, with weights based on the standard error of the PM_{10} risk estimates.

For all of the FPM components that were examined, the predictive power increased when the log-transformed variables were used. Figure 1 shows the resulting difference in the PM_{10} mortality risk estimates (in percent per 10-μg/m^3 increase in PM_{10}) per 5th-to-95th percentile difference in the FPM component across the 60 NMMAPS MSAs for which speciation data were available. For example, for Ni and V, the PM_{10} risk coefficients (per 10 μg/m^3) were high (0.6) in the MSAs where Ni and V were significantly high (95th percentile), compared to the MSAs where Ni was low (5th percentile). These differences in magnitude were not small, as the nationwide combined estimate for the 90 MSAs in the NMMAPS study was 0.21. Ni and V,

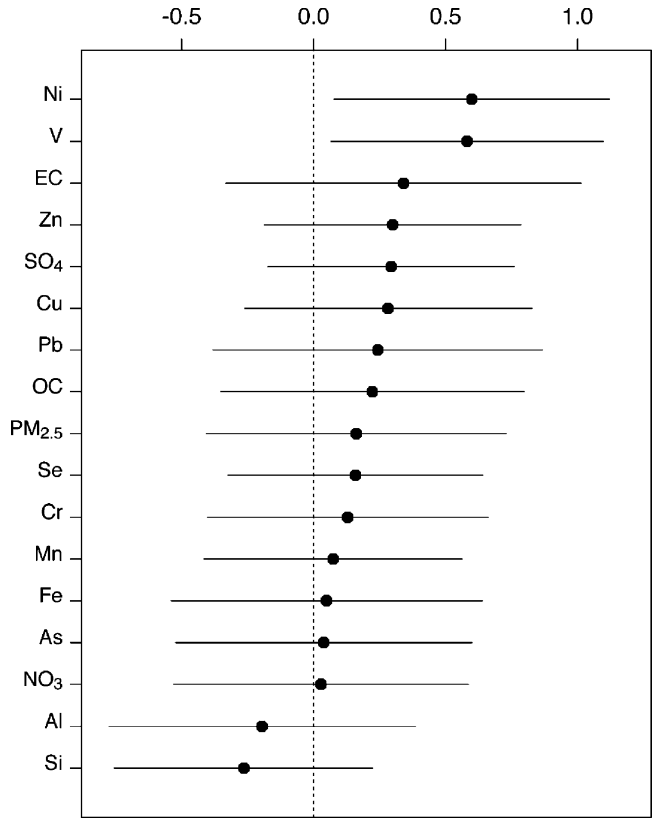

FIG. 1. Differences in mortality risk coefficients per the 5th-to-95th percentile difference in FPM and FPM component concentrations across NMMAP MSAs (for the 60 MSAs for which FPM speciation data were available). Reproduced with permission from Lippmann et al. (2006).

which are most strongly associated with residual oil combustion effluent, showed the strongest predictions of the variation in PM_{10} risk estimates across the NMMAPS MSAs, followed by elevated but nonsignificant increases above 0.21 that were associated with EC, Zn, SO_4^{2-}, Cu, Pb, and OC. The metals most closely associated with resuspended soil, i.e., Al and Si, had the lowest values, suggesting that they were unlikely to be influential on daily mortality. Thus, FPM components appear to explain some of the MSA-to-MSA variation in the NMMAPS PM_{10} daily mortality risk coefficients.

Dominici et al. (2007a) extended the analysis of the associations between Ni and V and daily mortality using U.S. EPA speciation data and NMMAPS mortality data to 69 MSAs and an additional 2 years (through 2005). They reported that their results were consistent with those of Lippmann et al. (2006), but noted that a sensitivity analyses that excluded the New York City (NYC) metropolitan region data yielded associations that were no longer statistically significant.

Aside from the strong influence of NYC, with its much higher levels of Ni and V than other U.S. cities, the results based on the NMMAPS data need to be interpreted with some caution, since they rely on comparisons between PM_{10}-associated daily mortality for the years 1987 through 1994 and FPM composition data for the years 2000 through 2003 or 2005. With the exception of Al and Si, which are present mostly in coarse mode dust particles, and Fe, which is present in appreciable amounts in both the fine and coarse modes, the PM components in Figure 1 are found primarily in the FPM range and their concentrations in PM_{10} and FPM would be similar at any given year. In this context, Dominici et al. (2007b) reported that both PM_{10}-associated daily mortality and $PM_{2.5}$-associated daily mortality in the NMMAPS data were statistically significant. In any case, there was, however, almost certainly, a significant temporal change in FPM composition between 1990 and 1995 due to a Clean Air Act mandated 50% reduction in SO_2 emissions from power plants. The reduction in emissions of SO_2 was almost certainly accompanied by some corresponding reductions in SO_4^{2-}, Se, and Ni from power plants. Some of the reduction of S emissions was due to reduced use of residual oil and reduced S in residual oil (accompanied by the known reduction in both V and Ni when S is reduced in residual oil at the refinery). Such reductions in S would be tied to reductions in V and Ni. For coal combustion plants, much of the reduction in S occurred due to fuel switching to low-S Western coal. These factors could have introduced some exposure characterization error in the mortality coefficients used in Figure 1. Such exposure errors tend to reduce coefficients of response and their statistical significance.

It should be noted that several of the components can come from different sources. For example, while most of the Ni and V is co-emitted by residual oil burning in ships and in power plants (less so in power plants now than a decade or more ago, when residual oil was more widely used in electricity generation), Ni (but not V) can also come from Ni smelters, and there can be local sources of both metals. Today, EC mostly comes from diesel emissions and biomass burning, but decades ago, there were far more emissions from coke ovens and steel mills, and thus EC did not represent vehicular emissions then as well as it may today. Sulfate is mostly from coal combustion plants, but it is also can result from SO_2 as a vehicular emission (Reponen et al., 2003; Riediker et al., 2004; Kweon et al., 2003), and in past decades sulfates were more prevalent emissions from steel and coke complexes, and from smelters. Se is mainly a coal plant emission, but is also emitted in smaller levels from residual oil. Zn in urban areas may derive primarily from two main sources—worn tires and diesel lubricating oil—and the chemical composition may differ, with more oxides in the Zn from tire wear; however, in some localities there may still be important Zn emissions from metal working.

TOXICOLOGY STUDIES

Because of their potential for oxidative activity and the production of reactive oxygen species (ROS), transition metals have long been suspected to be major components of ambient PM in producing adverse health effects. As described in previous section, the importance of transition metals in producing adverse

health effects was confirmed in the pseudo-intervention study of Utah Valley, which indicated that reduction in metals in PM associated with year-long closure of a local steel mill was associated with improved health conditions in the local population. The role of metals (e.g., V, Fe, Cu, Zn, and Ni) was further confirmed by later studies using human clinical as well as animal toxicology studies. This section will focus mainly on the evidence of this group of metals in producing adverse health effects in animals. The mechanisms involved are detailed in a later section of this review,

Concentrated Ambient Particles (CAPs) Inhalation Studies in Animals

As described in the review of the epidemiological studies, it is clear that FPM air pollution is causing cardiopulmonary effects. However, because of the very low ambient air concentration levels measured in these studies, particularly of the trace metal components, the biological plausibility of these epidemiologically demonstrated associations needs to be substantiated, preferably using animal toxicological studies. To date, while there are many toxicological studies that investigated the response of animals to ambient PM by inhalation or intratracheal instillation, only a few had investigated the contributions from specific air pollution components, either as an individual compound or as part of a mixture, in producing adverse health effects. It is, therefore, critical to systematically investigate the potential cardiopulmonary effects of components of ambient PM in different regions of the United States, since PM of different composition and from different sources may vary markedly in their potency for producing adverse health effects.

Since many ambient air pollutants may interact with each other, both chemically as well as biologically, it is very difficult to design an animal study to mimic ambient conditions and concentrations. Some, but not all, short-term studies with inhaled CAPs have found cardiopulmonary changes in rodents and dogs at high concentrations of FPM, as described in detail in the most recent Particulate Matter Criteria Document (PMCD) (U.S. EPA, 2004). These experimental toxicology studies, using "real-world" CAPs under controlled conditions, have provided some evidence that certain ambient PM mixes or specific PM components may be responsible for reported health effects of ambient PM, suggesting that some of the PM components are more toxic than others. For example, in an attempt to demonstrate that CAPs inhalation can induce cardiopulmonary effects, Clarke et al. (2000) investigated pulmonary inflammatory and hematological responses of canines after exposure to Boston CAPs. For pulmonary inflammatory studies, normal dogs were exposed in pairs to either CAPs or filtered air (paired studies) for 6 h/day on 3 consecutive days. For hematological studies, dogs were exposed for 6 h/day for 3 consecutive days with one receiving CAPs while the other was simultaneously exposed to filtered air; crossover of exposure took place the following week (crossover studies). No statistical differences in biologic responses were found when all CAPs and all sham exposures were compared. However, the variability in biologic response was considerably higher with CAPs exposure. Subsequent exploratory graphical analyses and mixed linear regression analyses suggested associations between CAPs constituents and biologic responses. Factor analysis was applied to the compositional data from paired and crossover experiments to determine elements consistently associated with each other in CAPs samples. In paired experiments, four factors were identified; in crossover studies, six factors (V/Ni, S, Al/Si, Br, Na/Cl, and Cr) were observed. Increased bronchial alveolar lavage (BAL) neutrophil (PMN) percentage, total peripheral white blood cell (WBC) counts, circulating PMNs, and circulating lymphocytes (LYM) were associated with increases in the Al/Si factor. Increased PMNs and increased BAL macrophages were associated with the V/Ni factor. Increased BAL PMNs were associated with the Br/Pb factor when only the compositional data from the third day of CAPs exposure were used. Decreases in red blood cell (RBC) counts and hemoglobin levels were correlated with the sulfur factor. BAL or hematologic parameters were not associated with increases in total CAPs mass concentration. In terms of significant individual components, sulfate was associated with increased WBC; black carbon, Al, Mn, Si, Zn, Ti, V, Ni, and Fe were associated with increased PMN; Na was associated with increased LYM; and Al, Mn, and Si were associated with decreased LYM. These data suggest that specific components of CAPs may be responsible for biologic responses, but the lack of overall statistical significant alterations in pulmonary and systemic responses diminished the impact of this study.

The effects of inhaled CAPs on lung inflammation were studied by Saldiva et al. (2002). They exposed normal and bronchitic rats to Boston CAPs or filtered air for 5 h/day for 3 days. The CAPs produced significant pulmonary inflammation. Some CAPs components (V and Br) were significantly associated with increases in neutrophils in BALF and lung tissue.

Rhoden et al. (2002) reported that *N*-acetylcystine (NAC) can prevent lung inflammation due to CAPs inhalation. They also reported the results of regression analyses showing strong associations between increases in thiobarbituric reactive substances (TBARS) accumulation and the CAPs content of Al, Si, and Fe, and between BALF PMN count and Cr, Zn, and Na.

Morishita et al. (2004) exposed normal and allergic brown Norway rats to CAPs in a mobile laboratory in Detroit. The allergic rats had, compared to the normal rats, increased pulmonary retention of La, V, Mn, and S, as well as increased lung inflammation. Using source-apportionment analyses, Morishita et al. (2006) concluded that the pattern of the airway responses was likely associated with local refineries and incinerators, and independent of sulfate and PM2.5 mass.

Oxidative stress induced by exposure to high levels of metals in the CAPs could be responsible for changes in cardiac parameters. As demonstrated by Gurgueira et al. (2002), in vivo inhalation of CAPs can promote oxidative stress and tissue damage. In that study, adult Sprague-Dawley rats exposed to Boston CAPs at 300 $\mu g/m^3$ for 5 h showed significant oxidative stress

in the lung and heart but not in the liver. The increase in the lung concentrations of ROS upon exposure to CAPs was rapid, indicating an almost immediate effect of PM, or PM components, on the intracellular sources of free radicals. Furthermore, the transient nature of these increases points to a reversible interaction of PM components with cellular targets. Both observations are compatible with Fenton-type reactions catalyzed by transition metals, redox-cycling processes, or biochemical changes triggered by noncovalent binding to membrane receptors. Using single-component regression analysis, increases in chemiluminescence (an index of oxidant load) showed strong associations with the CAPs content of Fe, Mn, Cu, and Zn in the lung, and with Fe, Al, Si, and Ti in the heart. The oxidant stress imposed by 5-h exposure to CAPs was associated with slight, but significant, increases in the lung and heart water content, and with increased serum levels of lactate dehydrogenase (LDH), indicating mild damage to both tissues. In addition, CAPs inhalation also led to tissue-specific increases in the activities of the antioxidant enzymes superoxide dismutase and catalase, suggesting that CAPs exposure may also trigger adaptive responses.

Shukla et al. (2000) examined exposure of mice to CAPs in New York City at 250 $\mu g/m^3$, and also exposed murine C10 alveolar cells (1) to PM collected in New York City, (2) to ultrafine carbon black (uCB), and (3) to fine glass beads.

At 24 h after a 6-h exposure to the CAPs, lavaged lung tissues of the mice showed significant increases in steady-state messenger RNA (mRNA) levels of NF-κB-responsive cytokines, including tumor necrosis factor (TNF)-α, TNF-β, interferon (INF)-κ, and interleukin (IL)-6. Both the PM and the uCB increased activation of oxidant dependent NF-κB in the murine cells, but the glass beads did not. Although iron was present in the collected PM, pretreatment with the metal chelator deferoxamine had no effect on such activation, indicating that the iron was not responsible for the effects. The result was in agreement with earlier work in which production of TNF-α and IL-6 were not inhibited after pretreatment of an urban PM with the same chelator.

Shukla et al. (2000) concluded that, in concert, these studies indicate that iron-catalyzed generation of ROS may not be a predominant mechanism of $PM_{2.5}$-induced oxidant production in epithelial cells, a finding further supported by data showing increased and persistent oxidative stress induced by uCB, an iron-free component of PM.

Overall, the early CAPs inhalation studies described in 2004 PMCD (U.S. EPA, 2004) have not effectively addressed the inconsistency often observed within the multiple observations within the same study or across different studies. The inconsistency could be due to temporal and spatial variations in the PM exposure matrix, or due to the short-term exposure nature of these studies. With limited statistical power, these short-term CAPs inhalation studies were not able to delineate the specific characteristics of PM in producing toxicity and associated potential underlying mechanisms.

An in vivo study by Kodavanti et al. (2005), utilizing two different rat strains, made a number of important findings They exposed two different strains of rats (spontaneously hypertensive [SH] and Wistar-Kyoto [WKY]) to CAPs from ambient air in North Carolina, concentrated by a factor of 40 to 60 times. The CAPs were drawn from an area in reasonably close proximity to a major freeway near the intersection with another major road, suggesting that the effects seen might have been related to vehicular emissions.

By contrast, in a study using material extracted from filters in Utah Valley when a steel mill was in operation, metals levels were higher (Frampton, et al., 1999). In this latter study, levels of IL-6 and IL-8 were elevated in human lung epithelial cells when exposed to PM from the two years the steel mill was in operation, but not when exposed to PM from the same Utah Valley location, for the year that the mill was closed.

The lack of significant increase in activity of these cytokines is also in contrast to the in vivo inhalation findings in a study using CAPs from New York City by Shukla et al. (2000), and to the in vitro findings of Maciejczyk and Chen (2005), using CAPs from 40 miles north of New York. In both these locations, residual oil combustion effluent is a small but important source of fine PM, exhibited by elevated levels of V and Ni, but that is not the case in North Carolina.

Shukla et al. (2000) found no effects with 1-day exposures (4 h/day) where the effects were assessed immediately after exposure. However, for 2 days of exposures (4 h/day), effects were found, suggesting either that there were important differences between the composition on all six 1-day exposures, versus the seven 2-day exposures, or more likely that the effects took longer than 4 h to become manifest. The effects from the sets of 2-day exposures occurred when total fine PM was relatively low; the 2-day period with by far the highest PM was the only such period associated with no adverse health effects. The authors stated, "No biological effects correlated with CAP mass." On days with lower mass, when effects were observed, concentrations of three metals (Zn, Al, and Cu) were enriched severalfold, but organic carbon (OC) was increased to a lesser extent. The authors stated that these studies demonstrated a pattern of rat strain-specific pulmonary and systemic effects that were not linked to high mass, but rather appeared to be dependent on CAP chemical composition.

On the 2-day period with highest CAPs PM, "sulfate" was over 3 times higher than for the next highest 2-day period, and was 14 times higher than on one of the 2-day periods when effects are found. Absolute Zn concentration was virtually the same on the day with the highest CAPs PM but with no effects, as during one of the two-day periods when effects are found, and considerably higher than on the two other 2-day periods when effects are found. However, copper was the lower on the seven 2-day periods on the day with the highest CAPs PM, while OC was the highest on all of the two-day periods.

The authors suggested that on the 2-day period with the highest FPM ($\sim$2800 $\mu g/m^3$) and sulfate ($\sim$820 $\mu g/m^3$) these high

levels may have, in some way, masked the harmful effects of other constituents.

Batalha et al. (2002) exposed normal and bronchitic rats to CAPs in Boston and studied the effects of the exposures on the morphology of their small pulmonary arteries. Increases in CAPs exposures and the CAPs contents of Si, Pb, sulfate, EC, and OC were associated with reductions in the lumen/wall (L/W) ratios.

Wellenius et al. (2003) exposed dogs with vascular occluders around a coronary artery and tracheostomies to Boston CAPs inhaled into the trachea. Cardiac function was measured and there were no associations of CAPs mass or number concentrations with heart rate (HR). There were CAPs-associated ST-segment elevations that were significantly associated with the concentration of Si, and Pb.

After the release of the 2004 PMCD (U.S. EPA, 2004), a series of longer term FPM CAPs inhalation studies were conducted at New York University (NYU). Because these studies collected both long-term electrocardiographic (ECG) data and simultaneous data on FPM composition, such studies can have more power to identify possible causal components of ambient FPM. The NYU subchronic CAPs inhalation studies involved a series of experiments that were used to study both the acute and cumulative effects of daily inhalation exposures to inertially concentrated ambient-air FPM (CAPs) in Sterling Forest, NY, in a mouse model of atherosclerosis. The results of the first of these studies, involving 5 to 6 mo of warm-season daily exposures (5 days/wk, 6 h/day, to an average CAPs concentration of 110 $\mu g/m^3$), were described by multiple authors (Chen & Hwang, 2005; Chen Nadziejko, 2005; Gunnison & Chen, 2005; Hwang et al., 2005; Lippmann et al., 2005a, 2005b, 2005c; Maciejczyk & Chen, 2005; Veronesi et al., 2005). These papers documented CAPs exposure-associated acute and chronic effects on cardiac function, increased amounts of, and more invasive, aortic plaque, and changes in brain cell distribution and in gene expression markers, as well as data on the effects of daily CAPs exposures in vitro on Nf*k*B activation.

To investigate the contributions of PM components to cardiovascular effects, Lippmann et al. (2005c) used the 5 mo of daily 6-h source apportionments of Maciejczyk and Chen (2005), the continuous heart rate (HR) data for exposure days (weekdays only) used in Hwang et al. (2005), and the corresponding HR variability (HRV) data used in Chen and Hwang (2005) to determine the source-related PM2.5 components' associations with HR and HRV. They used HR and HRV data collected on normal (C57) mice and a murine model for atherosclerotic disease (ApoE-/-) (Chen & Hwang, 2005; Hwang et al., 2005). Daily 6-h FPM air samples were also collected and analyzed by x-ray fluorescence (XRF), permitting attribution to major PM source categories (secondary sulfate, suspended soil, residual oil combustion, and a remainder category, which was largely due to long-range transported motor vehicle traffic). They examined associations between these FPM components and both HR and HRV for three different daily time periods: (1) during exposure; (2) the afternoon following exposure; and (3) late at night. For HR, there were significant transient associations ($p \leq .01$) for secondary sulfate during exposure, and for residual oil combustion (predominantly V and Ni) in the afternoon. For HRV, there were comparable associations with suspended soil (predominantly Si, Al, Ca) in the afternoon and for both residual oil combustion and traffic (Br, Fe, elemental carbon) late at night. The biological bases for these various associations and their temporal lags are not known at this time, but may have something to do with the differential solubility of the PM components at the respiratory epithelia, and their access to cells that release mediators that reach the cardiovascular system. Further research that can elucidate the underlying processes is clearly needed.

In a subchronic CAPs inhalation study (Lippmann et al., 2006), there was a dramatic change in cardiac function in the fall months in the mouse model of atherosclerosis, As shown in Figure 2, the 14 days with northwest winds carried more Ni, Cr, and Fe, but less of the other elemental tracers, than the 89 days with winds from all other directions, and were associated with significant increases in HR and significant decreases in HRV (Lippmann et al., 2006). V did not rise on the 14 days with unusually high levels of Ni, Cr, and Fe in this mouse study. Back trajectory analyses from Sterling Forest for the 14 days with northwest winds led through lightly populated areas to Sudbury, Ontario, which is the location of the largest Ni smelter in North America.

One important parameter that was not addressed in the study just described, but could influence metals' ability in mediating biological response, is the extent of soluble metal components present in the PM mass. In a study that exposed spontaneously hypertensive (SH) and normal-tensive (Wistar Kyoto, WKY) rats to North Carolina CAPs, plasma fibrinogen levels in SH rats were better correlated with the levels of water soluble metals, particularly zinc, than mass and other components of the CAPs (Kodavanti et al., 2005). This study demonstrated that stain-specific systemic effects were not linked to high mass but appear to be dependent on CAP chemical composition.

Showing that relatively rural air, far from major traffic emissions, can cause increases in arterial plaque, for example, in ApoE -/- mice exposed to CAPs $\times$ 10, versus those exposed to filtered air, is a significant finding; as is the finding that specific cardiac effects in such mice could be traced to high Ni concentrations on 14 days where wind back trajectories showed the air parcel passed over a specific Canadian Ni smelter. However, it is nevertheless important to keep in mind that findings in genetically engineered mice are not necessarily reflecting what will happen in humans.

Analogous Studies in Humans

With regard to whether the HRV findings in CAPs exposed mice are applicable to humans, two studies have been done where ambient air analyses were used to examine which types or sources of emissions or air parcels were associated with reduced HRV, and which also gave some insight as to which types of PM

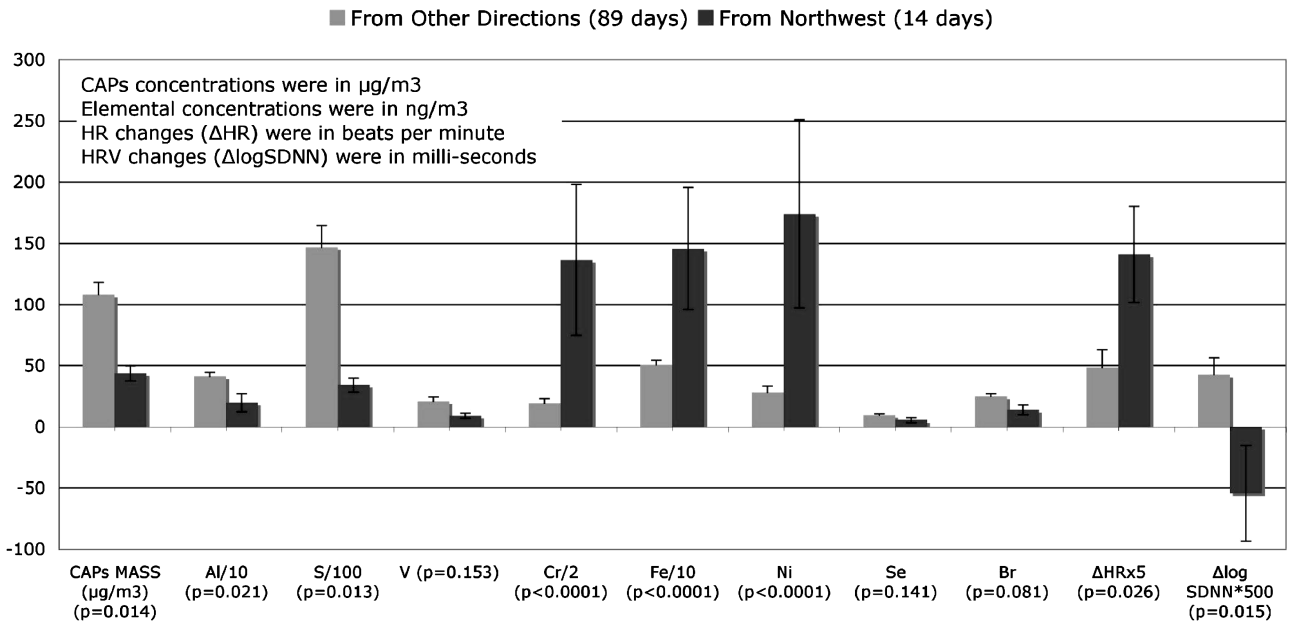

FIG. 2. Average elemental concentrations and HR and HRV for 14 days when winds were from the northwest (right bar) and that for the 89 days with winds from all other directions and the differences in heart rates of $ApoE^{-/-}$ mice exposed to CAPs and filtered air. CAPs concentrations were in $\mu g/m^3$, elemental concentrations were in ng/m^3, HR in beats/min, HRV (as log SDNN) in milliseconds. Error bars are $\pm$ SE. Reproduced with permission from Lippmann et al. (2006).

may be associated with, or not associated with, reduced HRV. In one, i.e., the Hong Kong sulfur-in-fuel intervention study described earlier, where Ni fell while Fe and Cr did not fall, it is possible that the large changes in Ni may account for at least some of the change in the intervention-related cardiovascular mortality in this study.

Another study also of interest in this context was that of Lippmann et al. (2006). It hypothesized that Ni may have been responsible for the notably high daily mortality associated with PM10 in New York City in the 90-city NMMAPS study, and in those 60 cities with speciation data, only Ni and V were significantly associated with NMMAPS mortality. The NMMAPS mortality coefficient for NYC was 3.8× higher than the average, and the Ni in NYC was 9.5× higher than the U.S. average.

Other Animal Inhalation Studies with Metals

In a study of Zhou et al. (2003), iron particles (median diameter = 72 nm) were inhaled for 6 h/day for 3 days by healthy Sprague Dawley rats at two concentrations, 57 and 90 $\mu g/m^3$. Compared to rats exposed to filtered air, no significant effects were observed at the lower rate of exposure. At the higher level, there was a significant decrease in total antioxidant power, as well as induction of ferritin, GST activity, increase in BAL protein count, and IL-1α levels in lungs, compared to either of the other groups (exposed to filtered air or to 57 $\mu g/m^3$). At the highest level of exposure, NF-κB binding activity was elevated by 1.3 times versus each of the other two groups, but significance was not reached. No changes in lactate dehydrogenase (LDH) activity, glutathione (GSH) or oxidized glutathione (GSSG), total cell number, cell viability, or cell differentials were found in either exposed group. The lack of LDH activity suggests that the cell injury implied by the increase in BAL protein count did not include significant cell lysis. The lack of change in GSH status suggests that oxidative stress in the animals exposed to either level of iron in this study can be controlled at these levels. The great majority of the iron produced and tested was in the form of iron oxides (Fe_2O_3), but it was nonetheless bioavailable in this form.

Fernandez et al. (2002) assessed the affects of two different CFAs on lung permeability in order to understand whether there might be adverse effects if municipal sewage sludge (MSS) were to be burned with coal. Mice were exposed, nose-only, to equal amounts of CFA, or of MSS with CFA, for 1 h daily for 24 consecutive days, at 1000 or at 3000 $\mu g/m^3$. Mice exposed to the MSS/CFA mixture exhibited significant increases in lung permeability and in BALF cell counts when compared to controls at both doses, with a larger increase at the higher dose, while the mice exposed to CFA alone did not exhibit such increases. The two different CFAs were very similar in pH and in size across the full range of sizes, starting in ultrafine (most of each sample was in the fine PM size range, but a small portion of each sample was in the coarse range). They reported that levels of Fe were higher in the CFA than in the MSS/CFA mixture. Zn

levels were higher in the MSS/CFA mixture, however, and were several times higher in two specific size ranges: ultrafine and submicrometer. Because these were the only major differences they were able to find between the two CFAs, they concluded that it was the higher Zn levels in the MSS/CFA mixture, but not higher Fe levels (found in the CFA), that caused increases in the measures of lung injury.

Ambient PM Instillation Studies

Besides using CAPs inhalation study, which can be expensive and time-consuming, studies using collected urban PM for intratracheal instillation/aspiration (IT/IA) to healthy and compromised animals have also produced interesting information. Although there are many issues such as extrapolation and dosimetry that need to be addressed when IT is used in a toxicological study, the results of IT of ambient PM collected from different geographical areas can be used to support the hypothesis that PM composition is one of the most relevant parameters in ambient PM-associated health effects.

Because of the locations of the sources, PM collected in different regions has different chemical composition. Costa and Dreher (1997) instilled rats with ambient PM from St. Louis, MO, Washington, DC, Dusseldorf, Germany, and Ottawa, Canada, and three combustion source particles (domestic oil fly ash, DOFA; residual oil fly ash, ROFA; and coal fly ash, CFA) to test for toxicity. Animals were dosed with (1) an equal dose by mass (nominal 2.5 mg/rat) of each PM mixture, or with (2) doses based on normalization of each PM mass to a metal content of 46 μg/dose and 35.5 μg of total metals (Cu, Fe, V, Zn) for the ambient PM and ROFA comparison. Biomarkers of lung inflammation (lung infiltration of neutrophils and eosinophils), permeability (total protein and albumin), and cellular injury (lactic dehydrogenase, LDH), were increased with the Ottawa extract, eliciting notably stronger effects than ambient PM extracts from the other cities. Compared to combustion source particles, such as ROFA, the ambient PM extracts exhibited, on a per mass basis, much less potency in inducing inflammatory responses. However, when the exposures were normalized to match metals content, there was little difference between the ambient PM and ROFA effects. Interestingly, the most potent ambient PM (Ottawa) was both the freshest one (collected (3 yr versus 10 yr before) and had the highest bioavailable metal content of the ambient PM.

In addition to pulmonary effects, instillation of rats of Ottawa PM extracts at 2.5 mg also induced pronounced biphasic hypothermia, a severe drop in heart rate, and increased arrhythmias (Watkinson, et al., 2002a, 2002b) that were not seen with a comparable instilled dose of Mt. St. Helens volcanic ash (shown by many studies to be relatively inert toxicologically). The cardiovascular changes observed for the Ottawa particles were actually greater than those with the ROFA particles. These experiments indicate (a) that instillation of ambient air particles, albeit at a very high concentration, can produce cardiovascular effects, and (b) that exposures of equal mass dose to particle mixtures of differing composition did not produce the same cardiovascular effects, suggesting that PM composition rather than just mass was responsible for the observed effects.

Kennedy et al. (1998) reported a similar dose-dependent lung inflammation (i.e., an increase in protein and PMN in lavage fluid, proliferation of bronchiolar epithelium, and intra-alveolar hemorrhage) in rats instilled with aqueous-extracts of particles in TSP samples collected from Provo, UT. in 1982. The particulate extract mixture was comprised of 1.0 mg/g Zn, 0.04 mg/g Ni, 2.2 mg/g Fe, 0.01 mg/g V, 1.4 mg/g Cu, 1.7 mg/g Pb, and 78 mg/g SO_4^{2-} in 500 ml saline solution. Doses of 0, 150, 500, and 1500 μg were instilled, and effects were seen at 500 μg. This study also indicated that a metal constituent, in this case PM-associated Cu, was a plausible cause of the outcome based on IL-8 secretion and enhanced activation of the transcription factor NF-κB in cultured epithelium.

To determine whether any specific metal is responsible for the pulmonary reactivity, various metal salts, at the concentration of metal present in the soluble fraction of an urban air particulate sample EHC-93, collected in Ottawa, Ontario, Canada, were instilled into mouse lung (Adamson et al., 2000). After 3 days, only a solution containing all metals tested and that of a Zn salt alone induced an increase in inflammatory cells and protein in lung lavage fluid. These two solutions also increased DNA synthesis in lung cells at this time, indicating a reparative response. Other solutions, containing metals such as Cu, Fe, Al, Pb, Mg, or Ni, induced no changes in the preceding measurements at the EHC-93 dose level of metal. In a more extensive 28-day study, Zn salts induced rapid focal necrosis of Type 1 alveolar epithelial cells followed by inflammation and elevation of protein levels in lavage fluid over a 2-wk period. Following the injury, epithelial cell proliferation increased and focal fibrosis was seen at 4 wk. A solution containing all the other metals tested without the Zn component induced only minimal lung effects. The results indicate that the acute toxicity associated with EHC atmospheric dust is most likely the result of the level of soluble Zn in this PM sample. This suggests that a high soluble metal content of atmospheric dust, in this case the Zn level, may be a crucial factor in determining pulmonary cell reactivity to inhaled PM.

Some of the most convincing evidence to demonstrate that the lung dose of bioavailable transition metals, not just instilled PM mass, was the primary determinant of the acute inflammatory response was derived from a series of studies using ambient PM_{10} collected in the Utah valley (Frampton et al. 1999, Ghio & Devlin, 2001; Dye et al., 2001). Frampton et al. (1999) showed that the extract of PM_{10} collected during the strike (having the lowest metal content, specifically soluble Fe, Cu, Pb, and Zn), showed no cytoxicity, minimal induction of cytokines, and lowest oxidant generation ability compared to extracts from PM_{10} (collected before and after the strike) having higher metal content. In a parallel study, Ghio and Devlin (2001) showed that exposures of human lungs in vivo to aqueous extracts of PM_{10}

collected before closure and after reopening of the steel mill provoked a greater inflammatory response than PM_{10} extracts from filters taken during the plant shutdown (reviewed in the epidemiological study section). Menke et al. (2006) reported that even low contemporary blood Pb levels are associated with greater cardiovascular mortality risks.

To investigate the dose, time course, and the roles of specific metals, Dye et al. (2001) exposed Sprague-Dawley rats, by IT, with equivalent masses of aqueous extracts of the same Utah ambient PM_{10}, described above, at 0, 0.83, 3.3, 8.3, or 16 mg extract/kg body weight in 0.3 ml saline. Twenty-four hours after IT, rats exposed to extracts of PM_{10} collected when the plant was open developed significant pulmonary injury and neutrophilic inflammation. Additionally, 50% of rats exposed to these extracts had increased airway responsiveness to acetylcholine, compared to 17 and 25% of rats exposed to saline or to the extracts of PM_{10} collected when the plant was closed. By 96 h, these effects were largely resolved, except for increases in lung lavage fluid neutrophils and lymphocytes in rats exposed to PM_{10} extracts from prior to the plant closing. Analogous effects were observed with lung histologic assessment. Chemical analysis of extract solutions demonstrated that extracts of PM_{10}collected when the plant was open contained more sulfate, cationic salts (e.g., Ca, K, Mg), and certain metals (e.g., Cu, Zn, Fe, Pb, As, Mn, Ni). The strong qualitative coherence among these human epidemiological, clinical, and animal toxicological studies clearly showed that soluble metals can be the most important components related to PM exposure-related health outcomes.

By contrast, Urch et al. (2004, 2005) found that metals were not significantly associated with brachial arterial constriction and increased blood pressure, but that EC and OC were so associated. Lipfert et al. (2006) found V and Ni to be significantly associated with long-term mortality, but that the traffic density variable was more robust and had larger explanatory value. Other studies also point to traffic emissions and particular metals as both having significant associations with health endpoints (Janssen et al., 2002; Grahame and Hidy, 2004). However, studies done largely in the absence of any but light traffic tend to show only the effects of particular metals (Maciejczyk & Chen, 2005). It is important to recognize that studies that have the capability of examining higher levels of both metals and of vehicular emissions tend to find both of health importance, and if the exposure to vehicular emissions is of good quality, tend to find little else of health significance (Schwartz et al., 2005; Gold et al., 2005; Ebelt et al., 2005).

Mice with allergic airway disease were used to identify FPM composition as a causative factor in the increased prevalence and severity of allergic diseases in Hettstedt compared with Zerbst in Germany (Gavett et al., 2003). Samples of FPM collected from these areas were instilled into ovalbumin-allergic mice. FPM from both areas significantly increased lung injury parameters and proinflammatory cytokines, while FPM from Hettstedt, but not FPMfrom Zerbst or control filter extract, caused a significant increase in immediate responses to ovalbumin challenge when aspirated 2 h before challenge. Antigen-specific immunoglobulin (Ig) E was increased by Hettstedt FPM whether administered before sensitization or challenge. Airway responsiveness to methacholine aerosol and lung inflammatory cell numbers were significantly increased only in allergic mice exposed to Hettstedt FPM before challenge. Since samples of FPM collected in Hettstedt in 1999 had severalfold higher levels of Zn, Mg, Pb, Cu, Cd, and As versus samples from Zerbst, it is possible that the metal composition of ambient FPMinfluences the severity of allergic respiratory disease.

More recently, in a European Union (EU) project entitled "Respiratory Allergy and Inflammation Due to Ambient Particles" (RAIAP), the role of physical and chemical composition of PM on release of cytokines of cells in vitro, on respiratory inflammation in vivo, and on adjuvant potency in allergy animal models was investigated (Steerenberg et al., 2003). Coarse (2.5–10 μm) and fine (0.15–2.5 μm) PM were collected during the spring, summer, and winter in Rome, Italy; Oslo, Norway; Lodz, Poland; and Amsterdam, the Netherlands. Primary rat alveolar macrophages and human epithelial cells (A549) were used for in vitro studies. For in vivo exposure, male BALB/*c* mice and Wistar rats were exposed by IT to these ambient PM suspensions (1.0 and 2.5 mg) as well as to saline and Ottawa EHC-99 dust. Inflammatory and airway allergic markers were evaluated. The results of this study showed that by clustering chemical constituents of PM based on the overall response pattern in the bioassays, five distinct groups could be identified. The clusters containing Ti, As Cd, Zn, Pb, Hg, and organics, derived from traffic, industrial combustion and/or incinerators, and combustion of black and brown coal/wood smoke, were associated primarily with adjuvant activity for respiratory allergy, whereas clusters of crustal of material (containing Ca, Al, Mg, Fe, Ba, Cu, Cr) and sea spray (Na, Cl) were predominantly associated with measures for inflammation and acute toxicity. The cluster of secondary inorganic aerosol and long-range transport aerosol (containing V, Ni, Se, nitrate, sulfate, ammonium and some organics) was exclusively associated with systemic allergy. This study showed that biological effects of PM can be linked to one or more PM emission sources, and that this linkage requires a wide range of bioassays.

Using projection-to-latent-surfaces (PLS) techniques to examine the relationships among sources, chemical composition, and toxicologic endpoints, Seagrave et al. (2006) instilled FPM collected from sites within the Southeastern Aerosol Research and Characterization (SEARCH) air monitoring network into rat lungs, and assessed general toxicity, acute cytotoxicity, and inflammation. The FPM samples were collected in four areas with differing sources of FPM, 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. The results of this study showed that urban sites with high contributions from vehicles and industry were most toxic. This study also showed that

the biological effects differ as a function of site and season. The PLS analysis based on chemical class indicated that PM containing metal oxides, transition metals (Pb, Mn, Cu, Se, Zn, and As), EC, OC, and hopanes/steranes was the most important predictor of cytotoxic and inflammatory responses. The PLS analysis also indicated that SO_4^{2-}, secondary organic aerosols, meat cooking, and vegetative detritus were not correlated with the biological responses. On the other hand, in PLS analysis based on the source apportionment, 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. This study supports the concept that specific constituents and/or sources of PM affect its toxicity.

Effects of ROFA and CFA on Pulmonary Responses in Animals

Major components of FPM are often derived from fossil fuel combustion. The largest contributions are generally attributable to sulfate and nitrate which are formed in the atmosphere from the oxidation of sulfur dioxide and nitrogen oxides in the combustion effluents. In addition, the mineral components of the fuels are emitted as the oxides in the FPM size range. Residual oil fly ash (ROFA) and coal fly ash (CFA), which are inorganic residues of combustion processes, contribute more than 2.5×10^5 tons annually to the ambient air PM burden in the United States (Costa & Dreher, 1997). Since the sulfate and nitrate ions, by themselves, are unlikely to be toxic at the concentrations that people inhale, it is logical, then, for toxicological studies to focus their investigation on the metal oxides portion of the combustion-derived PM in order to support the epidemiological findings as well as to delineate the plausible mechanisms associated with the observed adverse health effects induced by ambient FPM exposures. There are many hypotheses as to how ambient air PM exerts its toxicity. Because of the metals' ability to participate in Fenton-like chemical reactions to produce reactive species, the most frequently used combustion derived particles used to test the hypothesis that metals mediate the biological effects of air pollution is ROFA. ROFA is a complex mixture of sulfate, nitrate, and metals, such as Fe, V, and Ni, with the majority of these metals present in high concentrations as water-soluble salts.

After either IT or inhalation (IH), of ROFA, injury to the lung in the animal is evident within 24 h of exposure, with a dose-dependent recruitment of neutrophils, eosinophils, and monocytes into the airway (Prichard et al., 1996, Dreher et al., 1997). The peak of this influx occurs 18–24 h after exposure. The cellular influx persists 96 h later, and resolution occurs slowly (Prichard et al., 1996). Inflammatory lung injury after ROFA is accompanied by airway hyperreactivity (Gavett et al., 1997), an increase in susceptibility to infections (Prichard 1996, Antonini et al., 2002, 2004), and, at high concentration, noncardiogenic pulmonary edema (Watkinson et al., 1998). There are also effects of ROFA exposure on heart function, such as conductive and hypoxemic arrhythmias and cardiac-related deaths in monocrotaline-treated rats (Watkinson et al., 1998; Kodavanti et al., 1999), enhanced bradycardia, arrhythmias in ozone-exposed or cold-stressed animals (Watkinson et al., 2000a, 2000b, 2001; Campen et al. 2000), ST-segment depression in spontaneously hypertensive (SH) rats (Kodavanti et al. 2000), decreased heart rate variability (HRV) in rats with acute myocardial infarction (MI) (Wellenius et al., 2002), and cardiac inflammation, myocardial degeneration, fibrosis, and decreased granulated mast cells in SH rats (Kodavanti et al. 2003). An elevation in plasma concentrations of fibrinogen in exposed rats was also observed (Gardner, 2000; Kodavanti et al., 2002a).

Although qualitatively and quantitatively similar inflammatory responses were seen by IT or IH in an earlier study (Costa & Dreher, 1997), a more recent study showed that IT-ROFA mimicked IH in terms of lobar distribution of ROFA and injury biomarkers over 96 h, while morphological alterations and airway hyperresponsiveness (AHR) appeared to be more dependent on the method of administration (Costa et al., 2006). Moreover, in many of these studies, metal concentrations were several orders of magnitude higher than that measured in the ambient air. Interpretation and extrapolation of the results of these studies to ambient human exposure needs careful consideration. To date, there are more than 100 studies using ROFA as surrogate for ambient PM investigating its biological effects, the roles of its components, and associated mechanisms. In this review, only those studies dissecting specific contributions by transition metals relevant to ambient air pollution were cited.

It has long been recognized that oxygen-derived free radicals catalyzed by metals associated with air pollution particles could account for lung injury following exposure to air pollution particles. These free radicals can be detected using electron spin resonance (ESR). Twenty-four hours after IT of oil fly ash, prominent radical adduct ESR spectra could be detected in samples of lung lipid extracts (Kadiiska et al., 1997, 2004). Lipid extracts of lungs acquired from rats exposed to the soluble component of the oil fly ash demonstrated an ESR spectrum identical to those of animals instilled with air pollution particles. Furthermore, the ESR spectrum of lung lipid extracts from animals instilled with a mixture of V, Ni, and Fe sulfates resulted in the detection of a signal comparable to that exhibited by the soluble fraction of the oil fly ash. Individually, ESR spectra after instillation of vanadyl sulfate were more intense than those acquired from animals exposed to ferric sulfate, while Ni sulfate produced similar spectra as those produced by saline control. The results of this study supported a role for the individual metal components of the particle in free radical production.

To assess physical chemical factors that influence the pulmonary toxicity of ROFA constituents, Dreher et al. (1997) exposed rats to ROFA suspension, leachate, washed, neutralized suspension, neutralized leachate, neutralized leachate supernate,

and suspension + deferoxamine by IT at a dose of 2.5 mg ROFA/rat. The ROFA leachate, containing predominantly Fe, Ni, V, Ca, Mg, and sulfate, produced similar lung injury to that induced by ROFA suspension, indicating that the soluble components were primarily responsible for the effects. The inflammatory effects were abrogated by depletion of metals from the ROFA leachate by deferoxamine (a metal chelator). In addition, a solution containing a mixture of transition metal sulfate containing Fe, V, and Ni largely reproduced the lung injury induced by ROFA confirming that these transition metals were the most important components of ROFA in producing the adverse effects. Interestingly, neutralization of ROFA, soluble Ni, and transition metal sulfate mixtures, which produced fine precipitates in the solutions, led to the production of a more progressive acute lung injury in the case of ROFA particles and enhanced morbidity/mortality in the case of Ni and the transition metal sulfate mixtures. These findings provide direct evidence for the role of soluble transition metals in the pulmonary injury induced by ROFA, at least for exposures at relatively high doses of ROFA.

To further demonstrate how ROFA with differing V and Ni may differ in terms of their ability to cause in vivo acute pulmonary injury, male Sprague-Dawley (SD) rats were intratrachealy instilled with either saline or saline suspension of 10 ROFA samples collected at various sites within a power plant (<3.0 μm mass median aerodynamic diameter) at three concentrations (0.833, 3.33, or 8.33 mg/kg) (Kodavanti et al. 1998). After 24 h, ROFA containing the highest concentrations of water-leachable Fe, V, and Ni or V and Ni caused the largest increase in these biochemical indices of lung injury, while ROFA containing primarily soluble V caused more dramatic neutrophil influx. It appears that while V was responsible for the recruitment of neutrophils, Ni was responsible for increased pulmonary permeability, suggesting that the potency and the mechanism of pulmonary injury will differ between emissions containing V and Ni.

To investigate the host responsiveness to air pollution particle exposure, BAL inflammatory markers in normotensive (Wistar Kyoto, WKY) and SH rats were examined after a single IT exposure to either saline or ROFA (0.0, 0.83 or 3.33 mg/kg) and BAL lung injury markers were measured at 24 and 96 h post-IT (Kodavanti et al. 2001). Rats were also IT instilled with 0.0 or 1.5 μmol/kg of either VSO_4 or $NiSO_4$ $6H_2O$ in saline (equivalent to a dose of 2–3 mg ROFA), and assessed at 6 and 24 h post IT. ROFA-induced increases in BALF markers of inflammation were generally greater in SH rats than in WKY rats and had resolved by 96 h post IT in both strains. In response to a single metal IT exposure, both the onset and the duration of inflammatory response were metal and strain dependent. The V-induced increases in BALF protein and LDH peaked at 6 h post IT, and returned to control by 24 h in WKY rats. In SH rats, BALF protein and LDH were not affected by V. Ni caused BALF protein to increase in both strains at 6 and 24 h; however, the control values at 24 h were high in SH rats, and were not distinguishable from exposed rats. The Ni-induced increase in LDH activity was progressive over a 24-h time period (WKY > SH). The number of macrophages decreased following V and Ni exposure at 6 h, and this decrease was reversed by 24 h in both strains. V caused BALF neutrophils to increase only in WKY rats. The Ni-induced increase in BALF neutrophils was more dramatic and progressive than that of V, but was similar in both strains. Lung histology similarly revealed more severe and persistent edema, perivascular and peribronchiolar inflammation, and hemorrhage in Ni-than in V-exposed rats. This effect of Ni appeared slightly more severe in SH than in WKY rats. This study showed that inflammatory response to metallic constituents of ROFA is both strain and dose dependent, and that V caused pulmonary injury only in WKY rats, whereas Ni was toxic to both strains. In subsequent studies (Kodavanti et al. 2002b; Wu et al., 2003), however, Zn was found to be the responsible component in a batch of different oil combustion emission particles.

In another study investigating the role of soluble metal components in the toxicity of emission source particles, Gavett et al. (1997) investigated the effects of two ROFA samples of equivalent diameters, but having different metal and sulfate content, on pulmonary responses in SD rats. One ROFA sample had higher saline-leachable sulfate, Ni, V, and Fe, whereas the other sample had a higher Zn content. At a dose of 2.5 mg, 4 of 24 rats exposed to high-Zn ROFA suspension or supernatant had died 4 days post IT while none had occurred in high-Ni, -V, and -Fe groups. Pathological indices, such as alveolitis, early fibrotic changes, and perivascular edema, were greater in both high-Zn suspension and supernatant exposed groups than the other ROFA. In surviving rats, exposures to high-Zn ROFA also worsened the baseline pulmonary function parameters and airway hyperresponsiveness (AHR) to acetylcholine as well as BAL neutrophils. This study confirmed the finding of an earlier study in guinea pigs that soluble forms of Zn are capable of producing a greater pulmonary response than other sulfated metals in combustion generated particles (Amdur et al., 1978).

AHR induced by ROFA and its soluble components has also been observed in mice (Hamada et al. 2002). In this study, mice were exposed to aerosolized soluble leachate of ROFA (ROFA-s). AHR to acetylcholine challenge occurred in a time- and dose-dependent manner after exposure to ROFA-s with peak at 48 h post IH exposure. AHR was accompanied by an earlier onset of BAL neutrophils, which was maximal at 12 h after exposure. The AHR caused by ROFA-s was reproduced by a mixture of its major metal components (Ni, V, Zn, Co, Mn, Cu) but not by any individual metal alone. Intraperitoneal pretreatment of mice with the antioxidant dimethylthiourea abrogated ROFA-s-mediated AHR confirming the role of ROS in metal induced inflammation. Interestingly, ROFA-s had no effect on AHR of 2-wk-old mice, in contrast to the AHR seen in 3- and 8-wk-old mice. This study also found that ROFA treatment does not initiate neurogenic inflammation because ROFA-s-mediated AHR was unchanged in neurokinin-1 receptor knockout mice and in mice treated with an neurokinin antagonist.

To further investigate the manner in which ROFA mediates airway sensitization and lung injury in a Brown Norway (BN) rat model of house dust mite (HDM) allergy, Lambert et al. (1999, 2000) instilled BN rats IT with 200 or 1000 mg ROFA 3 days prior to local sensitization with 10 mg HDM and they were challenged with 10 mg HDM 14 days later. In addition, to determine whether the metals present in ROFA mediated this effect, BN rats were also dosed IT with either ROFA (1000 μg) or acidified saline + $NiSO_4$ (105.12 μg), VSO_4 (98.2 μg), $FeSO_4$ (58.49 μg), or a mixture (Mix) of each metal. Immunological endpoints were examined at 2, 7, and 14 days after sensitization, and at 2 and 7 days after challenge (16 and 21 days postsensitization, respectively). ROFA treatment augmented antigen-induced AHR and antigen-specific immunoglobulin E levels. Lymphocyte proliferation to antigen was enhanced at days 7 and 21 in the bronchial lymphocytes of ROFA-treated groups. BAL eosinophil numbers, LDH, total protein, and interleukin 10 (IL-10) were also significantly increased. HDM-specific IgE was higher in the serum of the ROFA, Ni, V, and Mix groups than in the HDM group after challenge, and antigen-induced AHR responses were increased in the Ni group. Lymphocyte proliferation to antigen was increased in the ROFA, Ni, and V groups compared to controls. Total protein and eosinophil peroxidase levels were elevated in the Fe group, and eosinophil numbers in the BALF were increased in the ROFA and Fe groups compared to HDM control. Cytokine IL-5 and IL-13 mRNA expression was also increased in the lung tissue of all metal- and ROFA-treated groups, while BALF IL-10 was elevated in the Fe and Mix groups, and IL-6 and TNF-alpha were elevated in the metal and ROFA-treated groups compared to controls. These results suggest that ROFA's metallic constituents mediate enhancement of sensitization to HDM, and that pulmonary inflammation may play a role in this adjuvant effect.

Genotoxic Effects of Transition Metals in ROFA

Using DNA microarray technology, Nadadur et al. (2000) measured the gene expression profile of SD rats 3 and 24 h after IT instillation with either saline, ROFA (3.3 mg/kg), $NiSO_4$ (1. 3 μmol/kg), or VSO_4 (2.2 μmol/kg). The microarray used in this study consisted of 27 genes representing inflammatory and anti-inflammatory cytokines, growth factors, adhesion molecules, stress proteins, transcription factors, and antioxidant enzymes; 3 negative controls; and 2 blank spots. A twofold induction of IL-6 and TIMP-1 was seen at 24 h post ROFA or Ni exposure. The pulmonary expressions of cellular fibronectin (cFn-EIIIA), ICAM-1, IL-1beta, and iNOS genes were also increased 24 h post ROFA, V, or Ni exposure. In a subsequent study using 84-gene microarrays, increased expression (1.5- to 3-fold) of stress response, inflammatory, and repair-related genes and also genes involved in vascular contractility and thrombogenic activity was found to be constituent specific and time dependent (Nadadur & Kodavanti, 2002). Three groups of genes—inflammation, remodeling, and stress-response—were induced in response to exposures to ROFA, Ni, and V. ROFA-induced expression of platelet-derived growth factor-A (PDGF-A), transforming growth factor (TGF-beta), E-selectin, and TIMP-2 was observed at 3 and 24 h postexposure, and the fold induction was similar for both time points studied. Increased expression of inducible form of nitric oxide synthase (iNOS) and increased induction of MAPP were observed at 3 h post ROFA exposure and returned to basal levels by 24 h. ROFA-induced expression of the cytokines, IL-5, IL-6, RANTES, and the adhesion molecule ICAM-1 was observed at 24 h postexposure. Ni-induced rat lung injury appeared to involve the expression of genes involved in stress response (HO-2, hsp70, TIMP-2) and cell adhesion (E-selectin, C-Fn, ICAM-1). Ni-induced overexpression of TIMP-2, E-selectin, hsp70, and C-Fn continued to be observed at 24 h. IL-6 was the only cytokine found induced at 24 h. Along with E-selectin, VCAM-1 was another adhesion molecule found to be induced at 24 h. The cardiac-specific proteins cardiac b-myosin and thrombomodulin (TM) were also found to be induced by Ni at 24 h. V-induced lung injury was associated with increased expression of the chemokine MIP-2, adhesion molecule E-selectin, and C-Fn at 3 h, with MIP-2 overexpression continuing even at 24 h. ET-1 and EST-1 were two additional messages that were found induced by V at 24 h. This study indicated that pulmonary injury caused by ROFA is different from that of its two toxic metal components, Ni and V, involving different initiation, progression, and resolution of complex inflammatory processes. Although BAL inflammatory markers (neutrophil influx, protein leakage, etc.) were similar in ROFA- or metal-exposed animals (Dreher et al., 1997; Kodavanti et al., 1997), the gene expression profile studies suggested that there were more complex interactions between metal constituents than previous studies had implicated.

Cardiovascular Effects of Transition Metals in ROFA

While studies of instilled ROFA demonstrated immediate and delayed responses, consisting of bradycardia, hypothermia, and arrhythmogenesis in conscious, unrestrained rats (Watkinson et al., 1998; Campen et al., 2000), further study of instilled ROFA-associated transition metals showed that Fe caused little response, whereas V caused marked bradycardia, arrhythmogenesis, and hypothermia immediately following instillation and lasting approximately 6 h. Ni caused no immediate response, but induced a delayed bradycardia, arrhythmogenesis, and hypothermia that began approximately 24 h after instillation and lasted for several days. When instilled in combination, Ni appeared to exacerbate the immediate effects of V, whereas Fe attenuated them.

In another study, Campen et al. (2001) examined responses to these metals in conscious rats by whole-body inhalation exposure. The authors tried to ensure valid dosimetric comparisons with the instillation studies, by using concentrations of V and Ni ranging from 0.3 to 2.4 mg/m^3. The concentrations used incorporated estimates of total inhalation dose derived using different ventilatory parameters. Heart rate (HR), core temperature, and electrocardiographic (ECG) data were measured

continuously throughout the exposure. Animals were exposed to aerosolized Ni, V, or Ni + V for 6 h per day for 4 days, after which serum and bronchoalveolar lavage samples were taken. While Ni caused delayed bradycardia, hypothermia, and arhythmogenesis at concentrations >1.2 mg/m^3, V failed to induce any significant change in HR or core temperature, even at the highest concentration. When combined, Ni and V produced observable delayed bradycardia and hypothermia at 0.5 mg/m^3 and potentiated these responses at 1.3 mg/m^3 to a greater degree than were produced by the highest concentration of Ni (2.1 mg/m^3) alone. The results are suggestive of a possible synergistic relationship between inhaled Ni and V, although these studies were performed at metal concentrations orders of magnitude greater than their typical ambient concentrations.

In a second study using dogs with preexisting cardiovascular disease, Muggenburg et al. (2003) evaluated the effects of short-term inhalation exposure (oral inhalation for 3 h on each of 3 successive days) to aerosols of transition metals. Heart rate and the ECG readings were studied in conscious beagle dogs (selected for having preexisting cardiovascular disease) that inhaled respirable particles of oxide and sulfate forms of transition metals (Mn, Ni, V, Fe, Cu oxides, and Ni and V sulfates) at concentrations of 0.05 mg/m^3. Such concentrations are 2 to 4 orders of magnitude higher than for typical ambient U.S. levels (usually 0.1 to 1.0 μg/m^3 for such metals). No significant effects of exposure to the transition metal aerosols were observed. The discrepancy between the results of Muggenburg et al. and those of Godleski et al. leaves open major questions about PM effects on the cardiovascular system of the dog. The use of ROFA samples from different sources may have accounted for the differences in response that were reported.

Kodavanti et al. (2003) exposed male SD, WKY, and SH male rats to IH of Boston ROFA, which contained bioavailable zinc (Zn) at doses of 2, 5, or 10 mg/m^3, for 6 h/day for 4 consecutive days. A second exposure paradigm used exposure to 10 mg/m^3 ROFA for 6 h/day, 1 day/wk, for 4 or 16 consecutive weeks. Cardiovascular effects were not seen in SD and SH rats with the acute or chronic exposure, but WKY rats from the 16-wk exposure group had cardiac lesions consisting of chronic active inflammation, multifocal myocardial degeneration, fibrosis, and decreased numbers of granulated mast cells. These results suggest that myocardial injury in sensitive rats can be caused by long-term inhalation of high concentrations of ROFA.

One possible mechanism for inhaled metals to cause cardiac effects is through the disruption of metal homeostasis in the body. For example, Zn, one of the components of ambient PM and ROFA, is functionally important to numerous biochemical processes in the body. Zinc has been shown to produce not only lung inflammation but also functional changes in the lung of animals and humans (Amdur & Chen, 1989; Fine et al., 1997). The inflammatory changes in the lung could lead to subsequent systemic inflammation, and alteration of circulatory functions. Indeed, IT exposure of male WKY rats with a high dose (131 μg/kg, approximately 1000$\times$ higher than that exists in the ambient air) of zinc sulfate induced significant pulmonary inflammation and injury, which were noted up to 48 h postexposure and were accompanied by persistently increased gene expressions of tissue factor (TF) and plasminogen activator-inhibitor-1 (PAI-1), but not thrombomodulin (TM), starting at 4 h postexposure. Plasma Zn increased to approximately 20% at 1 and 4 h postexposure with concomitant decline in the lung levels. At 24 and 48 h postexposure, Zn levels rose significantly (approximately 35%) in the liver. At these time points, plasma and liver levels of Cu and Se also increased significantly, suggesting systemic disturbance in essential metals. Similar temporal increases in expressions of TF, PAI-1, and TM mRNA were seen in cardiac tissues. Marked induction of metallothionein-1 (MT-1) and zinc transporter-2 (ZT-2) mRNA in lung, heart, and liver suggested a systemic metal sequestration response. As expected, with a high dose of Zn instillation, many biochemical processes such as those involved in kinases, mitochondrial functions, Ca homeostasis, and ion channels were affected. In contrast to extensive pulmonary edema and inflammation, only mild, and focal acute, myocardial lesions developed in a few Zn-exposed rats; no histological evidence showed increased deposition of fibrin or disappearance of troponin. At 24 and 48 h postexposure to Zn, increases occurred in levels of systemic fibrinogen and the activated partial thromboplastin time. These data suggest systemic metal homeostasis could be disrupted with exposure to a high dose of transition metal with subsequent extrapulmonary effects (Gilmour et al., 2006a, 2006b).

IN VITRO STUDIES AND MECHANISMS OF METAL-INDUCED BIOLOGICAL EFFECTS

In Vitro CAPs Source Apportionment Studies

Becker et al. (2005) investigated whether compositional changes in ambient PM in different seasons altered cellular response in normal human bronchial epithelial (NHBE) cells and alveolar macrophages (AMs). They exposed the cells to equal masses of coarse PM, FPM, and ultrafine PM from Chapel Hill, NC, during October 2001 (fall) and January (winter), April (spring), and July (summer) 2002. Coarse PM was more potent in inducing cytokines, but not ROSs, than were fine or ultrafine PM. In AMs, the October coarse PM was the most potent stimulator for IL-6 release, whereas the July PM consistently stimulated the highest ROS production measured by dichlorofluorescein acetate and dihydrorhodamine 123 (DHR). In NHBE cells, the January and the October PM were consistently the strongest stimulators for IL-8 and ROS, respectively. The July PM increased only ROS measured by DHR. Principal-component analysis on elemental constituents of PM of all size fractions identified two factors, Cr/Al/Si/Ti/Fe/Cu and Zn/As/V/Ni/Pb/Se, with only the first factor correlating with IL-6/IL-8 release. Among the elements in the first factor, Fe and Si correlated with IL-6 release, whereas Cr correlated with IL-8 release. These positive correlations were confirmed in additional experiments with PM from all 12 mo. These results indicate that elemental constituents of PM

may in part account for the seasonal variations in PM-induced adverse health effects related to lung inflammation. In contrast, in a study exposing healthy human volunteers to CAPs × 10 in the same area of North Carolina, no increases in IL-6 or IL-8 were found (Ghio, et al., 2000), suggesting that these in vitro effects at high dosages may not have transferability to humans inhaling CAPs at lower doses.

In conjunction with an animal inhalation study to investigate subchronic health effects of ambient FPM, a parallel in vitro study was conducted to address the relationship of FPM characteristics to the cellular response of human bronchial epithelial cells. In this simultaneous study, an in vitro exposure technique was used to compare the daily variations of the responses of cells to CAPs collected from a rural area upwind of New York City & the period of 9 a.m. to 3 p.m. on weekdays only, March–September, 2003 (Maciejczyk and Chen, 2005). Chemical composition data for CAPs were modeled using factor analysis with Varimax orthogonal rotation to determine four particle source categories contributing significant amounts of mass to CAPs at Sterling Forest (Tuxedo, NY). The source categories were: (1) regional secondary sulfate, characterized by high S, Si, and OC; (2) resuspended soil, characterized by high concentrations of Ca, Fe, Al, and Si; (3) ROFA emissions from ships (especially in the Port of New York) and power plants of the eastern United States, identified by the presence of V, Ni, and Se; and (4) unknown other sources. While the residual oil factor doesn't reflect other sources, the miscellaneous category does. One source in the unknown or miscellaneous category could have been fresh coal plant emissions, given the very high Se levels, and a prominent wind direction from the northeast, where a coal-fired power plant is located ∼20 miles from Sterling Forest. Also, the sulfate category contains secondary organics from distant upwind sources (Lee et al., 2003). To estimate the mass contributions of each individual source category, the CAPs mass concentration was regressed against the factor scores. Regional sulfate was the largest contributor to mass (65%), followed by soil (20%), residual oil combustion (2%), and the other sources contributing 13%. BEAS-2B, an airway epithelial cell line, stably transfected with NF-κB luciferase reporter gene, was exposed to FPM CAPs samples collected daily using a Biosampler (SKC, Eighty Four, PA). The NF-κB assay results found that only the ROFA category and Ni and V among the individual components, were significantly correlated with NF-κB.

The Roles of Reactive Oxygen Species

Airborne PM contains transition metals, such as Fe (most abundant), Cu, Ni, V, Zn, and Co. These metals are capable of catalyzing the one-electron reductions of molecular oxygen (O) necessary to generate reactive oxygen species (ROS). These reactions can be demonstrated by the Fe-catalyzed Haber–Weiss reactions (or Fenton reaction):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + HO^- \quad [1]$$

$$Fe^{2+} + O_2 + H^+ \rightarrow Fe^{3+} + O_2^- \quad [2]$$

$$HO_2^- + O_2^- + H^+ \rightarrow O_2 + H_2O_2 \quad [3]$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + \bullet OOH + H^+ \quad [4]$$

$$\text{Reductant}^n + Fe^{3+} \rightarrow \text{Reductant}^{n+1} + Fe^{2+} \quad [5]$$

Fe will continue to participate in the redox cycle in the reactions just shown, as long as there is sufficient O_2 or H_2O_2 and reductants. The hydroxyl radicals (OH, • OOH) and superoxide anion (O_2^-), along with H_2O_2, would react with many biological molecules to active cellular signals as well as produce cellular damage.

Upon deposition, soluble metals from inhaled PM will dissolve into the fluid lining of the airway lumen, where they can directly react with biological molecules extracellularly (acting as reductants in the preceding reactions) to produce ROS. For example, ascorbic acid in the human lung epithelial lining fluid can react with Fe(III) to cause single-strand breaks in supercoiled plasmid DNA (Smith & Aust, 1997). The free radicals causing the DNA damage in a PM_{10} suspension can be inhibited by mannitol, a hydroxyl radical scavenger (Gilmour et al., 1996; Donaldson et al., 1997; Li et al., 1997). Since the clear supernatant of the centrifuged PM_{10} suspension contains all of the suspension activity, the free radical activity is derived either from a fraction that is not centrifugable (10 min at 13,000 rpm on a bench centrifuge), or the radical-generating system products, most likely soluble metals, are released into solution (Gilmour et al., 1996; Donaldson et al., 1997; Li et al., 1997).

There are many different methods that can be used to ascertain that ROS are the responsible agents in producing adverse health effects. The ability of PM to produce ROS can also be assessed by measuring the electron spin resonance (ESR) spectrum of radical adducts. The chloroform extracts from rat lungs instilled IT with ROFA gave ESR spectra consistent with a carbon-centered radical adduct, while those spectra from lungs instilled with saline revealed a much weaker signal (Kadiiska et al., 1997). These signals were reproduced by instilling animals IT with the soluble fraction of the oil fly ash, which contains soluble metal compounds. The same signal was observed after instillation of V, Ni, and Fe sulfates, VOS_4 alone, or a mixture.

PM's ability to produce intracellular ROS can be measured using an intracellular fluorecent dye dichlorofluorescin (DCFH), an intracellular dye that fluoresces upon oxidation by ROS. Incubation of ultrafine carbon black (ufCB) with DCFH in the absence of cells generated significantly more ROS than larger sized CB (Wilson et al., 2002). With addition of either $CuSO_4$, $FeSO_4$, or $FeCl_3$, the ROS generation in the presence of ufCB was enhanced in a potentiative manner. When instilled IT in rats, the mixture of ufCB and $FeCl_3$ induced a significant neutrophil influx, indicating that ultrafine PM and iron salts interact in a potentiative manner to generate ROS and subsequent lung inflammation. Here and elsewhere, please note the dose involved.

Alternatively, the role of ROS in producing lung inflammation can be imdicated by using free-radical scavengers, such as dimethylthiourea (DMTU); antioxidants, such as glutathione or

N-acetylcysteine (NAC); or antioxidant enzymes, such as superoxide dismutase (SOD). The diminished response to PM after treatment with these antioxidants indicates the involvement of ROS. Using DMTU to inhibit ROS production, Dye et al. (1997) had shown that systemic administration of DMTU impeded development of the cellular inflammatory response to ROFA, but it did not ameliorate biochemical alterations in BAL fluid. In a subsequent study, it was determined that oxidant generation, possibly induced by soluble V compounds in ROFA, is responsible for the subsequent rat tracheal epithelial cells gene expression, inflammatory cytokine production (MIP-2 and IL-6), and cytotoxicity (Dye et al., 1999).

PM and its components can also stimulate resident or newly recruited alveolar macrophages (AM) or polymorphonuclear leukocytes (PMNs) to undergo oxidative stress, resulting in ROS production and subsequent cell response and injury. For example, AM chemiluminescence (CL) signals in vitro were shown to be greatest with ROFA containing primarily soluble V, and were less with ROFA containing Ni plus V (Kodavanti et al., 1998). In a study using PM from divergent sources (one natural dust, two types of oil fly ash, two types of coal fly ash, five different ambient air samples, and one carbon black sample), the CL responses in isolated PMN were significantly correlated with the insoluble Si, Fe, Mn, Ti, and Co content of the PM, but not with V, Cr, Ni, and Cu. Interestingly, pretreatment of the PM with the metal ion chelator deferoxamine did not affect CL activities.

The fact that ROS production can be induced by some metals, but not by others, could be due to the ability of cells to mobilize these metals. Size-fractionated CFA, in particles ($<$2.5, 2.5–10, and $<$10 μm) of bituminous b (Utah coal), bituminous c (Illinois coal), and lignite (Dakota coal), were used to compare the amount of Fe mobilization in A549 cells, a lung epithelial cell line (Smith et al., 1998). The amount of Fe A549 mobilized into cells was dependent on the type of coal used to generate the fly ash (Utah coal $>$ Illinois coal $=$ Dakota coal) but not related to the total amount of Fe present in the PM. Ferritin (an Fe storage protein) levels in A549 cells increased by as much as 11.9 fold in cells treated with CFA (Utah $>$ Illinois $>$ Dakota). More ferritin was induced in cells treated with the $<$2.5 μm fraction than with the $>$2.5 μm fractions. In another study, Mossbauer spectroscopy of a fly ash sample showed that the bioavailable iron was associated with the glassy aluminosilicate fraction of the particles (Ball et al., 2000). As with the bioavailability of Fe, there was an inverse correlation between the production of IL-8 and CFA particle size, with the Utah CFA being the most potent (Smith et al., 2000). Furthermore, treatment with a soluble form of iron, ferric ammonium citrate (FAC), mimicked the IL-8 level increase observed with CFA and was abrogated by the metal chelator desferoxamine (DEF) or by a free radical scavanger, tetramethylthiourea or dimethyl sulfoxide. Sulfate, the end product of fossil fuel combustion, could be the ligand that facilitates the Fe cellular mobilization (Ghio et al., 1999d). However, in a study utilizing 30 rural Western dusts, 2 CFAs, and V as a positive control, Veranth et al. (2006) found that the CFAs caused lower release of cytokines than all of the Western dusts, in an in vitro study using human lung epithelial cells. Instead, release of IL-6 and release of IL-8 were most highly correlated with the EC and low volatile OC portions of the dusts, suggesting the importance of vehicular emissions for the results, rather than surface iron on the CFAs. These findings parallel those of Ghio et al. (2000), who found no increases in IL-6 and IL-8 in healthy human volunteers exposed to CAPs ($\times$10) in Chapel Hill, NC.

Veranth et al. (2006) exposed human lung epithelial cells (BEAS-2B) to 30 different Western dusts, 2 CFAs, and V as a positive control. They found that several of the rural dusts induced IL-6 and IL-8 production in vitro, but that the two CFAs induced very low levels. One of the dusts produced higher IL-6 levels than the V control. Levels of IL-6 and IL-8 were most highly correlated with EC and low-volatility OC contents of the dusts. Thus, this study suggested that, even in rural dusts, vehicular emissions could cause inflammatory responses and can rival the effects of V in this regard. These findings are consistent with those of several other studies, noted earlier, finding effects due to vehicular emissions and to ROFA (inflammatory effects, or other effects), but not from other sources or factors.

In a high-dose installation study that compared the effects of ROFA, DOFA (distillate oil fly ash), and CFA on six different measures of inflammation and lung toxicity, Costa and Dreher (1997) found that CFA was considerably less toxic than the other two fly ashes. The metal content of the CFAs was far lower than either of the other fly ashes, a likely explanation for the differences in toxicity (Table 1).

Fe, an essential element in normal cell function, is one of the most tightly regulated metals in living cells. Disruption of Fe homeostasis could have devastating consequence in a biological system. Using ROFA and colloidal iron oxide, Ghio et al. (1997b, 1998a, 1998b, 1998c, 1999d) have shown that exposures disrupted Fe homeostasis and induced the production of ROS in vivo and in vitro, and that metal-chelating agents usually decreased the response and injury, indicating the important role that Fe plays in metal-induced lung injury. However, in a mixture of many soluble metals, such as that in the ambient PM or ROFA, the role of Fe is not as clear. For example, treatment of a ROFA suspension with DEF was not effective in blocking leachable metal-induced acute lung injury (Dreher et al., 1997). Dreher et al. (1997) indicated that DEF could chelate $Fe^{(III)}$ and $V^{(II)}$, but not $Ni^{(II)}$, suggesting that Ni played a role in the observed lung injury.

The in vitro study of Carter et al. (1997), using human airway epithelial cells, found that 2-h or 24-h exposures of ROFA increased concentrations of the cytokines IL-8, IL-6, and TNF-α, likely via expression of NF-κB. There was evidence of a dose-response function, and the longer exposure time created larger responses. V compounds, but not Ni and Fe compounds, mimicked these effects of ROFA. Use of the metal chelator deferoxamine or the free-radical scavenger DMTU inhibited the ROFA-induced cytokine responses.

Metals such as V and Ni may exert their toxic effects by interfering with Fe uptake and regulation (Salnikow et al., 2004). Coexposure to Fe, in either ferric or ferrous form, and Ni completely inhibited IL-8 production in 1HAEo$^-$ cells (a simian 40 virus-transformed lung cell line) and had no effect on "hypoxia-like" stress caused by Ni, suggesting the existence of two different pathways for the induction "hypoxia-like" stress and IL-8 production. The effect of Ni was not related to the blocking of Fe entry into cells, since the level of intracellular Fe was not affected by coexposure with Ni.

In subsequent studies, Ni was found to induce hypoxia in A549 cells (a simian 40 virus-transformed alveolar cell line) by stabilizing hypoxic inhibition factor-1 (HIF-1alpha) (Davidson et al., 2005) through the inhibition of HIF-prolyl hydroxylases (PHD's) (Davidson et al., 2006). Ni treatment was further shown to decrease both mitochondrial and cytosolic aconitase (c-aconitase) activity in A549 cells (Chen et al., 2005). Cytosolic aconitase was converted to iron-regulatory protein 1, a form critical for the regulation of cellular iron homeostasis. The increased activity of iron-regulatory protein 1 after Ni exposure stabilized and increased transferrin receptor (Tfr) mRNA and antagonized the Fe-induced ferritin light-chain protein synthesis. Exposure of A549 cells to soluble Ni decreased total cellular Fe by about 40%, a decrease that was likely mediated through the divalent metal transporter-1 (DMT-1). Similar interference of Fe uptake by V was observed using homozygous Belgrade rats, which are functionally deficient in DMT1 (Ghio et al., 2005). This disturbance of cellular Fe homeostasis by Ni and V may have a great impact on the ability of the cell to regulate a variety of cell functions, as well as creating a state of hypoxia in cells under normal oxygen tension.

An in vitro study by Ghio et al. (1999b) found that catalytically active metals, found in both soluble and insoluble fractions of PM from filters collected from North Provo, UT, in the 1980s, caused oxidative stress, increased influx of neutrophils, increased lavage protein levels, and increased IL-8 release. Concentrations of ionizable metals were higher in the soluble than in the insoluble fractions, but in most cases higher by factors of between 2.5 and 4. The metals included Zn, Ni, Fe, V, Cu, and Pb.

Intracellular Signaling Mechanisms

Many studies have shown that metals in ambient air PM altered intracellular redox state with subsequent modulation of the activity of several transcription factors, including NF-κB and AP-1, a critical step in the induction of a variety of proinflammatory cytokine and adhesion-molecule genes (PMCD, U.S. EPA, 2004). Other pathways such as apoptosis, activation of sensory nerve receptors, and processes involving matrix metalloproteinases were proposed (PMCD, U.S. EPA, 2004). One particular intracellular signaling pathway that can lead to diverse cellular responses, such as cell growth, differentiation, proliferation, apoptosis, and stress responses to environmental stimuli, is the phosphorylation-dependent mitogen-activated protein kinase (MAPK). The effects of As, Cr, Cu, Fe, Ni, V, and Zn on MAPK, extracellular receptor kinase (ERK), c-jun N-terminal kinase (JNK), and P38 in BEAS cells were investigated by Samet et al. (summarized in PMCD, U.S. EPA, 2004). Noncytotoxic concentrations of As, V, and Zn induced a rapid phosphorylation of MAPK in BEAS cells. Activity assays confirmed marked activation of ERK, JNK, and P38 in BEAS cells exposed to As, V, and Zn. Cr and Cu exposure resulted in a relatively small activation of MAPK, whereas Fe and Ni did not activate MAPK. Similarly, the transcription factors c-Jun and ATF-2, substrates of JNK and P38, respectively, were markedly phosphorylated in BEAS cells treated with As, Cr, Cu, V, and Zn. The same acute exposure to As, V, or Zn that activated MAPK was sufficient to induce a subsequent increase in IL-8 protein expression in BEAS cells. In a recent study, mutation of the activating protein (AP)-1 response element in an IL-8 promoter-enhanced green fluorescent protein construct reduced Zn^{2+}-induced IL-8 promoter activity. Moreover, Zn^{2+} exposure of BEAS-2B cells induced the phosphorylation of the AP-1 proteins c-Fos and c-Jun as well as the phosphorylation of ERK, JNK, and p38 MAPKs, whereas inhibition of ERK or JNK activity blocked IL-8 mRNA and protein expression (Kim et al., 2006). Zn^{2+}-induced cyclooxygenase 2 (COX-2) expression is also found to be involved in the p38 and EGFR kinase-mediated Akt activation and the PI3K/Akt signaling pathway plays a central role in this event (Wu et al., 2005). These data suggest that MAPK may mediate metal-induced expression of inflammatory proteins in human bronchial epithelial cells.

Release of inflammatory cytokines and mediators is the major consequence of cell activation by ROS, and is dependent upon the type of PM used in the experiments. In A549 cells, SiO_2 and Ni_3S_2 caused dose-dependent acute toxicity and apototic changes and increased IL-8 levels, in contrast to the response to diesel soots (Seagrave & Nikula, 2000). Expression of MIP-2 and IL-6 genes was significantly upregulated by ROFA exposure in rat tracheal epithelial cells, whereas gene expression of iNOS was maximally increased 24 h postexposure. V, but not Ni, appeared to be mediating the effects of ROFA on gene expression. Treatment with dimethylthiourea inhibited both ROFA- and V-induced gene expression in a dose-dependent manner (Dye et al., 1999).

Activation of MAPK pathways also occurred in vascular endothelial cells (Li et al., 2006). The production of extracellular H_2O_2 and activation of extracellular signal-regulated kinases1/2 (ERK1/2) and p38 MAPKs in human pulmonary artery endothelial cells (HPAEC) treated with urban particles (UP; SRM1648) and the effects of H_2O_2 on vasoconstriction in pulmonary artery ring and isolated perfused lung were investigated. Within minutes after UP treatment, HPAEC increased H_2O_2 production, which could be inhibited by diphenyleneiodonium (DPI), apocynin (APO), and sodium azide (NaN_3). The water-soluble fraction of UP as well as its two transition metal components, Cu and V, also stimulated H_2O_2 production. NaN_3 inhibited H_2O_2 production stimulated by Cu and V, whereas DPI and

APO inhibited only Cu-stimulated H_2O_2 production. Inhibitors of other H_2O_2-producing enzymes, including *N*-omega-methyl-L-argnine, indomethacin, allopurinol, cimetidine, rotenone, and antimycin, had no effects. DPI, but not NaN_3, attenuated UP-induced pulmonary vasoconstriction and phosphorylation of ERK1/2 and p38 MAPKs. Knockdown of p47phox gene expression by small interfering RNA attenuated UP-induced H_2O_2 production and phosphorylation of ERK1/2 and p38 MAPKs. Intravascular administration of H_2O_2 generated by glucose oxidase increased pulmonary artery pressure. The endothelial oxidative stress may be an important mechanism for PM-induced acute cardiovascular health effects.

DISCUSSION AND CONCLUSIONS

Considering the recent increase in epidemiological and toxicological research that has considered the influence of FPM composition on health-related responses, it seems ever clearer that spatial or temporal differences in FPM composition may mean that, for the same ambient level of FPM mass, the health implications of exposure to ambient air depend on the PM composition. Therefore, the mass-based ambient standards may provide a different level of protection at different places, and at different times at the same place, depending on the source mix contributing to the ambient mass concentration.

The Nature and Uses of the Studies Summarized in this Review Article

This review of the effects of metallic elements within ambient air PM on human health is one of three separate reviews on PM components that can influence human health. The other two cover other inorganic compounds (Schlesinger, 2007) and carbonaceous PM components (Mauderly & Chow, 2008). There are, inevitably, some overlaps in coverage among these three reviews since the effects observed in all studies of CAPs and ambient PM mixtures are likely to be influenced by components in more than one or both of the other categories covered in these review papers. As a result, evidence of significant associations between one or more components of the mixture in a multipollutant regression analysis may not provide clear evidence of causality because there is often confounding by differential exposure misclassification and measurement errors of the various components as represented by concentrations at central monitoring sites. For example, nonreactive species in fine PM, such as sulfate, Si, Al, Ca, and most of the Fe, can have indoor concentrations that are similar to those present outdoors, while reactive species such as sulfuric acid and semivolatile species such as nitrate and many organics may be much lower indoors than outdoors.

The substantially increasing number of studies of ambient PM mixtures that rely on data on PM component concentrations have provided valuable new opportunities to explore the separate and combined influences of those measured components on health-related responses. In this article, we have discussed where the influences of metallic elements have been reported, along with, in most cases, the potential influences of components of the other two categories.

Conclusions about causality of any specific PM components, or of source-related mixtures, must be tentative at this time. What the insights provided by the studies reviewed here do provide is a better basis for the design of future studies whose results may be more definitive.

Some General Caveats and Limitations of the Analyses Herein

The measurement of at least some of the PM components in the ambient air mixtures was a precondition for including a paper in this review. Thus, we generally only included discussions of effects associated with concentrations of metals. Even when quantitative data on metallic component concentrations were available, there were additional constraints on their utility as indicators of exposure to potentially causal components. While metals are very useful tracers for source attribution, the measurements by XRF for many of them are often below the lower limits of detection. When greater sensitivity and precision are needed, the more sensitive and more expensive measurements by ICP-MS can be made (Herner et al., 2006). However, both analytic methods provide no information on their chemical or physical form or solubility in biological fluids. Solubility, in turn, may depend not only on the associated cations and particle size, but also on whether they exist as dry particles, or as particles within aqueous droplets and, if so, on the pH of those droplets. In most cases, ionic forms of the metals will be most bioavailable, and therefore most likely to affect cells and organs beyond their deposition sites in the lung airways.

Where Do Metals Fit in the Larger Picture of PM-Associated Health Effects?

There is clearly emerging evidence that the inhalation of some metals in ambient air PM is associated with adverse health effects at concentrations near or not much higher than current ambient levels. These include Ni, V, and Pb, and suggestive evidence exists for others, such as Zn. There is also a rapidly growing literature implicating motor-vehicle-related pollution in human health effects, as indexed by EC, OC, and ultrafine particle number. However, there are also metals in motor vehicle exhaust whose role, if any, in association of their concentrations in ambient air with human health effects has not been determined. Furthermore, there is evidence that adverse health effects are significantly associated with aerosol acidity, which could be due to its irritancy, or to its role in solubilizing metals within the particles. Thus, for a more holistic evaluation of current knowledge on the health effects attributable to ambient air PM, it is important to consult the other two reviews in this series (Schlesinger, 2006, and Mauderly and Chow, 2008), as well as this one.

Are There Specific Metals That Can Account, at Least in Part, for Health Effects Associated With FPM Mass or FPM Source Categories?

Toxicological studies investigating the biological effects of constituents of ambient air have not, with one exception, identified an individual metal as being a likely cause for an effect of concern with respect to human health. The one exception is the demonstration that acute peaks of Ni within FPM CAPs were significantly associated with short-term increases in heart rate (HR) and decreases in heart-rate variability (HRV) in a mouse model of atherosclerosis (Lippmann et al., 2006). Lippmann et al. (2006) also discussed evidence that both Ni and V, which, together, are markers for the residual oil combustion source, have been significantly associated with excess daily mortality in time-series mortality studies. In these time-series studies the separate influences of Ni and V cannot be determined because their air concentrations are usually highly correlated. In a previous subchronic (6-mo) CAPs exposure studies in this animal model at NYU, we reported that total FPM was associated with effects on HR and HRV, as well as on the progression, during exposure, on aortic plaque growth and invasiveness, and on the gene expression and brain cell distribution (Lippmann et al., 2005). However, it is not known whether Ni, or any other specific component of the FPM CAPS, played any major role in these effects of prolonged series of daily exposures. Furthermore, there have been no previous studies of people in urban areas, or in working populations, that have related Ni exposure to cardiovascular disease.

If there are health-related effects of specific metals, other than the effects of Ni in ambient FPM on cardiac function, they are not yet known. However, it is important to remember that the absence of evidence for effects of specific metals does not demonstrate an absence of effects, and that both Ni and V were more closely associated with mortality than other metals in the NMMAPS reanalyses (Lippmann et al., 2006; Dominici et al., 2007), and in the Hong Kong sulfur-in-fuel intervention study (Hedley et al., 2002, 2004).

Are There Specific Source Categories That Can Account, at Least in Part, for Health Effects Associated With FPM Metals?

It is known is that ROFA, which is a mixture that is notably high in the content of Ni and V, as compared to other metals, and Utah Valley dust, which is a mixture enriched in steel mill emissions, were more toxic than other source-related mixtures that have been tested in laboratory animals in vivo or in cells in vitro. For pulmonary system responses, it appears, from such tests, that V may play a prominent role, and that the effects may depend on interactions among the metals. By contrast, other source-related mixtures, such as coal combustion effluents, that are notable for their content of Se, Fe, and Mn, and resuspended soil that contains more refractory metals, have been found to be less toxic.

For residual oil combustion effluents, the results of the analyses of the associations between annual average FPM component concentrations and average daily mortality coefficients in 60 NMMAPS MSAs, discussed earlier, and shown in Figure 1, are broadly consistent with the Hong Kong experience involving a switch to low-sulfur fuel. In both populations, the concentrations of Ni and V in ambient air PM were associated with significant differences in mortality rates, while all other measured PM components were not. Both Ni and V are present at relatively high concentrations in residual oil combustion effluents from oil-fired power plants and/or from ocean-going ship boilers consuming high sulfur fuel when operating in or near port cities, For example, in 2002, 93% of the U.S. emissions of Ni were in states that border the Atlantic and Gulf coasts and California, and 3% more was from states that border the Great Lakes, leaving only 4% for the other 25 states (2002 National Emission Inventory, Version 1, February 9, 2006, release). There are similar percentages for V. Thus, residual oil combustion effluents, as a source-related mixture, or of the Ni and/or the V in that mixture, may be responsible for a disproportionate contribution to excess FPM-associated mortality in coastal cities. These results alone do not indicate the relative contributions of Ni and V to the overall mortality impact, or whether they have differential effects on mortality due to cardiovascular and/or respiratory causes. However, these findings, when considered together with the statistically significant short-term cardiac function changes in mice that were associated with 6-h average concentrations of Ni below 200 ng/m^3, when the V concentrations were below 10 ng/m^3, suggest that Ni is the more likely causal factor for the effects associated with reduced mortality in humans in the NMMAPS, for which longer term Ni concentrations extended down from 19 ng/m^3 in NYC to a national average of 1.9 ng/m^3.

Addressing Research Needs Relating to Health Effects of Metals in Ambient Air Particulate Matter

There are many reasons why past research has not resolved the roles that metals may play in the health-related effects of ambient air PM. These are: (1) Concentrations of metals in ambient air PM generally range from a few μg/m^3 in some refractory metals to less than 10 ng/m^3 for transition metals that are known to generate ROS, raising the issue of biological plausibility; (2) epidemiologic research opportunities have been limited because of the paucity of data on the concentrations of PM components—even now, when there are several years of FPM speciation data available for many U.S. cities, they are mostly limited to every third or sixth day; (3) few toxicologists or clinical researchers have had the resources needed to perform CAPs studies that include speciation data on the PM in the exposure samples; (4) controlled exposures to pure compounds at concentrations of environmental relevance have been uniformly negative, even when sensitive animal models were used; (5) there is a lack of studies defining the relationship between personal exposure and ambient air levels for most metal species; and (6) most controlled exposure studies have been limited to one or

a few days, which may not be sufficient to elicit responses of concern.

The subchronic CAPs inhalation studies at NYU suggest that CAPs overall, and Ni in particular, can yield evidence that current levels of ambient air concentrations produce health effects of interest in terms of public health. Furthermore, there are many toxicology studies of ROFA cited earlier that are buttressed by epidemiological studies (Hedley et al., 2002, 2004; Janssen et al., 2002; Grahame & Hidy, 2004; Lipfert et al., 2006), suggesting a line of continuity between both types of studies with regard to damage from the combination of V and Ni. There are also some studies showing oxidative stress and DNA damage associated with V but not with Ni (Sorensen et al., 2005). If the inhalation of Ni, or Ni in combination with V, at current, relatively low, ambient air concentrations, does appreciably affect cardiac function and mortality in humans, one may wonder why the effects of such exposures have not previously been recognized. One reason may be that the increment in cardiovascular mortality that they may have produced is a relatively small part of the very large cardiovascular mortality. Also, the statistically significant transient and progressive changes that Ni produced in cardiovascular function in the ApoE-/- mice were relatively subtle, required advanced analytical techniques for their detection, and are unlikely to be detected in the kinds of short-term exposure studies that have previously been undertaken in laboratory animals.

In addition, the exact physical and chemical characteristics of Ni bearing ambient PM have not been determined. Based on the previous work at NYU, the fact that the potency of ultrafine Zn particles with a thin coating of sulfuric acid is much greater than for uncoated particles (Amdur & Chen, 1989) raises the likelihood that a specific form of a metal that has not been reproduced in the laboratory could be responsible for the observed biological effects.

Many studies have used ROFA as a surrogate for ambient PM in various in vivo and in vitro experiments. ROFA contains many soluble metals, and since it is clear, from this review, that they interact with each other, chemically and biologically, it should not be surprising that there are inconsistent and confusing results. Although ROFA was useful in providing plausible evidence that metals are important in eliciting adverse cardiopulmonary effects, it should not be the focus of future studies. The experimental in vitro design of Maciejczyk and Chen (2005), if performed in parallel to an animal inhalation studies and/or human clinical studies, could provide a better understanding of the source profile that may contribute to the adverse effects seen in animals and humans.

Signal transduction studies are very useful in dissecting the specific biological pathways leading to injury and repair. In most cases, it is much easier to obtain useful information using a single compound. For ambient PM, which consists of multiple chemical species, the usefulness of this type of study is not clear. On the other hand, the use of microarray technology, which measures endpoints further downstream from the initiating events (e.g., ROS production upon contact with PM), to investigate global gene expression levels could yield insights into how genes are interacting with complex PM exposures.

Much of the remaining skepticism concerning the biological plausibility of the premature mortality and increased morbidity that has been associated with ambient air FPM has been due to the paucity of exposure-response data in laboratory studies involving FPM inhalation, and the heretofore seemingly impossible task of identifying any specific causal components. The subchronic CAPs inhalation studies that were performed in Sterling Forest (Tuxedo, NY) (Lippmann et al., 2005b, 2006; Sun et al., 2005) helped to establish such plausibility, and have also developed a mechanistic base for the initiation and progression of effects attributable the long-range transported aerosol in the northeastern United States (Sun et al., 2005). The consistency in these analyses and reexaminations of available data lead to the conclusions that: (1) Ni is a particularly influential component of ambient FPM in terms of cardiac responses to the inhalation of ambient air FPM; (2) further research is needed on the specific influences of both Ni and V, which are both generally most closely associated with residual oil combustion effluents, on both acute and chronic respiratory and cardiovascular health effects; and (3) further research is also needed on the currently unknown impacts of other toxic metals in ambient air.

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