Fine Particulate N-Nitroso and Nitrite Organic Compounds in the Atmosphere

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ABSTRACT

Photochemistry between gas phase organic compounds, NO_x and ozone is expected to result in the formation of nitrogen containing compounds of toxicological importance. Classes of compounds which may form include nitro-, N-nitroso-, nitrate- and nitrite-substituted organic compounds. Many of these compounds are labile, semi-volatile organic compounds in equilibrium between the gas and particulate phases in the atmosphere. The phase distribution of these potentially toxic semi-volatile organic compounds can be determined using diffusion denuder sampling technology. The total concentration of N-nitroso compounds in a collected sample is determined using N-nitroso specific denitrosation reactions followed by detection of the NO produced with a chemiluminescence detector. Denitrosation chemistry can also be used to determine nitrite compounds. Differentiation between total N-nitroso- and nitrite-containing compounds is accomplished using sulfamic acid as a nitrite specific reagent. Specific nitro and N-nitroso organic compounds are detected using supercritical fluid chromatography coupled to the nitro- and nitroso-specific chemiluminescence detector. These analytical techniques for the sampling and determination of total N-nitroso and nitrite material have been used for the quantification of these compounds in both fine particles and gas phase in samples collected in Provo, UT. The results indicate that the majority of the N-nitroso and nitrite organic compounds present in fine particulate matter in the urban area studied are semi-volatile organic compounds which are lost from particles during sampling. Furthermore, the concentrations of these fine particulate compounds are comparable to the concentrations of gas phase N-nitroso and nitrite organic species. Detailed analyses of nitro-, nitrite- and N-nitroso-organic semi-volatile compounds can be expected to improve our understanding of the etiology of observed health effects associated with exposure to ambient fine particles.

INTRODUCTION

Past studies have shown that about one-third of the mass of fine particulate matter (dia. < 2.5 μm) collected on filters in western urban areas[1-2] is carbonaceous material. In the eastern United States sulfate is the major component of filter collected airborne fine particles. However, filter collected organic material comprises

about one-fourth of the fine particulate mass.[3-4] In the northwest, organic material is the dominant fine particulate component.[5] In previous studies of fine particles, the composition of particulate organic material has been significantly underestimated due to losses from the semi-volatile organic fraction during sample collection, i.e. a "negative sampling artifact".[6-9]

To avoid sampling artifact problems in the sampling of particulate phase organic compounds, we have constructed and tested a sampling system employing diffusion denuders, filters, and sorbent beds, the BOSS (BYU Organic Sampling System).[8] The data obtained to date with the BOSS show that particulate phase organic compounds in both urban and rural areas have been significantly underestimated by collection of particles with only a filter. The collection of gas phase compounds by a quartz filter may produce a small "positive" artifact,[6-7,10-13] but a much larger negative error results from the loss of 20-80% of the particulate phase organic material during sampling.[6-7,9,14-15] This includes the loss of about 90% of the semi-volatile organic material.[6-7,9,14] This sampling artifact must be considered in the collection of particulate organic material.

The importance, with respect to potential health effects, of the particulate organic compounds which have not been identified in past studies where particles are collected on a filter will be dependent on the chemical composition and the size distribution of the particulate organic compounds, both those lost from the particles during sampling and those remaining on the particles after sampling. We have developed[9] and tested[6-7,9,14] a high-volume, multi-component diffusion denuder sampling system for the determination of the size distribution and chemical composition of fine particulate organic compounds without sampling artifacts. The new sampling system, BIG BOSS,[9] uses a variety of size selective virtual impactor inlets to control the particle size of the particles introduced to the diffusion denuder sampler.[9,16] The BIG BOSS sampling system has been used for the characterization of alkanes, organic acids and polycyclic aromatic compounds in fine particulate carbonaceous material as a function of particle size in brief sampling programs conducted at Provo, UT,[9] Los Angeles, CA,[7,9,14] and at the western edge of the Grand Canyon.[6] In each of these studies, an average of 40% of the total fine carbonaceous material is lost from the particles during sampling. About 40% of the carbonaceous material remaining on the particles is soot. Hence, about half of the fine particulate non-soot organic material is lost from particles during sampling. The chemical composition of this semi-volatile organic material lost from fine particles during sample collection includes paraffinic compounds, organic acids and esters, and polar (hydroxy- and nitro-substituted compounds) and non-polar aromatic compounds.[6-7,9,14] It can be anticipated that some of these, as yet unidentified, compounds will be of toxicological importance.[17]

The particulate SVOC which have not been measured in past studies of fine particles in the atmosphere will contribute to health effects associated with exposure to fine particles. The significant under-measurement of these compounds in past studies means that our current assessment of the role of these compounds in exacerbation of health problems in populations exposed to particulate matter will err on the side of underestimating the importance of this class of pollutants.

Data from three different areas point to the need for detailed chemical and toxicological characterization of fine particulate semi-volatile organic material. Epidemiological studies completed during the past decade[18-19] have shown that there are significant health effects associated with exposure to respirable particles at concentrations substantially below the current PM_{10} standard. The physical-chemical basis of these effects is not currently understood. An early hypothesis suggested that the health effect was due to exposure to acid sulfate compounds.[20] However, this hypothesis does not appear to explain more recent epidemiological results.[18,21-22] The body of recent epidemiological data has resulted in a current, ongoing review of the PM NAAQS by EPA. It can be expected that this review will result in the implementation of some form of a fine particulate NAAQS.

Recent toxicological studies of respirable particulate matter have focused on the evaluation of exposure to genotoxic compounds.[23-26] The most extensive of these studies have been those conducted under the umbrella of the Integrated Air Cancer Project of EPA.[25,27] The emphasis in these studies has been on source apportionment of exposure in urban communities to pollutants from vehicle emissions and wood combustion.[27] The studies have included the determination of exposure to airborne mutagens.[25] Results of the IACP studies have shown that significant concentrations of both gas and particulate phase mutagens are present in the urban atmosphere and that the greatest exposure to these mutagens is associated with the products of photochemistry of NO_x and automotive and wood-smoke emissions.[28-29] Laboratory studies have shown that irradiation of simple hydrocarbons in the presence of NO, NO_2 and SO_2 results in a significant increase in gas phase mutagens.[30-32] The compounds responsible for the mutagenicity have not been identified. The principal mutagen formed in the toluene studies, PAN, accounted for about 30% of the mutagenicity.[32] Kamens et al.[33-34] have shown that nitrated mutagenic compounds are rapidly formed in wood smoke in the presence of O_3 and NO_2, and a large increase in both gas and particulate phase mutagenicity results from this wood smoke chemistry.[35-36] Similar changes have been seen in studies of auto emissions.[35,37] Detailed data on the contribution of semi-volatile particulate organic material to the toxicological character of urban fine particles are needed.

The photochemistry between gas phase organic compounds, NO_x and ozone

is expected to result in the formation of nitrogen containing compounds of toxicological importance. Many of these compounds will be semi-volatile organic compounds in equilibria between the gas and particulate phases in the atmosphere. These compounds may be detected using supercritical fluid chromatography, SFC, coupled to nitrogen, nitro- and nitroso-specific chemiluminescence detectors.[38] The instrument has been used for the characterization of these classes of compounds in environmental tobacco smoke and explosive combustion samples[38-39] and should be suitable for the identification of the photochemical products of NO_x in an urban atmosphere. Detailed analyses of nitrogen, nitro- and nitroso-organic semi-volatile compounds would improve our understanding of the etiology of observed health effects associated with exposure to ambient fine particles.

Mutagenicity assays are used as an indicator of genotoxicity and potential carcinogenicity of airborne pollutants.[24-25,40-41] Data on the mutagenicity of wood smoke, vehicle emissions and environmental tobacco smoke, and the chemical reactivity of these emissions suggest that the mutagenicity of combustion emissions increases with increasing residence time in the atmosphere, in both the gas and particulate phases. This increase in mutagenicity appears to be due to the products of reactions of NO_x with organic compounds. Current data on the mutagenicity of fine particles is limited to stable compounds which are retained by particles during sampling. However, the high concentrations of gas phase mutagens which are present in urban atmospheres suggests that significant amounts of mutagenic SVOC are also present in atmospheric particles. Because of their lower molecular weight, many of these compounds will be in equilibria between the gas and particulate phase and will be lost from particles during sampling. In addition, the concentrations of these compounds present in fine particles would be expected to be dependent on the water content of the particles, and hence on the relative humidity. They may, therefore, play a significant role in the observed health effects associated with exposure to fine particles.[42] Identification of the mutagenicity and the compound classes responsible for the mutagenic effect of these semi-volatile particulate pollutants can be expected to significantly increase our understanding of the health effects associated with exposure to these secondary products of combustion emissions.

Supercritical fluid separation techniques coupled to mutagenicity assays[38,43-44] have been developed. The separation processes do not chemically alter the organic compounds present in either the gas or particulate phases during the sample preparation and separation steps leading up to the mutagenicity assay. The technique couples SFC separation of material with microsuspension assays for Salmonella strains.[41] This technique allows the determination of the fractions separated from collected environmental samples which are responsible for genotoxicity. Parallel fractions from the chromatographic system are interfaced to an FID for detection of

all compounds and to a chemiluminescence detector for the specific identification of separated N-, nitro- and N-nitroso-containing organic compounds. The separated material can also be directly coupled to a mass spectrometer for compound identification.[45] This technique thus allows direct comparison of compound identity and concentration to biological activity.

We have initiated a research program to identify genotoxic, fine particulate SVOC formed as secondary photochemical products of urban emissions, and establish their concentrations in fine particulate matter in urban areas as a function of particle size. Particulate SVOC constitute a major fraction of fine particles in the atmosphere, have essentially not been measured by techniques used in most past environmental studies, and are possibly associated with unexplained health effects of atmospheric fine particles. The underlying hypothesis of the proposed research is that photochemical reactions among volatile organic compounds and NO_x in urban emissions result in the formation of secondary toxic semi-volatile nitro-, nitrite- and N-nitroso-organic compounds which are present predominantly in fine particles in the atmosphere and are associated with the previously observed exacerbation of health effects associated with exposure to fine particles. This report describes the development of analytical techniques for the specific determination of nitrite- and N-nitroso organic compounds in collected samples and initial experiments on the determination of these compounds in the Provo, UT urban area.

DETERMINATION OF N-NITROSO ORGANIC COMPOUNDS

Detection of SFC Separated Compounds with a Chemiluminescence Detector.

Two complementary analytical techniques are being used for the detection and determination of N-nitroso organic compounds. The first is the use of capillary column supercritical fluid chromatography using a chemiluminescence detector (TEA, Thermedics Inc., Model 543). The TEA is a gas phase chemiluminescence detector which was initially developed for the detection of N-nitroso-containing compounds.[46] This detector has also found use for the detection of nitro-containing compounds.[47-48] When pyrolyzed at temperatures of 350°C to 800°C, such compounds release the nitrosyl radical, NO. N-nitroso compounds are easily cleaved at 350°C, whereas the C-nitro compounds are usually cleaved at much higher temperatures of 700°C to 800°C. The nitrosyl radical is drawn by vacuum into a reaction chamber where it is reacted with ozone to form electronically excited nitrogen dioxide. The chemiluminescence decay of the excited state NO_2 is detected with a photomultiplier tube. Thus, analysis at 400°C allows the determination of N-nitroso containing compounds and analysis at 800°C allows the determination of both nitro- and N-nitroso-containing compounds. The technique has been used for the analysis of gas phase N-nitroso compounds in ambient air,[49] for the determination of both gas

phase[50] and particulate phase N-nitrosamines[39,44] in environmental tobacco smoke, and for the detection of nitro-organic compounds in ambient particulate matter[51] and environmental tobacco smoke.[39] The identification of N-nitroso compounds can be complicated by the presence of other compounds which will pyrolyze at 400° C to produce the ·NO radical.[38-39]

The detector response for a synthetic mixture of nitrogen containing explosives has been studied.[52] A chromatogram of the mixture with a pyrolysis temperature of 800° C is shown in Figure 1. Included in the mixture in the order of elution in Figure 1 are (1)-(3) the three isomers of nitrotoluene, (4) nitroglycerine, (5) 2,6-dinitrotoluene, (6) N-nitrosodiphenylamine(NNDPA), (7) 2,4-dinitrotoluene, (8) 1-nitronaphthalene-d_p, (9) pentaerythritol tetranitrate (PETN), (10) 2-nitronaphthalene, (11) 2,4,6-trinitrotoluene, (12) 2-nitrodiphenylamine, (13) 1,3,5-trinitrobenzene, (14) tetryl, (15) cyclotrimethylene trinitramine (RDX), (16) 1-nitronaphthalene, and (17) HMX. With a pyrolysis temperature of 450° C, the signal for the N-nitroso compound, NNDPA, is identical to that seen at 800° C, Figure 2. While the response is not detectable for most of the remaining compounds at 400° C, a measurable, but reduced response remains for the hydroxylated nitro and nitrate compounds, nitroglycerine (NG) and PETN, Figure 2. The response of these two compounds is very temperature dependent and the peak for both is increased at a pyrolysis temperature of 450° C compared to 400° C, Figure 2. In addition, some response is seen for some of the simpler nitro containing compounds at a pyrolysis temperature of 450° C. These results are significant with respect to use of the chemiluminescence detector for the detection of nitro and nitroso containing compounds in atmospheric samples because hydroxylated nitro containing compounds are present and suspected to be toxic.[17] While it is possible to distinguish between N-nitroso containing compounds and those nitro containing compounds which show some pyrolysis response near 400° C by determining the effect of temperature on the signal response, a more straightforward identification would be desirable.

Determination of N-Nitroso and Nitrite Compounds by Chemical Denitrosation and Chemiluminescence Detection.

N-Nitroso compounds are catalytically and selectively cleaved in a mixture of nitro and N-nitroso compounds to release NO in anhydrous acetic acid - HBr solutions.[53-56] The released NO may then be detected from its chemiluminescence, Figure 3. The instrument for this analysis is shown schematically in Figure 4.[52] The denitrosation reagent is placed in the reaction vial. A stable baseline is established with a flow of He through line B. The extract of the environmental sample to be analyzed is loaded in line A and then swept into the reaction vial by changing the position of the switching value. The NO released by the denitrosation reaction is

then swept into the TEA detector where it is detected by the chemiluminescence reaction with O_3, Figure 4. We have evaluated the analysis of N-nitroso compounds in both the synthetic explosive standard shown in Figure 1 and in collected atmospheric particulate matter.[52] The response of the detector in this analysis is linear over a wide dynamic analysis range, with a detection limit of 0.8 pmol.[52] The concentration of total N-nitroso containing compounds may be determined in an extract solution from a collected atmospheric sample using the analytical system shown in Figure 4. Conversely, the identity of an N-nitroso compound separated in an SFC analysis may be confirmed by analysis of acetic acid-HBr treated and untreated aliquots of the sample, Figure 1. As shown in Figure 1, treatment of the complex explosive mixture with the denitrosating agent results in complete disappearance of the NNDPA peak. The thermally labile NG and PETN compounds are, however, unaffected by the treatment, Figures 1 and 2. The NNDPA compound is the only compound in the mixture so affected.

In addition to denitrosation of N-nitroso compounds[52] the acetic acid-HBr solution will also react with nitrite compounds to release NO. However, nitrite compounds will react specifically with sulfamic acid to produce nitrogen,[57] e.g.,

$$NH_2SO_3H + HNO_2 = N_2 + SO_4^{2-} + H_2O + 2H^+$$

The denitrosation response of standard solutions of N-nitroso compounds is unaffected by the addition of sulfamic acid.[52] Thus, direct denitrosation analysis of an extract of an atmospheric sample yields the sum of N-nitroso and nitrite compounds in the sample. Analysis after treatment with sulfamic acid gives the concentration of only N-nitroso compounds.

Total N-Nitroso and Nitrite Containing Compounds in Atmospheric Fine Particulate Matter.

Brigham Young University has developed diffusion denuder sampling systems for the collection and determination of total semi-volatile and non-volatile particulate organic compounds[7-8] and for the size dependent chemical characterization of these particulate organic compounds.[6-7,9,14] The analytical procedures described above have been used for the determination of both gas and fine particulate phase N-nitroso and nitrite compounds on five samples collected in Provo, UT using the BOSS shown schematically in Figure 5. All sampling systems are preceded with a 2.5 μm virtual impactor inlet.[9,16] Flow through each of the sampling systems is nominally 200 sLpm. The components and purpose of each of the samplers of the BOSS, Figure 5, are:

System 1. A charcoal impregnated filter, CIF, diffusion denuder[8-9] is followed by a filter pack containing a quartz filter, followed by an XAD-II sorbent bed.[9] The denuder removes gas phase organic compounds. The quartz filter after the denuder collects fine (<2.5μm) particles. The organic compounds collected by the XAD sorbent bed in this sampler are semi-volatile organic compounds lost from the particles during sampling and a small amount of gas phase organic material not collected by the diffusion denuder.

System 2. A quartz filter is followed by a CIF diffusion denuder, and an XAD-II sorbent bed. The quartz filter collects particles and any gas phase organic compounds which can be absorbed by quartz, both those originally in the gas phase and those lost from the particles during sampling. The denuder then removes gas phase compounds passing the quartz filter. Any gas phase compounds not removed by the denuder are then collected by the XAD sorbent bed. This system is used to independently determine the gas phase organic compounds not collected by the denuder.

Sampling Systems 1 and 2 of the BOSS, Figure 5, provide the data needed for the calculation of particulate organic compound concentrations. The concentration of any particulate organic compound or of total particulate carbonaceous material is calculated from Equation (1) (see Figure 5 for sample notation):

$$C_{Total} = Q_1 + (X_1 - X_2)/E_{XAD} \qquad (1)$$

where Q and X refer to the concentrations of the organic compound or organic material collected by the indicated quartz filter and XAD sorbent beds, respectively. E_{XAD} is the efficiency of collection of organic compounds by the XAD sorbent bed (nominally expected to be 80%).[9,11] The first term in Equation (1) gives the material found on the quartz filter after the denuder. The second term in Equation (1) is the semi-volatile organic compounds lost from the particles during sampling. Comparison of the carbonaceous material on the first quartz filters in both Systems 1 and 2 allows estimates to be made of the absorption of gas phase compounds by a quartz filter.[6,8,11] Determination of the gas phase concentration of an organic compound requires a third sampling system, Figure 5:

System 3. A quartz filter, followed by an XAD sorbent bed. The sum of the amounts of organic material found on these two components is the total amount of the organic material. The concentration of the organic material present in the atmosphere in the gas phase is then calculated as the difference between the total found with System 3 and the particulate phase concentration of the compound as determined from Equation (1).

Four 70 hour samples and one 30 hour (sample D) sample were collected using the BOSS as shown in Figure 5 in Provo, UT during March and April, 1996. Replicate data were obtained for each sample. The collected Q_1, Q_2, Q_3, X_1, X_2 and X_3 samples were all analyzed for total N-nitroso and nitrite compounds using the procedure illustrated in Figures 3 and 4. The quartz filters and XAD samples were each three times extracted with a mixed (1/1 volume) dichloromethane/methanol solvent by sonication.[9] Blank quartz filter and XAD samples were analyzed by the same procedure as for the collected samples. Negligible N-nitroso- or nitrite-material was found in the blank samples. The results of these analyses are given in Figures 6 and 7. The precision of the sampling and analysis as determined from the collocated data was ±10% for both N-nitroso and nitrite compounds.

As indicated in Figures 6 and 7, particulate and gas phase N-nitroso and nitrite compounds were present in all collected samples. A significant fraction of the total particulate N-nitroso and nitrite compounds were semi-volatile organic compounds which were lost from the particles during sampling and subsequently captured by the X_1 sorbent bed, Figure 5.

Some N-nitroso and nitrite compounds were present in the X_2 sorbent bed, indicating the presence of gas phase N-nitroso and nitrite material not removed by the denuder. The efficiency of the denuder was calculated by comparing the results for X_1, X_2 and X_3. The efficiency varied from 55-82% (61% average) for the removal of gas phase N-nitroso and nitrite material in the denuder.

As shown in Figures 6 and 7, the concentrations of N-nitroso and nitrite material in the gas phase in equilibrium with the fine particulate N-nitroso material is comparable to that found in the particles. An important point to be emphasized is that the concentrations of particulate semi-volatile N-nitroso and nitrite compounds would have been significantly underestimated based on only the quartz filter data from Systems 2 or 3. The semi-volatile N-nitroso and nitrite compounds which are removed from the particles during the sample collection are lost over a time period much shorter than the sampling time. The concentrations of N-nitroso and nitrite material on the filters before, Q_2 in Figure 5, and after, Q_1 in Figure 5, the denuder were comparable for all samples. The concentrations of gas phase N-nitrosodimethylamine (NDMA) which might be present in the sampled atmosphere based on literature data[49] is of the order of 0.2 to 0.5 nmol m^{-3}. The concentration of particulate phase semi-volatile N-nitroso material is comparable.

Preliminary experiments indicate that use of the BOSS diffusion denuder minimizes the artifact formation of N-nitroso compounds during sampling. In two experiments, quartz filters before (Q_2 and Q_3) and after (Q_1) the diffusion denuder

were spiked with diethylamine prior to sample collection. In both experiments, the concentration of N-nitroso compounds observed on the spiked quartz filters located before the denuder and on subsequent XAD sorbent beds were higher than that found on collocated unspiked samples. In contrast, for the quartz filter and associated XAD sorbent bed after the denuder, N-nitroso compounds found on both spiked and unspiked samples were the same. The denuder is expected to remove $NO_2(g)$, $HNO_2(g)$, $O_3(g)$ and related compounds which could react during sample collection to form N-nitroso compounds not originally present in the atmosphere.[58]

Work is continuing on the SFC/chemiluminescence detection of the collected N-nitroso and nitrite compounds, and on the identification of the amounts of nitro containing compounds which are also present in the collected samples. Work planned for the future includes identification of the major N-nitroso, nitrite and nitro compounds present in collected samples and validation studies of the stability of the compounds during sample collection and extraction. Finally, studies are planned to couple SFC/chemiluminescence analysis with mutagenicity assays[44] to identify the toxic nitro and N-nitroso compounds in atmospheric particulate matter.

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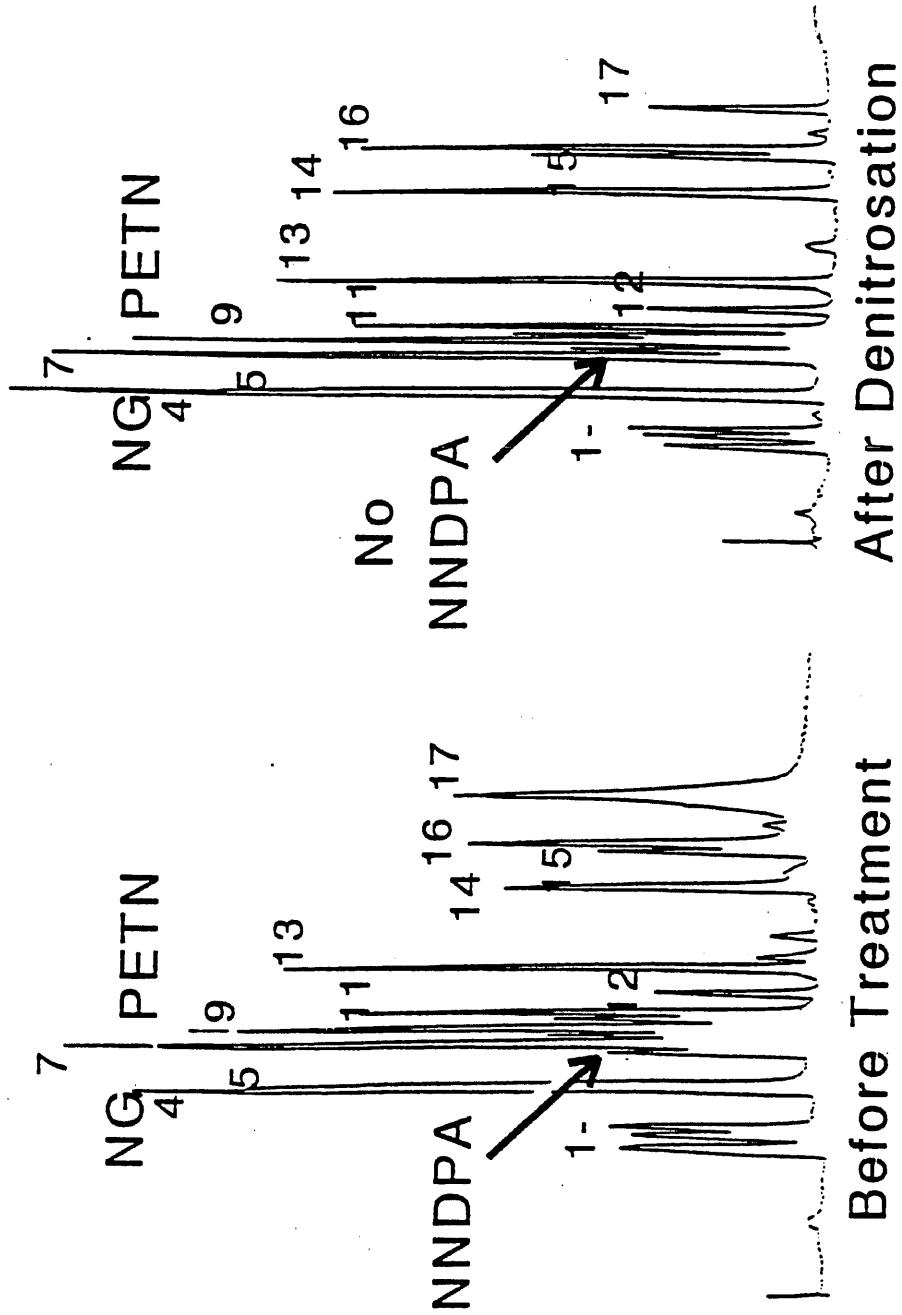

Figure 1. SFC chromatograms of a standard explosive mixture using chemiluminescence detection at a pyrolysis temperature of 800°C without and with pre-treatment of the sample with the acetic acid-HBr denitrosation reagent.

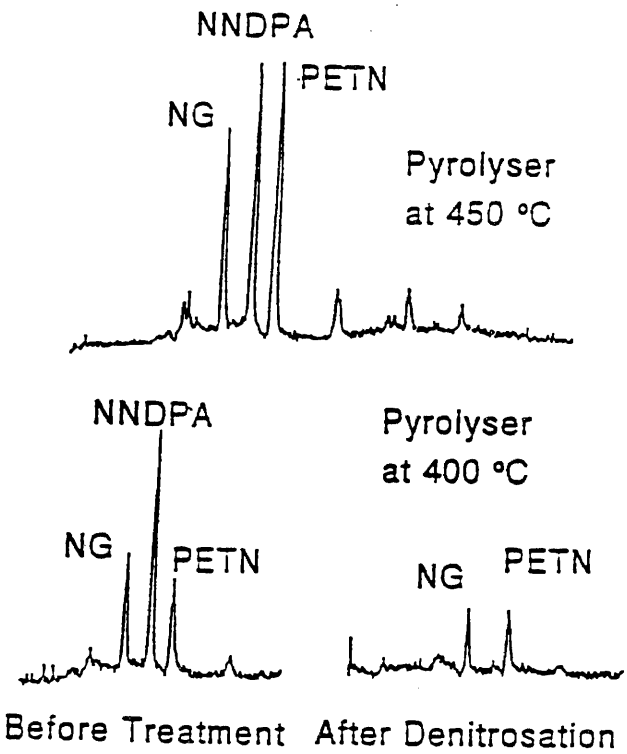

Figure 2. SFC chromatograms of a standard explosive mixture using chemiluminescence detection at a pyrolysis temperature of 450 and 400°C and at a pyrolysis temperature of 400°C after treatment of the sample with the acetic acid-HBr denitrosation reagent.

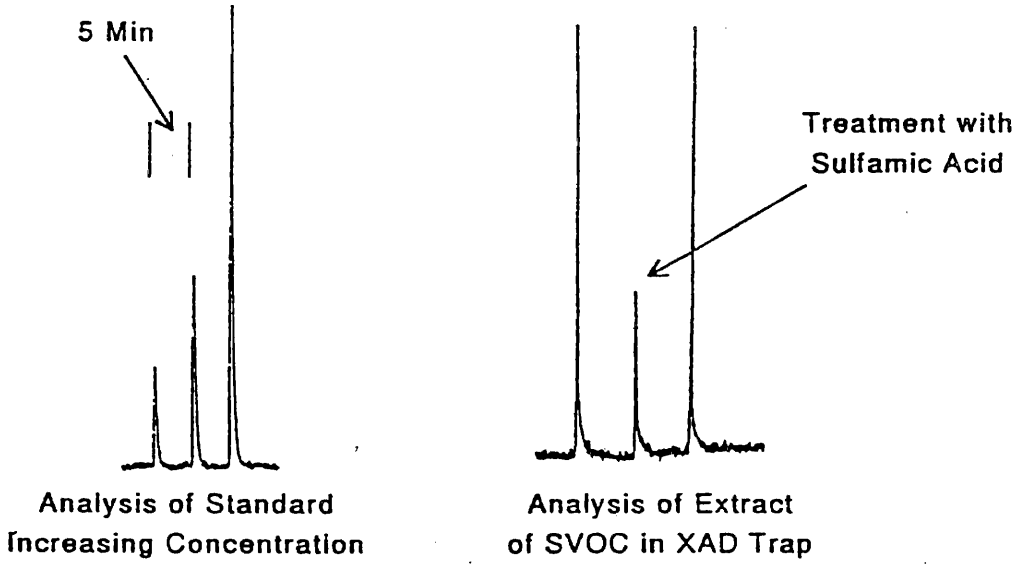

Figure 3. Repeat denitrosation analysis of an XAD extract of collected SVOC without and with treatment of the extract solution with sulfamic acid.

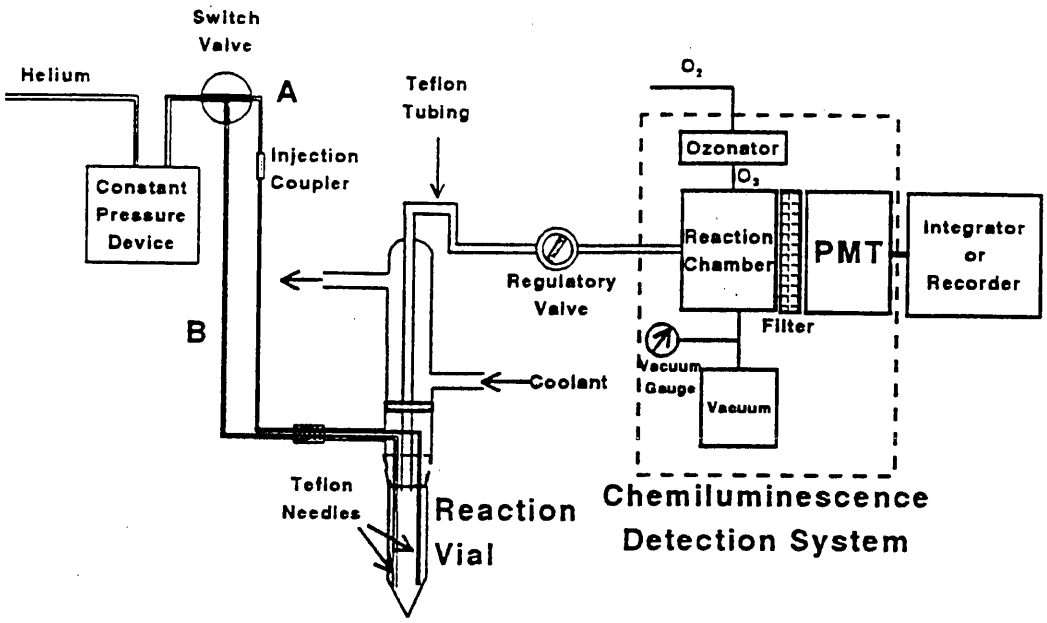

Figure 4. Schematic of the denitrosation analysis system.

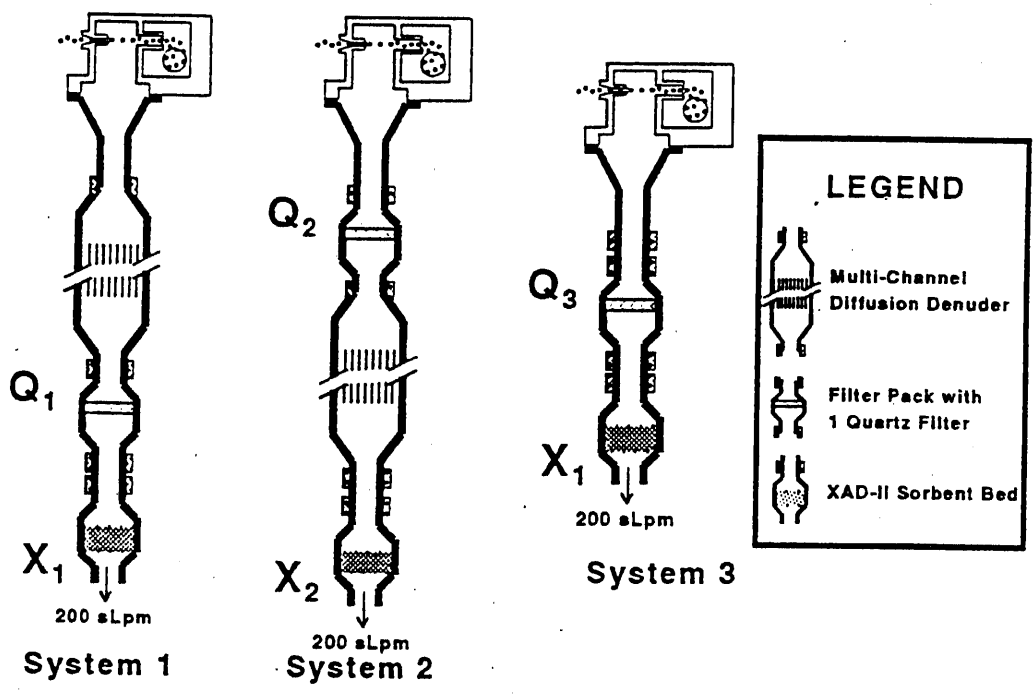

Figure 5. Schematic of the BOSS sampling system for the determination of gas and fine particulate N-nitroso and nitrite compounds.

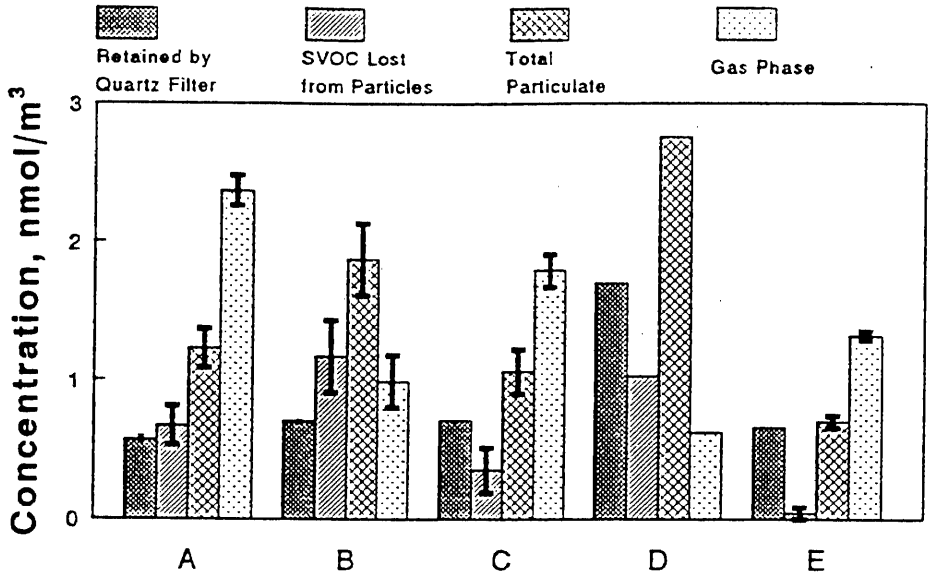

Figure 6. Concentration of N-nitroso material retained by particles on a quartz filter during sampling, lost from particles during sampling (SVOC Lost), and total particulate and gas phase N-nitroso material for each sample.

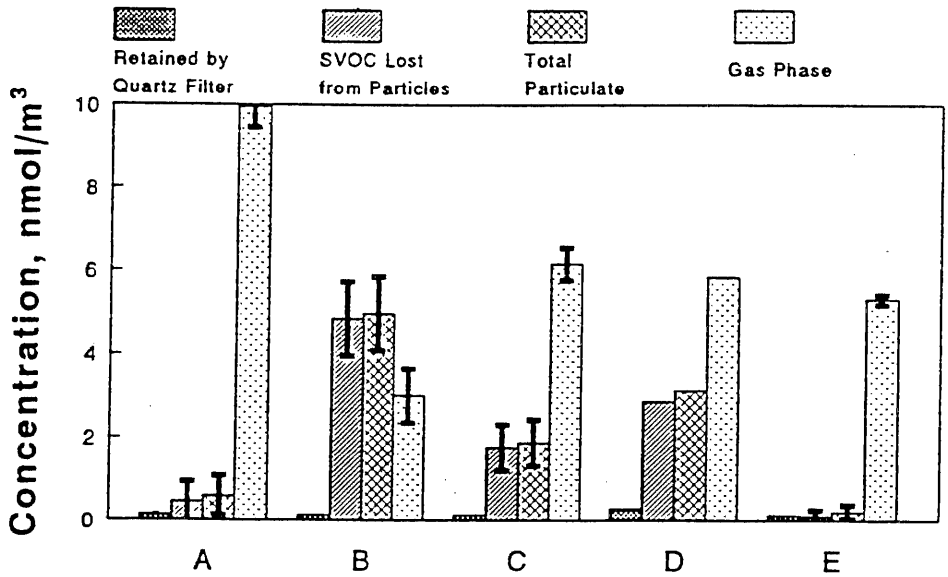

Figure 7. Concentration of nitrite material retained by particles on a quartz filter during sampling, lost from particles during sampling (SVOC Lost), and total particulate and gas phase nitrite material for each sample.

FACTORS INFLUENCING PARTICLE TOXICITY: PARTICLE SIZE AND COMPOSITION[1]

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Introduction. There is increasing interest in factors which contribute to the inherent toxicity of airborne particulate material (PM). This growing concern about particulate toxicity is driven, to a large extent, by epidemiological studies demonstrating an association between low ambient levels (20-40 $\mu g/m^3$) of respirable airborne particulate (PM10) and increased mortality and morbidity in the populations of several cities in the US, Canada, South America and Europe. A key issue arising from these epidemiological findings is the biological plausibility of adverse effects at such low concentrations of particulate. The major components of ambient PM10 mass determined at various urban and rural sites across the US include: sulfate, carbon (organic and elemental) and minerals, with nitrates found also as a significant component of ambient PM in the Western US. When considering the concentrations of ambient PM components it becomes clear that at present there is limited clinical or animal toxicology data to explain the epidemiological findings.

The following presents some of the key factors relating to PM composition and size characteristics which existing data suggest contribute to the PM bioactivity with an emphasis placed on ambient airborne particulates. To the extent studies on other particulate materials, considered primarily as occupational hazards, provide insight into characteristics contributing to ambient PM toxicity, they are also discussed. Specific knowledge gaps and opportunities for future research are identified.

Particle Size. An aerosol's aerodynamic particle size and size distribution are critical factors influencing the extent to which particles penetrate into the respiratory tract and how and where they deposit. Aerodynamic size is influenced by the geometric size as well as properties such as particle density and shape. Particle deposition in the respiratory tract occurs by mechanisms of interception, impaction, sedimentation, diffusion and electrostatic precipitation. The relative contribution of each deposition mechanism to the fraction of inhaled particles deposited varies for each region of the respiratory tract (extrathoracic, tracheobronchial or alveolar). Another physicochemical characteristic that influences particle size and deposition is hygroscopicity. Clearance of deposited particles depends on the initial deposition site, physicochemical properties of the particles such as solubility, translocation mechanisms such as mucociliary transport and endocytosis by macrophages or epithelial cells, and on the time since initial deposition.

Ambient PM can be broadly divided into coarse and fine modes based on size, mechanism of formation and composition. The demarcation between the fine and coarse modes lies in the size range of 1-3 μm. The fine particles can be further classified as nuclei mode and accumulation mode particles. As one would expect, fine particles account for most of the surface area and number of particles in ambient PM. Particle size is a key factor in particle dosimetry in the respiratory tract. Particle composition is also related to particle size. Differences in composition due to differences the in sources of the two particle modes may influence their inherent toxicity. Fine mode particles are typically formed from combustion processes as well as atmospheric reactions involving SO_2, nitrates and organics. In contrast, coarse mode particles are typically formed by mechanical disruption, evaporation and suspension processes and include to varying degrees soil dust, combustion ashes and metal oxides and minerals associated with earth's crust.

Ultrafine particles. There is growing interest in the potential contribution of ultrafine particles to the toxicity of inhaled ambient PM. Studies in laboratory animals have shown that low solubility ultrafine particles (diameter <0.05 μm) are more inflammatory in rodent lungs than larger size particles of the same composition. For example, ultrafine particles of titanium dioxide, carbon black or diesel soot have been associated with acute and chronic inflammatory responses and, upon prolonged exposure, neoplastic effects in the rat lung. When particle dose in these studies is considered as mass/lung, the ultrafine particles can be seen to produce adverse effects at mass doses well below those required for coarse particles (Driscoll, 1996). Interestingly, rat lung inflammation and neoplastic responses after exposure to poorly soluble particles are correlated with lung dose in particle surface area, which is very high for ultrafine particles (Driscoll, 1996). It should be noted that in these studies the ultrafine particles formed agglomerates of 0.1-0.3 μm in size and did not exist as singlet particles. Also, the ultrafine particle exposures eliciting adverse effects, while in the range of those which could occur occupationally (3-10 mg/m^3), were well above those which might occur environmentally. Studies with sulfuric acid have also indicated a particle size dependence for some effects. For example, exposure of animals to acid droplets 0.3 μm in size increased macrophage phagocytosis while exposure to ultrafine acid droplets produced a decrease in phagocytic activity (Chen, 1992a). In these studies the ultrafine particles also cause a more persistent decrease in macrophage intracellular pH than the larger acid particles.

The mechanism underlying the greater toxicity of poorly soluble ultrafine aggregate particles is unclear at present. Ultrafine particles are more rapidly transferred to the interstitium than fine (0.3 μm) size particles of the same composition and ultrafines exhibit a greater accumulation in the regional lymph nodes and a greater retention in the lung (Driscoll and Maurer, 1991; Oberdorster et al., 1992). Further, studies on cell activation and inflammatory mediator release indicate that ultrafine aggregate particles are remarkably more effective in vivo than their larger size counterparts in activating macrophages to release potent proinflammatory cytokines (e.g., TNFα, macrophage inflammatory protein 2) (Driscoll and Maurer, 1991; Driscoll et al., 1994).

Ultrafine particles as singlets appear to show a markedly greater toxicity than the ultrafine aggregate particles. Studies have demonstrated Teflon^R fume to be acutely toxic in laboratory animals and in some situations to produce a "fume fever" like response in humans. The adverse effects of these Teflon^R fumes was associated with the ultrafine particles and aging of the aerosol which likely allowed for greater aggregation decreased toxicity. Studies by Johnston et al. (1996) have demonstrated that a 15 minute exposure of rats to ultrafine singlet Teflon^R particles at levels of 4050 μg/m^3 ($\sim$5 x 105 particles/cm^3) produced severe lung hemorrhage, edema and inflammation associated with a high mortality rate. The ultrafine particle were reported to be widely distributed in rat lung tissue; being present in epithelial cells, endothelial cells and the interstitium shortly after exposure. Acute exposure to ultrafine singlet Teflon^R particles rapidly increased expression of several pro-inflammatory cytokines and anti-oxidant molecules (Johnston et al., 1996). The cytokine findings are interesting since several of the cytokines whose expression was increased are upregulated by oxidative stress. Importantly, the studies on ultrafine singlet particles demonstrate significant toxic effects at mass exposure level in the range of ambient PM10.

Particle Size: Data Gaps and Research Opportunities. As discussed above, clear differences exist in the sources and composition of coarse and fine mode ambient PM. While there is increasing attention to fine mode particles, there remains a need to better define the inherent toxicity of particles in both the coarse and fine modes and to determine potential interactions between coarse and fine particle components. Regarding coarse mode PM candidate causal components include airborne allergens which are well established as factors in human morbidity. Additionally, coarse mode particles can contain transition metals such as iron as well as inflammatory mineral particles such as various polymorphs of crystalline silica; the extent to which these and other components of coarse PM particles contribute to adverse response to ambient PM is not well understood.

Regarding ultrafine particles and potential human health risks, a key question relates to the ambient concentration of ultrafine particles and their composition. To date toxicology studies on poorly soluble ultrafine singlet Teflon^R particles in healthy laboratory animals have demonstrated severe lung toxicity at exposure levels of $\sim 40\ \mu g/m3$. One may expect that adverse responses would be observed at even lower concentrations in compromised animals. However, although there are ambient sources of ultrafine particles, there are limited data on the concentrations and composition of ambient ultrafines. This type of information will be important to further establish the environmental relevance of ultrafine particles and to define the conditions of potential human exposure as well as the types of materials which should be studied.

Establish that ultrafine singlet particles other than Teflon^R are highly toxic. To further assess the significance of ultrafine particles to potential ambient PM health effects, there is a need to evaluate the toxicity of ultrafine particles which are environmentally relevant. If other non-Teflon^R particles are similarly active at exposures near those expected for ambient ultrafines, then additional studies to fully characterize the nature of the response (dose and duration, effects on tissues outside the lung) would be clearly warranted.

Mechanisms of ultrafine particle toxicity. Studies on ultrafine aggregate particles indicate they have a marked effect on macrophage release of pro-inflammatory cytokines, that they are rapidly interstitialized and have a prolonged retention. Similar studies on ultrafine singlets indicate that shortly after exposure a wide distribution of the particles, is present in epithelial cells, the interstitium, and endothelial cells, and that ultrafine exposure results in a rapid induction of a number of inflammatory and anti-oxidant genes. At present there is no information on the key cellular targets of ultrafine particle effects, the mechanisms by which they cause cell activation, or if they may act on tissues outside the lung, these areas require further investigation. In addition, while some studies have suggested particle number or particle surface area (discussed below) may be key in ultrafine particle toxicity, potential mechanisms underlying number or surface area dependent effects are not understood.

Dosimetry. In considering exposure-dose-response relationships for fine particles, at present it is not clear which dosimeter (e.g., mass, surface area, particle number) is most appropriate for assessing fine particle exposure as it relates to potential toxicity. Moreover, if the biologically-effective dose resulting from inhaled particles is defined as a time integral of the

total inhaled particle number, surface area or mass, questions remain about how the dose should be normalized (e.g., per ventilatory unit or per critical cell type) and what time interval is appropriate to characterize acute versus chronic effects. For example, a steady-state retained dose may be most appropriate to characterize chronic responses such as the increase in morbidity observed in the epidemiology studies on PM. Choice of the dosimeter would be aided by additional studies that enhance the understanding of particle characteristics related to important target-tissue response mechanisms.

Interactions. The fine particle component of ambient particulate provides a large surface area onto which materials may become absorbed (vapors, gases) and react resulting in the formation of toxic materials such as reactive oxygen species. Additionally, fine particles may act as a vehicle to deliver surface adsorbed materials to sites in the lung they would not otherwise reach in significant concentration. For example the transport of water soluble gases on the surface of hygroscopic particles (Wilson, 1996). The surface of fine particles may also serve as a site for reactions between components of ambient air pollution and endogenous materials once a particle has deposited. The possibility that fine particles act to promote reactions between components of ambient pollution and deliver the products to the deep lung needs to be investigated. This information will provide insights in to potential mechanism of toxicity as well as guide development of better approaches for generating test aerosols which most closely reflect ambient PM.

Particle Composition. Adverse health effects, even for materials considered to be of high inherent toxicity, have typically not been observed in controlled studies at exposure levels in the range of 20-30 $\mu g/m^3$. An exception to this are bacterial endotoxin and ultrafine particles which are discussed below.

The discontinuity between the toxicology data base and the epidemiological findings is likely the result of a number of factors. First, and most obvious, is that the clinical and laboratory animal models which have been used to study PM toxicity to date are not reflective of those sensitive individuals responding adversely to ambient PM. The apparent insensitivity of current models may reflect, at least in part, that we are not characterizing the responses most closely related to processes underlying PM associated morbidity and mortality (e.g., cardiac physiology, oxidative stress). Secondly, differences in dosimetry likely result from the types of particles used in the controlled exposure studies versus those available in the ambient environment. Further, dramatic differences in inhalability and dosimetry exist between laboratory animals and humans. Choice of appropriate dose metrics to link exposure and response should be motivated by insight on mechanism(s) of dose delivery and bioactivity of the particles in question. Only a description of the factors that influence inhaled dose have been accomplished to a substantive degree for PM. An additional reason for the discontinuity between toxicology and epidemiology is that there may be aspects of ambient PM toxicology (e.g., specific components, interactive effects) which are not yet identified or have only recently begun to be studied which play a key role in adverse responses to ambient PM. Considering the role of particle composition in PM toxicity, factors which increasingly appear as important include: transition metals and their catalysis of oxidant generation, particle acidity and specifically, interactions between acid and particles; and

particle associated organics which may act directly as inflammatory agents or promote the activity of other materials (e.g., metals).

Transition Metals. Transition metals, when in the presence of an appropriate reductant, can cycle between valence states and promote the production of oxygen radicals via the Haber-Weiss reaction. The potential importance of transition metals and, specifically, iron in PM-associated oxidative stress and toxicity, has been established in studies on fibrous and nonfibrous mineral particles (reviewed in: Lund and Aust, 1990; Kamp et al, 1992). Many silicate minerals contain iron either as an integral component of their crystal structure, a substitute cation and/or a surface impurity (e.g., iron content of crocidolite and amosite can be up to 36% by weight and chrysotile can contain 2-3% iron by weight). In addition, crystalline silicates and other metal oxides can complex iron and other metals via interactions between the metal ions and functional groups such as silanols on the mineral surface. In this respect, a variety of silicates have been shown to accumulate iron in vitro and in vivo in a form which is redox-active (Ghio et al., 1992; Eborn and Aust, 1995). Studies in cell free systems have demonstrated that many minerals give rise to oxygen radicals when incubated in the presence of hydrogen peroxide through transition metal dependent reactions and, consequently, produce oxidative damage to critical biological molecules (e.g., DNA, lipid) (Lund and Aust, 1990; Kamp et al, 1992; Ghio et al., 1992, 1993; Driscoll, 1995). In agreement with these in vitro findings, in vivo laboratory animal studies have demonstrated that increasing or reducing the level of iron associated with silicate minerals correspondingly increases or decreases their toxicity (Ghio et al., 1992, 1993). Thus, the concept that iron (or other transition metals), present as an integral component of PM, or complexed to the surface of PM can play a key role in PM toxicity is firmly supported by studies on mineral particles.

Transition metals present in ambient PM include iron and, to a lesser extent, vanadium, nickel and copper. Several recent reports support a role for iron and other transition metals in the toxicity of ambient PM. Intratracheal instillation studies using residual oil fly ash and other PM have demonstrated an association between particulate iron content and the ability to elicit pulmonary inflammation and airway hyperreactivity in rats (Costa et al., 1994). Treating fly ash (acid washing) to reduce iron content attenuates its inflammatory activity in vivo (Tepper et al., 1994). In vitro studies have shown that when latex particles (used as a surrogate for a relatively innocuous particle) are complexed with iron, vanadium or nickel there is a significant increase particle-associated oxygen radical production in vitro and the iron complexed particles exhibit greater inflammatory activity in vivo (Meng et al., 1996; Ghio et al., 1996). Recent studies using ambient PM samples (Aust and Smith, 1996) indicate that, like crocidolite asbestos, iron can be mobilized from ambient PM using low molecular weight chelators and ambient PM was found to be more potent than asbestos in causing in vitro oxidant dependent damage to biomolecules. Other preliminary studies have shown residual oil fly ash can activate lung epithelial cells in vitro to release potent pro-inflammatory cytokines; an effect dependent, at least in part, on PM associated iron.

The above described findings demonstrate that metals associated with PM can promote the generation of reactive oxygen species, increase the inflammatory activity of PM, and stimulate

release of inflammatory cytokines, the latter being known to affect processes outside the lung. These effects could contribute to adverse responses in susceptible individuals, thus, the data indicate transition metals are potential factors in PM-induced toxicity. It should be noted, however, that these findings for metal-mediated effects have only been observed at relatively high doses in vivo and in vitro and their relevance to potential effects at ambient PM exposure levels has not been established. In addition, the mechanisms underlying the metal enhanced inflammatory activity of particles remains uncertain, as does the in vivo target(s) for these effects. It is possible that the metal catalyzed oxidants may elicit a cytotoxic action on lung cells. Alternatively, there is increasing evidence that oxidants in the lung and other tissues act as activation signals for production of pro-inflammatory cytokines and other mediators of potential important to adverse PM responses.

PM-Associated Acid. Clinical studies on acid aerosols have demonstrated changes in pulmonary function (e.g., bronchoconstriction, increased airway reactivity) as well as changes in mucociliary clearance (US EPA, 1989). Most studies have used acid sulfate aerosols with the effects being attributable to hydrogen ion concentration. For example, in healthy human subjects changes in lung function after acute sulfuric acid exposure are observed at acid aerosol concentrations $\geq$1-2 mg/m^3. Asthmatic subjects appear to be more sensitive to acid-induced alterations in pulmonary function (Koenig et al., 1989; Utell et al., 1989). Acceleration in mucociliary clearance after acute exposure to 0.1 mg/m^3 sulfuric acid has been reported (Leikauf et al., 1981). Studies involving bronchoalveolar lavage subsequent to acid exposure have revealed no evidence of lung inflammation after acute exposure to 2 mg/m^3 sulfuric acid. Thus, with the exception of the mucociliary clearance studies and some studies of asthmatics, the levels of acid eliciting responses in human clinical studies are markedly greater than those present environmentally.

As with the clinical studies, studies in laboratory animals have typically not demonstrated significant alterations in pulmonary function at near ambient exposure concentrations. However, changes in lung morphology (i.e., mucus cell hyperplasia) and host defense (i.e., altered mucociliary clearance, macrophage function) have been detected after acute or subchronic exposure to sulfuric acid concentrations of 125-300 μg/m^3. Although the mechanisms and, for some endpoints the toxicological significance, of these changes are presently not clear, as with the effects on mucociliary clearance in humans, these findings indicate sulfuric acid exposures at near ambient sulfate levels may alter the homeostasis of cells and tissues in the respiratory tracts of healthy animals. To the extent that some individuals may be more susceptible to acid effects or that interactions may exists between acid sulfates and nitrates and other components of ambient PM, these findings suggest a role for acid aerosols in ambient PM toxicity.

Recent studies indicate that acid particle size and acid coating of particles influences the activity of acid associated aerosols. Intracellular pH is known to be an important regulator of cell function and greater and more persistent reductions in the intracellular pH of lung macrophages have been demonstrated in guinea pigs exposed to ultrafine (0.04 μm) versus fine (0.3 μm) particles (Chen et al., 1992a). While the mechanism(s) underlying the greater effect of ultrafine acid particles on macrophage pH remain uncertain, studies suggest that both the total number

of acid (or acid coated) particles/cm^3 interacting with cells as well as the total mass concentration (μg/m^3) to a cell are important factors in acid effects on the lung (Chen et al., 1995). In addition to effects of ultrafine acid particles, an apparent synergistic effect has been demonstrated when acid is delivered to the lung as a coating on particles. Coating of metal oxide particles with sulfuric acid results in decrements in lung function (i.e., total lung volume, vital capacity, carbon monoxide diffusing capacity) and increases in airway hyperreactivity at acid concentrations of 20-30 μ/m^3; similar effects are not detected when the metal oxide or pure acid droplets are given alone (Chen et al., 1992b). In fact, an approximate 10 fold greater concentration of sulfuric acid droplets is required to elicit a similar respiratory tract effects as the acid coated particles. These studies provide evidence for synergistic interactions between acid and other components of ambient PM. It should be noted, however, that the metal oxide (i.e., ZnO) exposure concentrations in these studies were in the mg/m^3 range and greatly exceeded ambient particulate levels. Additionally, the total number of particles delivered to the lung was much greater for the acid coated particles than for the pure acid particles. Regarding the latter, the extent to which the total number of particles versus the total mass of particles is key to the response is unknown. Studies at lower exposure concentrations and with carrier particles more relevant to ambient PM should provide additional insights regarding the importance of acid: particle interactions to environmental health. It is also possible that acid coated particles may interact with other components of ambient air pollution to result in enhanced toxicity. For example, exposure of guinea pigs to ozone plus acid coated ultrafine particles was reported to result in greater impairment of lung function than exposure to uncoated particles and ozone or a similar dose of aqueous acid droplets and ozone.

PM-Associated Organics. Ambient PM contains a variety of organic materials some of which are derived from anthropogenic sources and others from natural sources. It has been clearly demonstrated that many organics extracted from ambient PM are genotoxic (U.S. EPA, 1982). Additionally, organic extracts from combustion source particles have been shown to be both genotoxic in vitro and tumorigenic in laboratory animal models. Compared to organic extracts of ambient or combustion source PM there is less information available on the genotoxicity of intact ambient PM particles. It should also be noted that combustion-derived particles have been shown to cause lung tumors in rats but only at extremely high concentrations relative to ambient PM levels (Driscoll, 1996). At present, no consistent association has been made between air pollution exposure and human lung cancer risk. In addition, it is unlikely that by virtue of their genotoxicity these materials contribute to acute effects of ambient PM.

Studies suggest organic components in ambient PM may contribute to acute toxicity through a direct inflammatory action or the ability to complex or interact with other components of ambient PM. Regarding the latter, organic compounds may influence the activity of PM through complexation of metals. Humic like substances (HLS) are organic acids present in terrestrial and aquatic environments forming from the composition of organic matter and may formed by incomplete combustion of fossil fuels. Because of their acidic functional groups, HLS can complex metal cations. Significant amounts of HLS were recently demonstrated in combustion products of coal, diesel, oil and wood and in PM collected on PM10 filters (Ghio et al., 1996). A significant correlation was observed between the amount of HLS in PM and metal content,

suggesting that the two may be associated. In addition, there was a correlation between HLS content, metal content and the ability of PM to catalyze production of reactive oxygen in vitro and to elicit neutrophilic inflammation in rat lungs. It is possible that by chelating metals in a manner which allows redox cycling HLS may contribute to the oxidant generating activity of ambient PM.

A preliminary report on the ability of PM to activate cytokine production in alveolar macrophage indicates that bacterial endotoxin may be an important inflammatory agent in ambient PM (Luster and Dong, 1996). Endotoxin, or lipopolysaccharide, is a component of the cell wall of gram-negative bacteria. Exposure of rat or human alveolar macrophages in vitro to ambient PM activated the production of tumor necrosis factor α (TNFα) and interleukin-6 (IL-6), key inflammatory and immunoregulatory cytokines. Treating the ambient PM to inactivate endotoxin blocked its in vitro macrophage activating effects. Subsequent tests of the PM demonstrated the presence of endotoxin. In vitro exposure of alveolar macrophages to diesel soot which did not contain endotoxin did not activate cytokine production. These findings suggest a significant inflammatory factor in ambient PM may be of microbial origin. In this respect, endotoxin is known to be a potent inflammatory agent in the respiratory tract of humans. Airborne levels of endotoxin of 10-50 ng/m^3 have been implicated in adverse pulmonary responses to a variety of organic dusts in an occupational setting (Jacobs, 1989). While environmental levels of endotoxin are well below the ng/m^3 range, it is possible sensitive individuals may respond to lower concentrations. Additionally, preliminary findings indicate that extremely low levels of endotoxin can potentiate chemically-induced liver injury. It is possible that endotoxin may be a factor in adverse responses to ambient PM among sensitive individuals.

Particle Composition: Data Gaps and Research Opportunities. There is a critical need to identify the components of ambient PM contributing to the adverse responses demonstrated in epidemiology studies. Understanding which components or interactions between components are key in the toxicity of PM will be useful in designing appropriate monitoring and/or potential control strategies. Some urgent research areas include the following:

Development and use of appropriate laboratory animal models. Several components of ambient PM have been implicated in contributing to its toxicity (e.g., transition metals, inorganic acid coated particles and, organic acids and biogenic organics). A persisting issue, however, is the disparity between exposure levels eliciting PM effects in controlled toxicology studies and the remarkably lower ambient exposure levels purportedly producing adverse effects. One reason for the disparity between the toxicology and epidemiology findings is likely the use of healthy animals in the former which are not reflective of susceptible individuals. To address this issue, there is a need to exploit existing models and develop/validate new models which better reflect those segments of the population responding adversely to ambient PM. In this respect, there is a need to better understand the characteristics/conditions of those responding. Using these "susceptibility" models studies can be conducted to assess whether components of ambient PM identified in healthy laboratory animals/humans as mediators of PM toxicity elicit responses at levels relevant to environmental exposure. Additionally, these models can be used to determine whether other components of ambient PM which are only weakly active or inactive in healthy

laboratory animals elicit significant effects in compromised models. Studies on concentrated ambient PM in healthy and diseased laboratory animals should also be useful in evaluating the responses to the composite of ambient PM and potentially to identification of the active components.

Determining dosimetry: critical toxicant-targets interactions. Cytotoxicity, inflammation, oxidant stress and altered lung cell function have all been reported as aspects of the respiratory tract response to relatively high concentrations ambient PM components. Defining the critical in vivo targets of effect (e.g., epithelial cells, macrophages, upper or lower airways etc.) and the nature of effects (e.g., oxidant production, cytokine release) will be key to developing more sensitive approaches And biomarkers to assess the adverse effects of PM in toxicology and clinical studies.

In order to understand important relationships between retained particle burdens and critical target tissue responses, characterization of both nonabsorptive (e.g. mucociliary transport of intact particles) and absorptive (e.g. dissolution) clearance processes and rates is required. Solubility is a key parameter to characterize as well as any composition factor that may relate to mechanisms of toxicity.

Up to this point most of the studies on ambient PM toxicity have focused on respiratory tract responses. However, it is becoming clear that alterations in the function of tissues outside the respiratory tract can occur secondary to the response to an inhaled material at the portal of entry. Increased attention should be given to characterizing responses outside the respiratory tract (e.g., heart, liver, spleen) since these may represent important secondary targets of PM exposure, particularly in sensitive individuals.

Definition of interactions between PM components. Studies by Chen and co-workers (1992 a,b) identified an apparent synergism between sulfuric acid and metal oxide particles and point to the potential importance of interactive effects. While the findings of Chen and co-workers are intriguing their relevance to ambient exposure conditions remains uncertain because of the concentration and the types of "carrier" particles used. Additional work is needed to determine if similar interactions occur when acid is associated with particles more relevant to ambient PM and when particulate exposures are near ambient levels. To the extent possible, the effects of acid coated aerosols should be examined in clinical studies to demonstrate human relevance. Here, as with the laboratory animal studies, it is important to generate the test atmospheres in a manner producing aerosols which as closely as possible exhibit characteristics of ambient PM. If the nature of the synergism between acid and particles are determined, this may provide insights into other important interactive effects.

The findings for acid coated particles suggest other interactions may exist within ambient PM that have been missed by studying single components or responses in healthy subjects/animals. Studies investigating other potential interactions within ambient PM should be pursued. For example, interactions between acids and metals associated with PM surfaces may be important. Previous studies have demonstrated that acid pH can promote mobilization of iron from particles

and increase irondependent oxidant generation. The demonstrated ability of acid and of acid coated particles to decrease the internal pH of lung cells suggests a testable hypothesis of how acid exposure could increase the toxicity of particle associated metals. Since chemical reactions between materials on the surface of particles may be key to synergistic effects of PM components, a better understanding of this chemistry as it relates to the formation of more toxic materials as well as the interactions between particle surfaces and host factors (cells, proteins, lipids) within the lung is needed.

Defining the contribution of microbial products to activity of ambient PM. In vitro studies have demonstrated that endotoxin in PM10 filter samples activates human and laboratory animal cells to produce potent proinflammatory cytokines. Studies have demonstrated endotoxin can cause adverse respiratory responses in humans at exposure of 10-50 ng/m3 and also may potentiate the toxicity of other chemicals. The degree to which endotoxin in ambient PM10 may contribute to pulmonary inflammation and other adverse responses to ambient PM needs further evaluation.

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THE SUBMICRON ATMOSPHERIC AEROSOL AS A CARRIER OF REACTIVE CHEMICAL SPECIES: CASE OF PEROXIDES

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Abstract

The submicron atmospheric aerosol carries short-lived, reactive chemical species, including hydrogen peroxide and organic peroxides, at concentrations as high as one millimolar in the associated water. This conclusion is based on equilibrium calculations and (limited) experimental data. Hydrogen peroxide and hydroxymethylhydroperoxide ($HOCH_2OOH$) are especially likely to be found in the aerosol (aqueous) phase because of their very high Henry's law constants. Aerosol phase concentrations of H_2O_2 fall within a range in which significant biochemical effects have been observed when cells are bathed with H_2O_2 solutions. This may help to explain the results of epidemiological studies that have shown that adverse health effects are associated with fine aerosols and/or sulfates. The submicron sulfate containing aerosol is itself frequently a product of chemical reactions involving H_2O_2, hence a surrogate for the peroxide and associated reactive species. That is, the epidemiological results may signal a response to atmospheric peroxides rather than to sulfates. This hypothesis supports reduction of the total submicron aerosol mass as a way to reduce adverse health effects because the total submicron mass is closely linked to the aqueous component that carries the reactive species. To test this hypothesis, studies are needed of the effects of exposures of cellular layers and/or animals to submicron H_2O_2 containing aerosols that also contain salts such as ammonium sulfate in the pH range 2 to 6.

Introduction

Recent epidemiological studies[1] indicate that increases in human mortality are associated with concentrations of sulfates and fine particles ($PM_{2.5}$) significantly lower than those previously thought to affect human health. If this evidence stands critical evaluation, the National Ambient Air Quality Standard (NAAQS) for particulate matter may need to be revised downward.

Although there have been significant advances in our understanding of the dynamics of the atmospheric aerosol, the effects of the aerosol on human health are poorly understood. There have been continuing efforts, so far without success, to identify biologically active chemical species (such as sulfuric acid) which may cause a disproportionate fraction of the health effects. This paper calls attention to certain rarely identified components of the submicron aerosol and provides supporting evidence for their involvement in the unexplained health effects.

In evaluating the health effects of the atmospheric aerosol, we should be careful not to rely too heavily on the chemical analysis of particulate matter collected routinely from the atmosphere. The chemical analysis is usually conducted long after the aerosol is sampled on filters or other sampling devices. Short-lived chemical species associated with an aerosol, including hydrogen peroxide, organic peroxides and free radicals, may be much more biochemically active than the components measured days or weeks later.[2] These components are generated in the atmosphere by chemical and physical processes that also drive the formation of the aerosol. Biochemically active components may be dissolved in water associated with the particles. In the next section we review the chemical properties of the submicron aerosol.

Chemical Properties of the Submicron Aerosol

The components of the submicron aerosol can be divided into the following categories:

(1) The primary component includes elemental (black) carbon and high molecular weight organic compounds directly emitted in aerosol form into the atmosphere. Also present may be metallic compounds from high temperature processes (smelting, welding, etc.). There may also be contributions from the smaller particles in soil dust and the marine aerosol near coastal sites.

(2) The secondary component results from atmospheric chemical reactions that produce inorganic ionic species of which the most important on a mass basis are NH_4^+, SO_4^{2-} and NO_3^-. Organic vapors also react in the atmosphere to form polar condensable products which appear in the aerosol. For example, cyclic olefins react with ozone to form condensable dicarboxylic acids. The secondary components normally reported in studies of atmospheric aerosol composition are relatively stable reaction products; they

have usually survived in the atmosphere and on filter or impactor substrates for many hours or days before chemical analysis.

(3) Water is a major component of the submicron aerosol in amounts that depend strongly on the relative humidity. Estimates are that the mass of water is about equal to that of the dry portion of the submicron aerosol at a relative humidity of 80%.[3] The water content also depends in a complex way on both the inorganic and organic constituents.[4] Atmospheric water concentrations are likely to range from 10 to 50 $\mu g/m^3$ for urban aerosols.

(4) The components mentioned above are the ones usually identified in discussions of the submicron aerosol. Each is present in the atmosphere at concentrations of the order of 10 $\mu g/m^3$. A fourth rarely mentioned constituent is a group of of short-lived reaction intermediates including peroxides and free radicals. Their concentrations have been measured many times in the gas phase in the atmosphere. Measured concentrations of H_2O_2, aldehydes and organic acids in Los Angeles rainwater correspond well to gas phase concentrations based on Henry's law calculations.[5] Some of the peroxides are highly soluble in water. The high solubility of H_2O_2 results from the hydrogen bonds between H_2O_2 and water molecules which are stronger than those between molecules of the separate species. For cloud water concentrations of 0.5 g/m^3, H_2O_2 is about equally distributed between the gas and aqueous phases. However, the total aerosol water concentration per unit volume of gas is many orders of magnitude less than in cloud water, so the fractions of the corresponding chemical species in the aerosol phase are much smaller in a given air mass. In the next section we review the factors which determine aerosol peroxide concentrations.

Aerosol Peroxide Concentrations

Little information is available on peroxide concentrations in the aerosol phase. The only measurements we have been able to find were exploratory results reported by Hewitt and Kok.[6] The sampling site was located in a forested area in Colorado exposed to air with two prevailing wind directions, westerly winds bringing in relatively unpolluted air with NO_x concentrations below 0.5 ppb and easterly air masses from the Denver/Boulder metropolitan area. Aerosol samples were collected on Teflon filters from an air flow rate of about 1 m^3/min and a sampling period of 3 h. Precautions were taken to minimize sampling artifacts. Hydrogen peroxide and methylhydroperoxide were reported to be present in the aerosol at concentrations of a few nanograms per cubic meter of air.

Although data on peroxide concentrations in the aerosol phase are limited, values can be estimated from measured gas phase concentrations and the corresponding Henry's law constants. In addition to H_2O_2, methylhydroperoxide (MHP, CH_3OOH) and hydroxymethylhydroperoxide (HMHP, $HOCH_2OOH$) have also been detected in the atmosphere in the ppb concentration range (Table 1). Values of the Henry's law constant

for these substances are given in Table 2. The solubility of HMHP is even higher than that of H_2O_2. Based on measured atmospheric (gas) concentrations and the corresponding Henry's law constants, millimolar gas phase concentrations were calculated for these species as shown in Table 2. The other organic peroxides do not have gas phase concentrations or Henry's law constants sufficiently large for such high concentration levels. The assumption that H_2O_2 is in equilibrium between the gas and aerosol phases was verified by calculations which took into account gas phase diffusion and chemical reaction with SO_2 in solution. Millimolar peroxide concentrations in the aerosol are consistent with the measurement of nanograms per cubic meter of air reported by Hewitt and Kok.[6]

These estimates of aerosol phase concentrations are based on Henry's law constants for water solutions of the peroxides. Aerosol phase aqueous solutions are likely to be fairly concentrated solutions of ammonium sulfate and nitrate. The measurements of Lind and Kok[7] indicate an increase in the solubility of hydrogen peroxide in ammonium sulfate solutions by a factor somewhat less than two for a saturated solution. These investigators ascribe the increased solubility to an association between H_2O_2 and ammonia or ammonium ion in solution. They also found that the solubility of H_2O_2 decreased as sulfuric acid concentrations were increased to 1 M.

The hydration of ions in aqueous solution is a well-known phenomenon which stabilizes the dissolved ions, both positive and negative. Positive ions show this effect more strongly than anions. Cations attract the negative ends of the water molecules binding several water molecules to form a stable hydrated ion. The dielectric constant of H_2O_2 is about the same as that of water, and H_2O_2 also behaves as an ionizing solvent. As a result, H_2O_2 forms adducts called peroxohydrates with many substances in which it acts like water of crystallization.[8] Two crystalline peroxohydrates of industrial importance are sodium carbonate peroxohydrate (SCP) and urea peroxohydrate (UP). The solubility of SCP ($Na_2CO_3 \cdot 1.5H_2O_2$) (MW157) is 154 grams per liter of water, approximately 1 M, at 20°C. Hence a saturated solution corresponds to a 1.5 M solution of H_2O_2. In the case of UP ($(NH_2)_2CO \cdot H_2O_2$) (MW94.1), the solubility is 800 grams per liter of water, about 8 M in H_2O_2.

If ions in an aqueous solution bind H_2O_2 more tightly than water, they would increase H_2O_2 solubility by serving as additional H_2O_2 carriers. The presence of additional ionic carriers would enhance H_2O_2 transport into the lung by the submicron aerosol. It might also explain the increase in solubility observed by Lind and Kok (1986) for H_2O_2 in ammonium sulfate solutions.

It is likely that the bonding of dissolved sodium carbonate with H_2O_2 is stronger than with water. This can be inferred from the manufacturing process for SCP, in which an aqueous solution or suspension of sodium carbonate and H_2O_2 is evaporated in a spray dryer to produce SCP.[8] The ability to drive off the water in the presence of the competing H_2O_2 indicates that the bond between the carbonate and H_2O_2 is significantly stronger than with water. A similar process used in the manufacture of UP starts with a

35% solution of H_2O_2 and powdered urea. Of course the ratio of H_2O_2 to water in the manufacturing processes is much higher than it is in the atmosphere. The peroxide/ammonium sulfate association is a sufficiently important issue for aerosol transport of reactive chemical species to warrant further experimental and theoretical study.

Peroxides also play a key role in the formation of sulfates through the conversion of atmospheric SO_2 to sulfates which are a major component of the submicron aerosol. This is discussed in the next section.

Aerosol Sulfate as a Surrogate for Hydrogen Peroxide

Hydrogen peroxide is the most important oxidant of SO_2 in the aqueous phase when the pH is less than about 5; this is frequently the case for the atmospheric aerosol as well as for clouds and fogs. The H_2O_2 is chiefly a product of photochemically driven gas phase chemical reactions, for example involving the hydroperoxyl radical:

$$HO_2^{\cdot} + HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \tag{1}$$

There may also be aqueous phase reactions which lead to H_2O_2 formation. A common route for sulfate formation involves the dissolution of SO_2 and H_2O_2 in aerosol particles and fog droplets. Once dissolved, the SO_2 is distributed among three chemical species: hydrated SO_2 ($SO_2 \bullet H_2O$), bisulfite ion (HSO_3^-), and sulfite ion (SO_3^{2-}). These three species containing sulfur in its +4 oxidative state are collectively known as S_{IV}. Their relative concentrations are dependent on the pH of the aqueous medium.

The mechanism of sulfite oxidation by H_2O_2 has been studied by many investigators.[9-13] The reaction rate is given by the expression

$$\frac{d[S_{VI}]}{dt} = k_0[H_2O_2]_{aq}[S_{IV}] \tag{2}$$

where $[S_{VI}]$ is the sulfate concentration and k_0 is an overall reaction rate coefficient defined by:[11,12]

$$k_0 = \frac{k_1 k_2 [H^+]}{(k_{-1} + k_2[H^+])\left(1 + \frac{[H^+]}{K_{A_1}} + \frac{K_{A_2}}{[H^+]}\right)} \tag{2a}$$

Above a pH of 1.5, k_0 decreases with increasing pH throughout the pH range of atmospheric aerosols. The pH dependence of the S_{IV} solubility can be expressed as

$$[S_{IV}] = H_{SO_2} p_{SO_2} \left[1 + \frac{K_{A_1}}{[H^+]} + \frac{K_{A_1} K_{A_2}}{[H^+]^2} \right] \tag{3}$$

where H_{SO_2} is the Henry's law coefficient.

For pH > 1, often the case for atmospheric aerosols, the rate of sulfate formation, Equation (1), becomes approximately independent of pH. Combining Equations (1), (2) and (3) in the pH independent limit

$$\frac{d[S_{VI}]}{dt} \approx \frac{k_1 k_2}{k_{-1}} H_{SO_2} H_{H_2O_2} K_{A_1} p_{SO_2} p_{H_2O_2} \tag{4}$$

where k_1, k_2 and k_{-1} are rate constants for individual reaction steps in the conversion of S_{IV} to S_{VI}, and H_{SO_2} and $H_{H_2O_2}$ are Henry's law constants for hydrated SO_2 and H_2O_2, respectively. The equilibrium constant $K_{A_1} = [H^+][HSO_3^-]/[SO_2 \bullet H_2O]$. The partial pressures p_{SO_2} and $p_{H_2O_2}$ are in atmospheres. The total rate of sulfate formation in the aqueous component is given by the product of Equation (4) and the liquid water content of the aerosol (or fog) assumed constant. According to Equation (4), the rate of sulfate formation is proportional to $p_{H_2O_2}$. The atmospheric sulfate concentration is proportional to the concentration of H_2O_2 integrated over the lifetime of the air parcel. The presence of ammonium sulfate in the aerosol aqueous phase increases $H_{H_2O_2}$ according to measurements cited above, and this is likely to increase the rate of sulfate formation. Sulfates and H_2O_2 are thus tightly coupled. Reactions in the aerosol phase that lead to sulfate production also result in the uptake of water, because of the hygroscopicity of the salts. In turn, this enhances the rate of aerosol sulfate formation.

Biochemical Effects of H_2O_2

Epidemiological studies have shown a link between fine particle aerosols, including sulfates, and enhanced mortality. In the last section, a close relationship between atmospheric sulfates and H_2O_2 was established based on well known chemical reactions. In this section, we review the biochemical effects of H_2O_2 in aqueous solutions.

As a defense mechanism against foreign organisms and other environmental challenges, hydroperoxides and radicals are introduced naturally into the alveolar region of the lung by phagocytic cells. Hydrogen peroxide and other species produced by phagocytes act to destroy the foreign matter, but may inadvertently injure normal tissue.

Aerosol particles, containing these peroxides are likely to induce a similar response, thus subjecting alveolar cells to releases of hydrogen peroxide.

The effects of hydrogen peroxide on biological systems have been studied by bathing cells with aqueous solutions with concentrations ranging from 20 μM to 1 mM corresponding to 0.2 to 10 ppb in the equilibrium gas phase, respectively. These gas phase concentrations fall within the range observed in atmospheric measurements (Table 1). The most notable effect of H_2O_2 is a concentration dependent inhibition of the adenosinetriphosphatase-synthase enzyme complex that phosphorylates ADP to ATP.[14,15] For alveolar type II cells, investigators have noted that at higher concentrations there was also relatively little recovery of cellular ATP levels over their incubation period and that this loss is likely to be only one of many oxidation-induced biochemical alterations that lead to cell death. The destruction of the alveolar epithelium by H_2O_2 exposure impairs lung function by allowing liquid into space allotted to gas exchange[16] and is considered an early symptom of adult respiratory distress syndrome.[17] Hyslop et al.[15] observed that increasing H_2O_2 exposure decreases cell survivability even after removal from exposure to a full growth medium. Table 3 summarizes the effects of hydrogen peroxide exposure.

The transport of H_2O_2 into the alveolar portion of the lung from the atmosphere can take place either by the gas or the particles. As a result of its high solubility and molecular diffusivity, most of the gas phase peroxide tends to be absorbed in the upper regions of the lung. The fraction which penetrates into the lower lung will deposit uniformly by molecular diffusion in the alveoli. The submicron aerosol particles on the other hand have a very low diffusion coefficient and can penetrate efficiently to the lower lung where about 20 to 30% of the particles deposit. However as the gas phase H_2O_2 is absorbed in the upper airways, aerosol phase H_2O_2 will tend to desorb. The problem is clearly very complex, and the details of the behavior of the peroxide containing aerosol in the lung, including absorption of gaseous peroxides, exchange of peroxides between gas and particles and absorption of water by the particles are beyond the scope of this paper. However, it is possible to estimate the rate of delivery of aerosol particles to the alveolar portion of the lung. For this purpose, we take the atmospheric concentration of submicron particles to be 5×10^4 particles/cc. About 20 m^3 of air are inhaled per day. If 25% of the inhaled particles deposit, the total deposition is about 2×10^{11} particles per day. There are of the order of 300×10^6 alveoli in the lung, corresponding to a deposition of the order of 10^2 to 10^3 particles per alveolus per day. The mass average submicron particle diameter is about 0.1 μm before inhalation, but may grow at the higher relative humidities in the lung.

The exposure of the alveolar membranes to H_2O_2 containing aerosols is therefore quite different from the biochemical studies referenced above in which peroxide solution was placed in contact with cells. Only a small fraction of the total alveolar surface will be covered by deposited particles. Studies are needed of the effects of exposures of cellular layers and/or animals to submicron H_2O_2 containing aerosols, that also contain salts such as ammonium sulfate in the pH range 2 to 6. The aerosol concentrations and

particle sizes should simulate atmospheric systems, that is, concentrations in excess of 10^4 particles/cc and particles smaller than 1 μm. Also needed are more data on peroxide concentrations in the atmospheric aerosol and on peroxide solubility in concentrated ammonium sulfate solutions at low pH.

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Table 1. Concentrations of Hydrogen Peroxide in the Gas Phase

Maximum Gas Phase Concentration (ppb)	Sampling site	Sampling period	Reference
10	-	before 1988	Sakugawa et al.[18]
4.85	Rubidoux, Claremont	1987	Downs et al.[19]
	Los Angeles, Long Beach, CA	1987	Drummond et al.[20]
1.5	Freising/Munich, Germany	May 1988	Hellpointner and Gäb[21]
3.0	Glendora, CA	-	Kok et al.[22]
1.72	Niwot Ridge, CO	Summer 1989	Watkins et al.[23]
5.3	Kinterbish, AL	Summer 1990	Watkins et al.[24]
2.5	George L. Smith III State Park, GA	July 1991	Lee et al.[25]
3.0	Atlanta, GA	August 1992	Lee et al.[26]
5.8	Kinterbish, AL	Summer 1992	Watkins et al.[24]
1.3	Yarmouth, Nova Scotia	August 1993	Lee et al.[26]

Table 2. Calculated Concentrations of Reactive Chemical Species in the Submicron Aerosol

Species	Maximum Gas Phase Concentration		Henry's Law Constant (22°C)		Maximum Aqueous Phase Concentration (mM)
	(ppb)	Reference	($M\ atm^{-1}$)	Reference	
H_2O_2	10	Sakugawa et al.[18]	1.02×10^5 (25°C)	Lind and Kok[7]	1
HMHP†	5	Lee et al.[25]	5×10^5	Zhou and Lee[27]	2.5
			2.38×10^6	Staffelbach and Kok[28]	11.9
MHP‡	0.7	Hewitt and Kok[6]	3.65×10^2	Lind and Kok[7]	0.26 μM
all others§	0.1	Hewitt and Kok[6]			

†hydroxymethylhydroperoxide ($HOCH_2OOH$)
‡methylhydroperoxide (CH_3OOH)
§includes ethylhydroperoxide and hydroxyethylhydroperoxide

Table 3. Effects on Cell Populations of Exposure to Hydrogen Peroxide

Dosage (mM)	Exposure time (min)	Effect	Reference
4.0 (apical)	60	50% loss of net active ion transport	Kim and Suh[16]
0.04 (basolateral)	60		
2.5 (apical)	60	50% loss in integrity of the alveolar cell monolayer	
0.06 (basolateral)	60		
0.5	2	60% loss of intracellular ATP	LaCagnin et al.[14]
0.11	60	50% loss of intracellular ATP	
0.5	10	55% decrease in cellular respiration	
variable	15	Decreased cell survivability after transfer to full growth medium	Hyslop et al.[15]

DEATH FROM INHALATION OF CONCENTRATED AMBIENT AIR PARTICLES IN ANIMAL MODELS OF PULMONARY DISEASE

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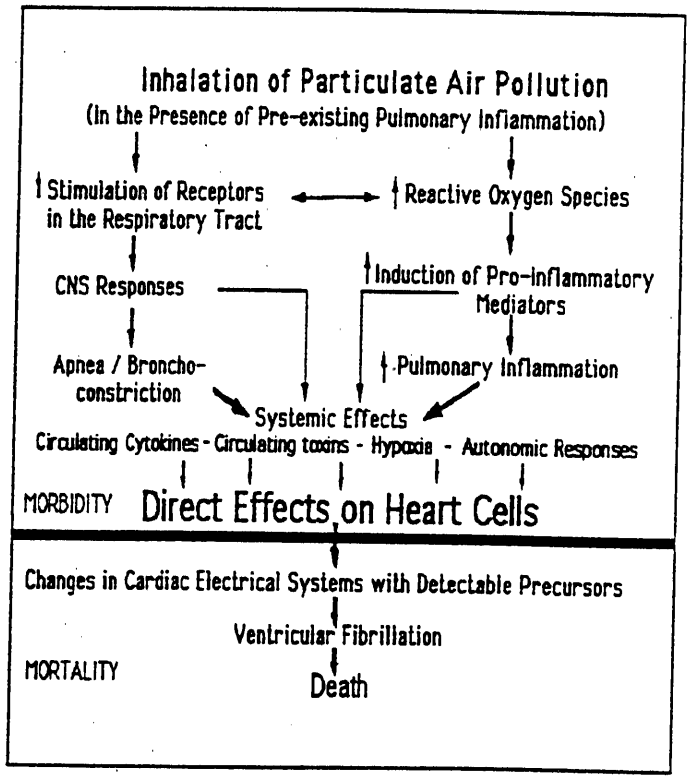
Introduction

Recent epidemiologic studies show that exposures to particulate air pollution far below the current National Ambient Air Quality Standards are associated with increased morbidity and mortality.[1-8] These epidemiologic studies offer important clues to potential mechanisms such as the fact that people with chronic respiratory disease are at greatest risk for increased respiratory symptoms and death associated with particulate air pollution.[9-12] Our objective is to test the biologic plausibility and the importance of several different pathophysiologic mechanisms of death relatable to ambient urban air particles using an animal model of chronic bronchitis. Health effects of ambient particles are best defined using "real world" ambient urban air particles unaltered by collection techniques. The use of concentrated ambient particles (CAPs) in animal exposures allows us to detect the modeled effect and to test mechanistic hypotheses under well-controlled conditions. Our studies utilize: 1) the Harvard Ambient Particulate Concentrator (HAPC), a newly developed device that can increase ambient particle concentrations by a factor of 30 without changing the physical or chemical characteristics of the particles,[13-14] 2) a typical Northeastern United States urban aerosol with a usual fine particle concentration range of 5-15 $\mu g/m^3$,[15] and 3) an animal model of chronic bronchitis used previously in our laboratory.[16-18] This model of chronic bronchitis includes significant inflammation,[16,18] airway hyper-responsiveness,[16] and increased deposition of inhaled particles in the altered airways.[17]

Our hypothesis is that ambient urban air particles are complex mixtures with intrinsic toxicity; in concert with pre-existing inflammation, particulate exposure results in stimulation of lung receptors and induction of pro-inflammatory mediators that lead to local and systemic effects, which ultimately account for the epidemiologic associations between adverse health effects and particulate air pollution. Hypothetical mechanistic pathways by which inhalation of ambient particles in urban air may lead to increased morbidity and mortality are outlined in the adjacent diagram. The study reported here concentrates upon the plausibility of death resulting from exposure of animals with chronic bronchitis to ambient air particles and investigates both the inflammatory and bronchoconstrictive response pathways.



Methods

Harvard/EPA Ambient Fine Particle Concentrator. The Harvard/EPA Ambient Fine Particle Concentrator (HAPC) was used to expose animals to concentrated ambient fine particles (in the size range 0.1-2.5 μm). This system has been described in detail elsewhere.[13-14] Briefly, the HAPC consists of the following components: 1) a high volume conventional impactor with a 2.5 μm cut-off size (separator), 2) a series of three virtual impactors with a 0.1 μm cut-off size (concentrators I, II, and III), and 3) an animal exposure chamber.

The first impactor is a high volume conventional impactor (Fractionating Sampler, Anderson Samplers) and removes particles larger than 2.5 μm operating at 1100 liters/minute, while particles smaller than 2.5 μm escape collection.[19] The deflected flow of the conventional impactor is drawn through a series of three virtual impactors. Each virtual impactor accelerates particles larger than 0.1 μm in a rectangular nozzle. These particles cross the deflected air streamlines, and enter a slit-shaped collection probe, while particles smaller than 0.1 μm follow the deflected streamlines. Particles in the size range 0.1-2.5 μm pass through the collection probe and are referred to as the minor flow (20% of the total flow). The minor flow of the virtual impactor contains the concentrated aerosol.[20] The total flow rate into the third stage is 50 liters/minute and concentrated particles are supplied to the exposure chamber at 10 liters/minute.

Animal Exposure Chambers. Two identical exposure chambers were used for the inhalation studies, one for a filtered air control groups and one for the exposure groups, respectively. The chambers had a rectangular shape, with dimensions 36 x 12 x 135 cm (hence the total chamber volume was 58 liters), and were made of plexiglass. The concentrated aerosol, exiting stage-III of the virtual impactor, was supplied to the animal chamber through a copper tube, 1.9 cm in diameter and 15 cm long. The aerosol flow rate in both chambers was 10 liters/minute. The chamber used for controls was sampling room air passing through a glass fiber filter (Gelman, Type A/E, Ann Arbor, MI) connected to the inlet of the chamber to remove particles. The filter efficiency range is 99.6-99.99%. The use of three virtual impactors results in a total pressure drop of 30 inches of H_2O (i.e., the concentrated aerosol is supplied to the animal exposure chamber at a pressure of 0.93 atmospheres). Thus, in order to account for possible effects of this negative pressure on the animal exposures, a valve was placed at the inlet of the control chamber to create the same pressure drop (i.e., 30 inches H_2O) as in the concentrated air chamber. Since the aerosol is supplied at 10 liters/minute, the mean residence time in the chambers is 5.8 minutes. This residence time results in minimal particle losses due to deposition on the chamber walls[14]. The average residence time of the sampled aerosol in the concentrating system (virtual impactors and transition pieces) is just a few seconds. Therefore, the aerosol residence time in the entire exposure unit is about 6 minutes, which gives an estimate of the time-constant of the system with regard to responsiveness to changes in the ambient concentration.

Particle Characterization During the Exposures. The animal exposures were conducted at the Harvard School of Public Health. Outdoor air was drawn through a manifold into the HAPC. The ambient levels of fine particulate mass ($PM_{2.5}$) were estimated using a collocated Harvard-Marple Impactor (HMI). The Harvard-Marple Impactor (HMI) has been designed and characterized to have a 50% aerodynamic diameter cutpoint of 2.5 μm at a flow rate of 4 liters/minute.[21] Sampling lasted for 24 hours (the first 6 hours of the exposure plus an additional 18 hours) to ensure sufficient particle loading for gravimetric analysis. Measurements of the concentrated aerosol were conducted by placing an isokinetic probe (a copper tube, 0.64 cm in diameter) inside the chamber and at a distance of 30 cm from the aerosol inlet and at about 20 cm upstream of the animal cages. The probe was connected to a 47 mm Teflon filter (Gelman, 0.2 μm pore, Ann Arbor, MI) and a flow-controlled pump sampling at 1 liter/minute. Fine

particulate mass concentrations (d_p<2.5 μm), measured with either the HMI or the isokinetic probe, were determined by weighing the filters on a Cahn 31 Electrobalance in a constant temperature and humidity controlled room.[22]

In addition to the integrated fine mass concentrations, ambient air samples were drawn through the TSI Scanning Mobility Particle Sizer (TSI SMPS, Model 3934, TSI Inc., St. Paul, MN) to determine the fine particle size distribution of the ambient aerosol. At least five samples per day were drawn at random time intervals throughout the three days of the exposures. The SMPS determined particle size distribution in time intervals of 5 minutes. Particle concentration as a function of particle size is first measured based on counts, and subsequently converted to a volume distribution. The rationale for the SMPS samples was to obtain qualitatively rather than quantitatively an estimate of the volume size distribution (in terms of VMD and GSD) of the aerosols during the exposures. The upper particle size measurable with the SMPS is about 1.0 μm. However, since the contribution to the ambient fine mass of particles in the range 1.0-2.5 μm is typically 10% or less,[23] the size distribution obtained with the SMPS was considered sufficiently accurate for the purposes of the experiment.

Chronic Bronchitis in Rats and Exposure Protocols. The animal model of chronic bronchitis is produced by exposing Charles River CD rats to 250 ppm SO_2 for 5 hours/day, 5 days/week for 6 weeks.[16-18] Bronchitic animals were used in these experiments within a week of completing the SO_2 exposure. Exposure groups included bronchitic rats exposed to filtered air or CAPs, and normal control animals exposed to filtered air or CAPs. A minimum of 10 rats were in each group. Concentration of outdoor air particles was approximately 30 fold. Exposures were for 6 hours/day on 3 consecutive days. Animals were observed throughout the exposure and returned to individual cages overnight. Complete autopsies were performed on animals dying spontaneously and on randomly selected survivors. Bronchoalveolar lavage (BAL) and collection of pulmonary tissues for histopathology were done on the remaining survivors.

Histopathology. At autopsy, the lungs were fixed with 2.5% glutaraldehyde via the airways at 20 cm of water constant pressure. Total lung volumes were determined by displacement, and the lungs were cut horizontally into 2 mm sections which were numbered and then 3 slices were randomly selected for processing by paraffin histology.

BAL. On the day following the last CAPs or filtered air exposure, rats were euthanized with an overdose of sodium pentobarbital (65 mg i.p., Anthony Products Company, Arcadia, CA) . Cells were collected by BAL with six 5-ml washes using endotoxin-free Dulbecco's phosphate-buffered saline (PBS). Fluid recovered from BAL was centrifuged (400 X *g*) at 4°C. Viability and total cell counts were determined by hemocytometer counts of small aliquots diluted in trypan blue solution. Cell type was determined from modified Wright-Giemsa-stained cytocentrifuge preparations. A total of 300 cells were counted per sample and the number of neutrophils and alveolar macrophages calculated as the total cell count times the percentage of the respective cell type in the BAL sample.

Statistical Analyses. Significance in mortality analysis was determined with the Log rank test.[24] Chi-squared analysis was used in the morphologic analysis of occurrence or non-occurrence of bronchoconstriction in airways. Unpaired Student's t-test was used to compare BAL cell counts and differentials among animals groups exposed to filtered air or CAPs.[25] Differences were considered significant when $p < 0.05$.

Results

Exposure data. The 24-hour average ambient $PM_{2.5}$ levels ranged from 7.9 to 11.3 μg/m^3

whereas the concentrated fine mass levels in the chamber ranged from 190.5 to 317.1 μg/m^3. At least a total of 60 μg of particulate matter was collected on the filters of the HMI and the isokinetic probe. Since the Cahn 31 Electrobalance is accurate within ±5 μg[22], the uncertainty in both ambient and concentrator levels was ±9%. The average concentration enrichment achieved by the HAPC was 27.7 (ranging from 26.1 to 30.5), a value very similar to that in the original characterization of the HAPC (the average concentration enrichment in the study by Sioutas et al.[14] was 26.3). The overall concentration enrichment is a product of the particle concentration enrichment in each individual virtual impactor stage by a factor of approximately 3.

Data from the SMPS showed that the VMD over the three days of exposure ranged from 0.31 to 0.46 μm, whereas the GSD varied from 1.9 to 2.3. The relatively similar size distributions of the ambient fine aerosols are mainly due to the stable meteorological conditions in Boston during the exposures. All three days were sunny and very clear, with the relative humidity ranging from 20-40% and the temperature ranging from 1 to 5°C.

Animal responses. Deaths occurred during exposure without visible change in behavior and also overnight. Only exposures to CAPs resulted in highly significant mortality in animals with chronic bronchitis. Animals with chronic bronchitis or controls had no deaths with exposure to filtered air. Normal control rats did not die with exposure to CAPs. During exposure either to CAPs or filtered air, the animals did not show signs of irritant inhalation such as rubbing their eyes or nose, coughing, or sneezing. The animals often slept in their cages and thus it required careful observation to determine whether animals were not responding or asleep.

Table 1. Mortality and Pathologic Findings in Rats with or without Chronic Bronchitis Following Exposure to Concentrated Ambient Air Particles for 6 hrs/day for 3 days

Parameter	Control No Lung Disease	Chronic Bronchitis
Concentration of Ambient Air Particles μg/m^3 ± SD	254 ± 45	272 ± 40
% Mortality	0 %	37%
Significance (Log Rank Test)	—	$p < 0.05$
Pathologic Findings on Death or Sacrifice	No inflammation No bronchoconstriction	Airway Inflammation Bronchoconstriction Pulmonary Vascular congestion
BAL Findings	no change compared to filtered air	↑ neutrophils 2X compared to filtered air

Pathologically, inflammation was seen in groups with pre-existing disease, but bronchitic rats exposed to CAPs also exhibited evidence of significant bronchoconstriction. The presence of bronchoconstriction was enumerated in histologic sections comparing the number of constricted airways in animals that died during the exposure with those who survived exposure but were

killed afterwards. Visible buckling of the epithelium was the only criteria used and is illustrated in photomicrographs in figure 1.

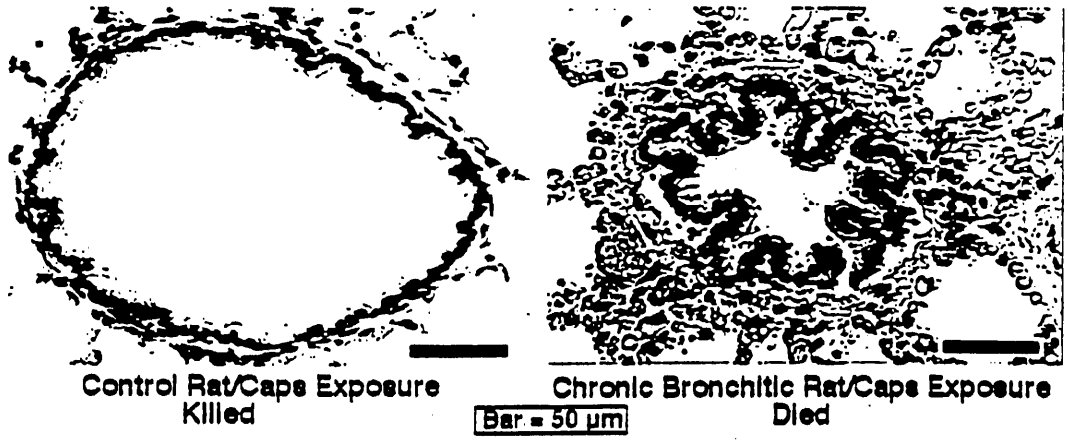


Figure 1. The illustrated airways were selected by matching the diameters of adjacent pulmonary arteries in which the artery wall was one-seventh of the diameter of the vessel. Thus, it was assumed that the arteries were not constricted and if either airway were not constricted, it is likely that these would be a similar diameter. The illustration shows very slight bucking of the epithelium in the CAPs-exposed control (considered positive in the quantification), but severe bronchoconstriction in the CAPs-exposed bronchitic animal that died during the exposure.

Table 2. Quantification of Bronchoconstriction in Animals That Died with CAPs Exposure Compared to Those Euthanized After the Third Day of Exposure.

Parameter	Control (No Lung Disease)		Chronic Bronchitis	
Percentage of airways with Bronchoconstriction**	Died —	Killed 7.1%	Died 80.6%	Killed 55.5%
Chi squared significance+ Died vs Killed	——		p = 0.02	
Chi squared significance++ Killed Cont vs Each	——		p = 0.0001	p = 0.0001

** Percentage of constricted airways enumerated in histologic section of lungs from animals that either died during the 3 day exposure period or were killed afterwards in control or chronnic bronchitis animals exposed to CAPs.
+ Significance of the number of constricted airways within each group of animals that either died during exposure or were killed afterwards.
++ Significance of the number of constricted airways detected in animals with chronic bronchitis who died or were killed after exposure to CAPs compared to control animals without pre-existing disease that were exposed to CAPs and then killed.

In BAL analysis of surviving animals, the rats with chronic bronchitis exposed to filtered air had significant increases compared to control rats. CAPs exposure further increased the absolute

number of BAL neutrophils from 2.26×10^4 (chronic bronchitis + air exposure) to 6.20×10^4 (chronic bronchitis + CAPs exposure).

Discussion

Toxicologic studies of single pollutants at higher than ambient concentrations in human exposures have not shown impressive alterations.[26-28] Other studies report changes in clearance or non-specific airways reactivity following sulfuric acid exposures.[29,30] However, no study has demonstrated pathological changes that might result in death. Animal studies have reported modest changes in clearance of particles from the lungs[31] or in airway responsiveness.[32] Long-term exposure of animals to respirable particles has not produced lethal consequences unless particle concentrations were excessive, resulting in overload conditions.[33-35] Unlike laboratory produced aerosols, ambient aerosols are complex mixtures of particles containing a variety of compounds whose interactions may be a critical feature. Although more complex exposures, such as diesel particles, mixtures of oxidant gases and sulfuric acid particles, or irradiated automobile exhaust have sometimes been used, rarely have inhalation studies attempted to reproduce the complex size distribution and particle composition characteristic of urban ambient particles. The majority of human and animal experiments have been carried out with relatively simple materials, such a sulfuric acid aerosols, silica, aluminum oxide, latex, iron oxide or carbon. Indeed, in our previous studies of particle deposition in rats with chronic bronchitis, the animals were exposed to aerosol particles of ^{99m}Technecium sulfur colloid at a high dose of 50 ± 5 mg/m^3 for 30 minutes (activity median aerodynamic diameter of 0.45 μm), and this exposure did not result in deaths of any animals.[17] To our knowledge, no one has ever exposed adequate populations of animal or human subjects including those with pre-existing disease to unaltered, concentrated ambient air particles as a means to test the hypothesis that air particles are intrinsically toxic.

SO_2 induced chronic bronchitis in rats is a disease model that has increased airway mucus,[36] increased inflammation,[18] and airway hyperresponsiveness to non-specific stimuli.[16] It is, thus, not surprising that we find both increasing inflammation and even morphologic evidence of bronchoconstriction in these animals exposed to CAPs. Early studies with this model have shown reversibility of these changes within a few weeks of cessation of SO_2 exposure.[36] In our experience, this disease model has very low spontaneous mortality, < 1%, (unpublished observations). Therefore, since CAPs have little effect on normal animals, the deaths of these animals with chronic bronchitis suggests that the presence of pre-existing disease may have substantial synergistic impact upon the inhalation of ambient particulate. In the companion paper from our laboratory,[26] similar increases in mortality, pulmonary inflammation, airway response, as well as extension of cytokine responses to the heart are described with a model of pulmonary inflammation and fuel oil fly ash particles.

Thus, we have shown significant mortality resulting from CAPs exposure in animals with chronic bronchitis, increased bronchoconstriction associated with these deaths in this group, and potentiation of acute inflammation in the chronic bronchitis group.

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THE "COHEN HYPOTHESIS" REVISITED: DUST RETENTION, AIRFLOW OBSTRUCTION, AND DISEASE RISK

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ABSTRACT

In 1978 Cohen advanced a mechanistic theory, based on epidemiologic observations, for which evidence gathered since has been supportive. Cohen's theory suggests that a toxic exposure (most commonly but not exclusively cigarette smoking) may in genetically susceptible individuals produce a fundamental lesion expressed as (but not restricted to) airways obstruction; this lesion may then result in accumulation of potential carcinogenic activity in airways and in the circulation as a consequence of impaired host defenses and reduced clearance. Although cancer risk was explained in the hypothesis, other health outcomes are also postulated. Tests of hypotheses based on this theory have confirmed an association between airways obstruction and indices of cancer risk (mortality, incidence, frequency of bronchial epithelial metaplasia in high risk groups). Cohen's theory predicts that individuals with airways obstruction should have higher detectable and biologically significant levels of biomarkers for exposure or effect, controlling for present smoking and intake of agents inhibiting carcinogenesis and restricting age and sex, than nonobstructed individuals. Cigarette smoking is the ideal exposure by which to test the hypothesis because it is more prevalent, readily quantifiable, and accessible in numbers than solely occupational exposures, and because it provides a generalizable model based on an extreme situation of carcinogenic burden. We proposed a test of this hypothesis in 1984, using cigarette smokers with and without airways obstruction. A limited pilot study performed in 1983 confirmed the feasibility of the protocol and provided data on group means and variances.

BACKGROUND

In 1978, Cohen proposed a schema by which the adverse effects of smoking may be mediated by a common pathway she termed "pulmonary dysfunction". (1) Pulmonary dysfunction is considered to be a functional lesion which occurs in the presence of a genetic susceptibility and results in a disturbance in host defense mechanisms that clear active substances that initiate carcinogenesis, fibrosis, and cell injury. These active substances may be other exogenous agents, inflammatory products, or endogenous agents. Their accumulation may have three effects: 1) further injury to pulmonary tissue, accelerating the process of airflow obstruction in a positive-feedback loop, 2) longer intrabronchial residence time of active carcinogens, predisposing to lung cancer, and perhaps 3) release or accumulation of the active substances into the circulation, where they may act on other target tissues (Figure 1). Several mechanisms might be involved (2). Since the Cohen hypothesis was proposed, evidence has appeared to suggest that potential initiating events could be cumulative and might demonstrate an internal dose-response relationship in the presence of organ clearance failures (3). Observed synergism between smoking and various dust exposures might also relate in part to ingestion by macrophages of carcinogen adsorbed onto the particulates and to the accelerated transport of such a carcinogen into a vulnerable phagocytic cell and its metabolism there (4). In some circumstances tumour promotion may be a permissive phenomenon (5,6), which, if prolonged, might enhance transformation and proliferation.

Cohen's theory was developed to explain epidemiologic observations relating cigarette smoking, and by extension other exposures: 1) Cigarette smoking is a risk factor in a variety of nonrespiratory outcomes (7). Throughout this proposed, `smoking' refers to cigarette smoking, and will be assumed to include pipes or cigars. 2) The first-order relatives of patients with either COPD or lung cancer show a common predisposition to airflow obstruction which persists after correction for cigarette smoking habits, age, sex, race, socioeconomic status and alpha-1-antitrypsin status. This suggests a common genetic susceptibility to COPD and lung cancer (8,9), a feature integral to Cohen's hypothesis. 3) After correction for smoking history, men are more susceptible to airflow obstruction than women following a significant period of cigarette smoking - further evidence of a constitutional, possibly genetic contribution to airflow obstruction. Comparable studies on lung cancer have not been reported (10,11). These studies support the Cohen theory as a general mechanism but do not address its implications for carcinogenesis associated with diminished clearance of carcinogens. Indeed, the theory may be generalized, as Cohen herself was inclined, to include other disease states in which an inhaled or endogenous activity may be included.

This theory suggests a plausible mechanism by which inhaled agents, such as cigarette smoke, vinyl chloride, coke oven emissions, and silica, exert numerous secondary effects and may amplify its primary effect by affecting its own clearance from the

pulmonary bed. It suggests a mechanisms for synergism between cigarette smoking and occupational exposures and the contribution of some occupational exposures, such as coke-oven emissions or silica, to risk of both cancer and airflow obstruction developing by different mechanisms (12). We have previously discussed the implications of this theory for silica-induced carcinogenesis (13,14). However, the theory may also be a useful concept in relating airways irritation, airflow obstruction, and mortality, especially for cancer.

If this theory is correct, certain hypotheses could be tested against the evidence:

1) Populations exposed to inhaled agents that produce airflow obstruction are likely to evidence preneoplastic changes in the bronchial epithelium and presumably vice versa if the mechanisms is a general one. Mittman's group reported that in a population of coke-oven workers the frequency of cellular metaplasia correlates with the degree of airflow obstruction measured (12).

2) Inhaled agents that are not intrinsically carcinogenic but that interfere with host defenses against chemical insults may increase the risk of lung cancer and possibly of cancer at distant sites: this suggests one mechanism for synergistic effects between cigarette-smoking and certain occupational exposures, including silica.

3) Individuals who have COPD might have increased evidence of activity in body fluids compared with either age-matched smokers or nonsmokers when current smoking is controlled.

4) Airflow obstruction and cancer risk would be associated after adjusting for smoking in other populations. Van Den Eeden and Friedman have independently confirmed the predicted association between airflow obstruction and lung cancer in other populations without familial relationship (15).

Reduced clearance of carcinogens would result in a level of accumulation of biological activity in bronchial secretions and the circulation sufficient to exert a specific biological effect (genotoxicity) reflecting potential carcinogenic burden. Biomarkers of genotoxicity may be used to indicate systemic burden of genotoxic activity (16).

The logic of Cohen's argument applied to occupational exposure to carcinogens as well, and particularly to synergism between smoking and occupational exposures (Figure 1).

It should be possible to make a direct test of the hypothesis for an association between impaired pulmonary function and carcinogenesis mediated by reduced clearance of exogenous and, perhaps, endogenous chemical activities that may variously initiate

carcinogenesis, induce vascular injury, or stimulate fibrogenesis. One approach is to test this hypothesis by assessing biomarker activity in body fluids of persons with and without impaired pulmonary function, of comparable age, by smoking status.

In 1984 we proposed such a study, using the then-available biomarkers of genotoxicity and mutagenic activity. Unfortunately, we were not able to conduct the study as planned. However, its relevance to the problem of particulate effects seems even greater today than when we first proposed the work over a decade ago.

Cigarette smoking would be the exposure of choice for this initial study because it is more prevalent, more readily quantifiable, and more consistently associated with elevated mutagenic activity than solely occupational exposures. Demonstration of an effect associated with cigarette smoking would support the Cohen hypothesis and provide a model that could later be extended to studies of occupational exposures and of interactive effects. Duration of smoking before the study would not be germane except as a determinant of obstructive airways disease because urine mutagenicity and the epithelial marker of the micronucleus assay do not reflect cumulative injury. Both are short-term reversible effects for which current smoking status only is significant.

A small pilot study was performed in San Diego in 1983 in preparation for a more comprehensive protocol. Data from this were used to refine the protocol and derive estimates of group mean differences and variance used in the sample-size calculations.

The research proposed is the simplest, most direct test of Cohen's hypothesis. The results, positive or negative, would have to be accounted for in any subsequent evaluation of this hypothesis. If positive, this study opens the way to further studies to characterize the possible mechanisms, compounds, and evaluate possible modification of the effect by diet or other interventions. Application to more narrowly occupational exposures would follow and to interactions between smoking and selected occupational exposures. Demonstration of the effect would be powerful evidence of previously undocumented mechanisms associated with cancer risk and synergism.

The principal limitations of the work are: 1) that a positive result cannot identify specific mechanisms nor define the functional lesions; it can only confirm that an effect exists, 2) a negative result cannot rule out local effects within the lung, such as altered bronchial epithelial or macrophage metabolism of carcinogens or prolonged survival of oxidant species.

There are a number of possible variations on the Cohen hypothesis that may apply to special problems. Silica-associated carcinogenesis may be of particular interest because the mechanisms by which silica exposure may impede clearance of other activities in the lung are obvious and visible in scale. (Figure 2) (13, 14, 17) The apparent inverse association between the frequencies of lung cancer and stomach

cancer has also been tentatively explained as a reflection of the disruption of normal pulmonary clearance of carcinogenic activity by cigarette smoking, leading to retention in the lungs and a higher risk of lung cancer rather than normal clearance to the throat, swallowing, and gastric exposure. (18) This speculative hypothesis, put forward in 1980, remains untested. Because the Cohen hypothesis is essentially a mechanism for amplification of a biological effect, it complements rather than competes with other proposed mechanisms in terms of explanatory power.

The Cohen hypothesis represents a possible conceptual approach to sorting out the connection between respiratory exposures and nonspecific health outcomes. It is long overdue for empirical validation and has been neglected for much too long. It is presented here as a postulated mechanism that may have significant explanatory power and that deserves more rigorous scrutiny.

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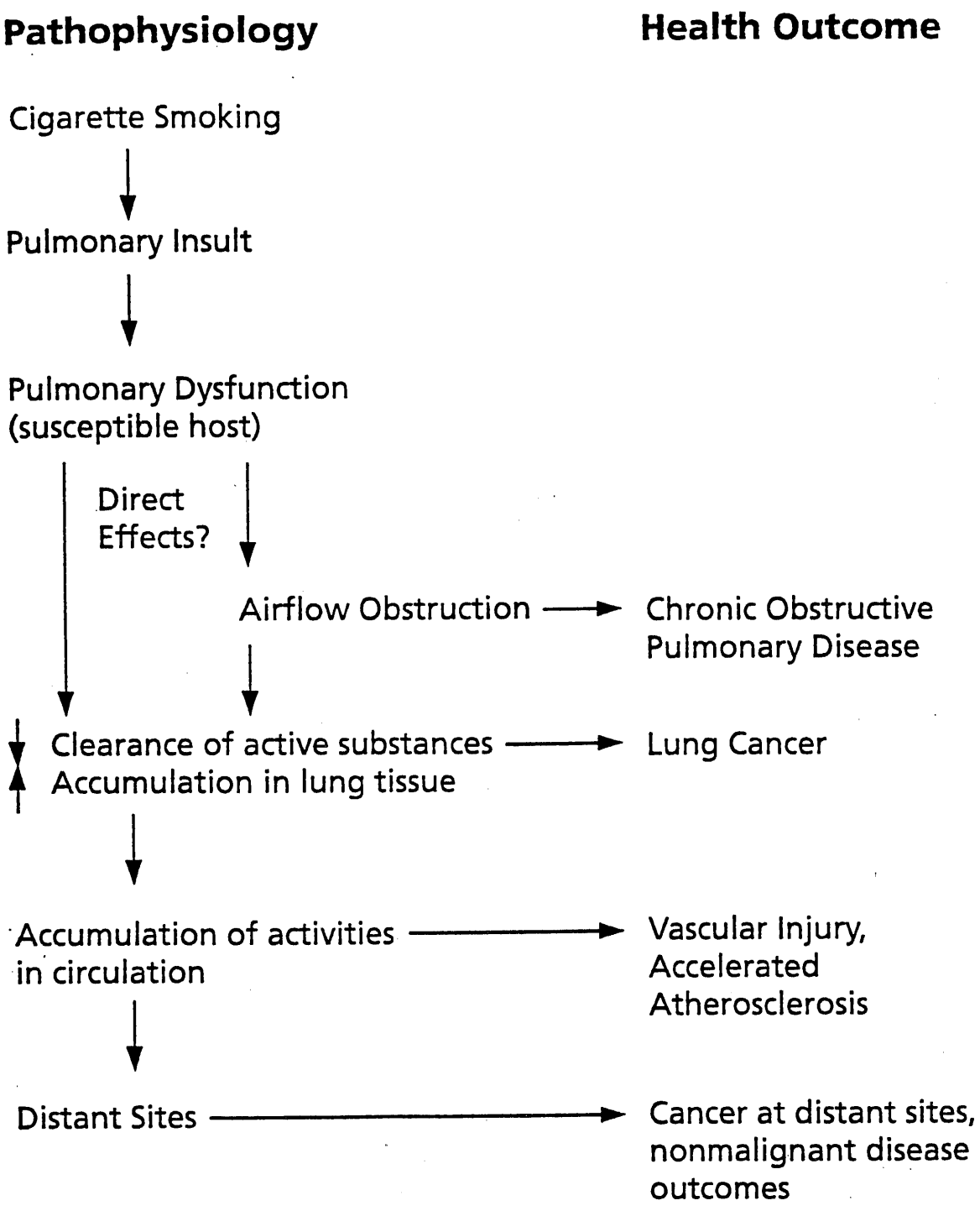
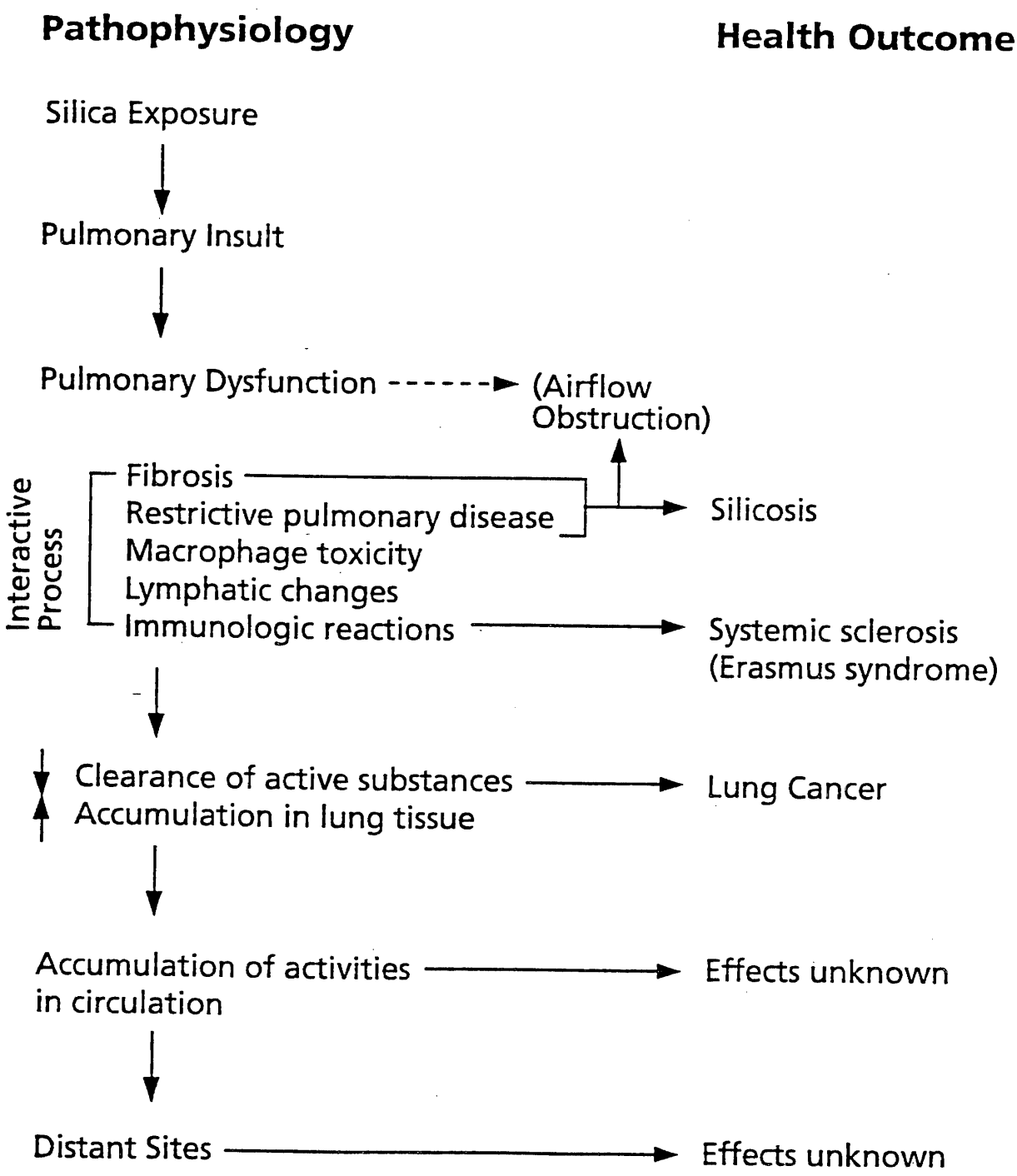
FIGURES

Figure 1. Schematic flow diagram of events in the lung predicted by the Cohen hypothesis, using the example of cigarette smoking.

Figure 2. Application of the Cohen hypothesis to silica exposure and risk of cancer.


Pathophysiology
Health Outcome
Cigarette Smoking
Pulmonary Insult
Pulmonary Dysfunction
(susceptible host)
Direct
Effects?
Airflow Obstruction
Chronic Obstructive
Pulmonary Disease
Clearance of active substances
Accumulation in lung tissue
Lung Cancer
Accumulation of activities
in circulation
Vascular Injury,
Accelerated
Atherosclerosis
Distant Sites
Cancer at distant sites,
nonmalignant disease
outcomes


Pathophysiology
Health Outcome
Silica Exposure
Pulmonary Insult
Pulmonary Dysfunction
(Airflow Obstruction)
Interactive Process
Fibrosis
Restrictive pulmonary disease
Macrophage toxicity
Lymphatic changes
Immunologic reactions
Silicosis
Systemic sclerosis (Erasmus syndrome)
Clearance of active substances
Accumulation in lung tissue
Lung Cancer
Accumulation of activities in circulation
Effects unknown
Distant Sites
Effects unknown

A Time-Series Analysis of Acidic PM and Daily Mortality and Morbidity in the Buffalo, NY Region.

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ABSTRACT

A key question in the particulate matter (PM)/health effect associations reported recently is: What characteristic(s) of PM is (are) responsible? A component which may provide one biologically plausible pathway to support the causality of these associations is aerosol acidity (H^+). An increasing number of observational studies have demonstrated associations between H^+ and increased adverse health effects in the U.S. and abroad. While studies have shown significant H^+ associations with increased morbidity in the United States, similar associations have yet to be shown with mortality.

We considered a two-and-a-half year record of daily H^+ measurements (May 1988-Oct. 1990) collected in the Buffalo, NY region in a time-series analysis of total and respiratory daily mortality and hospital admissions. Other pollutants considered included: PM_{10}, O_3, CO, SO_2, and NO_2. Various modeling techniques were used to control for confounding of effect estimates due to seasonality, weather and day-of-week effects. Of the pollutants considered, H^+ was most consistently associated ($p<0.05$) with adverse health effects. Relative risks (RR's) were estimated for increases equal to the maximum minus the mean concentration (e.g., 345 nmoles/m^3 for H^+, 65 $\mu g/m^3$ for PM_{10}). Respiratory hospital admissions RR estimates associated with H^+ ranged from 1.30-1.50, while those for respiratory mortality ranged from 1.48-1.60. Total mortality RR estimates for H^+ ranged from 1.21-1.25, while those for PM_{10} ranged from 1.11-1.12. Simultaneous regression with gaseous air pollutants had minimal effects on the H^+ RR's. These analyses indicate that H^+ may be a significant contributor to the acute adverse health effects previously associated with PM mass.

INTRODUCTION

In recent years, there have been growing concerns regarding the potential adverse effects of PM air pollution on human health. These concerns have stemmed from historical air pollution episodes that were found to be associated with increases in severe health outcomes, such as mortality and hospital admissions. The London smog episode of 1952, probably the best documented of such incidents, resulted in a peak respiratory hospital admissions rate of 460/day, while just prior to the fog the average

daily admission rate was 175/day (Her Majesty's Public Health Service, 1954). In addition, the 1962 London smog episode was shown to have elevated levels of H^+ (Commins and Walter, 1967). These episodes offer convincing evidence of the adverse impacts of acute environmental exposures on human health. However, the quantification of the air pollution-health effects relationship at more routine low level exposures is not as straightforward.

While various pollution indicators including: PM, sulfur dioxide (SO_2), and ozone (O_3), have been shown to be significantly correlated with increased morbidity and mortality, PM has recently been the focus of great interest (e.g. , Kinney and Ozkaynak, 1991; Schwartz, 1991; Thurston et al., 1992a). The Clean Air Act currently regulates all PM with an aerodynamic diameter under 10 microns (PM_{10}) as a criteria pollutant and has established National Ambient Air Quality Standards (NAAQS) for a 24 hour (150 $\mu g/m^3$) and an annual (50 $\mu g/m^3$) averaging period (Federal Register, 1987). However, there is concern that ambient PM_{10} concentrations below these levels are causing adverse health effects, prompting a review of the present standard (EPA Draft Particulate Matter Criteria Document, 1995).

Some have argued that any further PM controls should focus specifically on those chemical components of PM that are most responsible for the noted effects (e.g.,Thurston, 1995). Furthermore, identification of especially harmful chemical components would help provide the biological plausibility needed to support the causality of the observed PM/health effect associations. Two key PM components which have been suggested are: sulfates ($SO_4^=$) and strong aerosol acidity (H^+). Sulfate concentrations have traditionally been interpreted as an indicator of the acidic content of the PM. However, the $SO_4^=$ ion actually represents the sum of various forms: H_2SO_4, $(NH_4)HSO_4$ and $(NH_4)_2SO_4$, which range, respectively, from strongly to weakly acidic. The distribution of each component within the total sulfate content is highly variable, as is the overall level of acidity. A more direct measurement of acid aerosol exposure is the strong H^+ concentration of a PM sample, but this has not been widely measured spatially or over time to date, severely limiting analyses available to directly assess the potential health effects of acidic aerosols.

The biological plausibility of the acid aerosol component as a causal agent in the PM-mortality association is supported by a growing body of evidence from both animal toxicity and controlled human exposure studies. These studies have indicated that adverse health effects are a result of H^+ delivery to the respiratory tract. Acid aerosol exposures between 0.1-1.0 mg/m^3 have demonstrated lung clearance function and pulmonary mechanical effects in normal and asthmatic subjects (e.g. Leikauf, 1984; Koenig, 1983). Animal studies have also shown adverse effects such as clearance abnormalities (Chen and Schlesinger, 1983), airway hyperresponsiveness (Gearhart and Schlesinger, 1989), and changes in pulmonary function (Amdur and Chen, 1989). While the exact mechanism behind these responses to H^+ is not clear, Seaton et al. (1995) have proposed the hypothesis that ultrafine acid aerosols provoke alveolar inflammation, causing release of mediators that might induce attacks of acute respiratory illness in susceptible individuals.

Although limited, observational epidemiological evidence of increased health effects due to acid aerosol exposures have also begun to accumulate. London studies have indicated stronger associations between aerosol acidity and increases in mortality and morbidity than other PM measures (Thurston et al., 1989; Lippmann and Ito, 1995). In North America, summertime levels of H^+ and $SO_4^=$, in combination with O_3, have been significantly associated with acute increases in respiratory hospital admissions (Bates and Sitzo, 1987; Thurston et al., 1992a; Thurston et al., 1994). Additionally, exposures to a daily average of 0.5 $\mu g/m^3$ H^+ was found to be more significantly associated with asthma symptoms than other environmental factors (Ostro et al., 1991). In contrast, North American observational studies of an acid/mortality relationship have been more limited. As part of a larger air pollution investigation, Dockery et al. (1992) examined the influence of acid aerosol levels on mortality in two communities using a 9 month period of daily H^+ observations and were unable to detect significant associations. The conflicting results of these studies indicate a need to further evaluate the acid aerosol/health effect relationship in the U.S.

While biological plausibility is an important criteria in establishing causality, another equally important criteria is coherence (Hill, 1965). Coherence describes the logical inter-relationship between health indices. For example, episodic increases in hospital admissions would be expected if increases in mortality were observed. Bates discusses the importance of coherence in epidemiological investigations in his evaluation of the different health indices used in the many air pollution investigations (Bates, 1992). Bates notes that few studies discuss the inter-relationship between different health indices, and stresses the importance of internal coherence.

Therefore, the two objectives of this research were to first test the hypothesis that acid aerosols are a harmful component of PM, and second to test that the observed pollutant associations are coherent across both morbidity and mortality health effect outcomes at a single locale. To evaluate these objectives, a unique environmental/health effect outcome database was constructed for the Buffalo, NY region for a two and a half year period during 1988-1990. Pollutant/health effect associations were then estimated using time-series regression techniques.

MATERIALS AND METHODS

Data Collection

Hospital Admissions Data

Hospital admissions were used as an indicator of morbidity within populations in the Buffalo area (Erie, Niagara, Monroe, Orleans, Genesee, and Wyoming counties). This ensures that each patient was deemed ill enough to require admission by a physician. Daily hospital admissions data were obtained for the years 1988, 1989 and 1990 from the Statewide Planning and Research Cooperative System (SPARCS), a division of the New York State Department of Health (NYSDOH). These three years have been selected because this is the time period during which daily H^+ measurement were made as part of a NIEHS study. SPARCS is a comprehensive health care database which compiles hospital admissions statistics for all of NY state. All general hospitals in

the state are required to submit admissions and discharges data to SPARCS. Daily unscheduled hospital admissions for residents of the Buffalo area will be considered according to their primary diagnoses which are classified according to the 9th Revision of the International Classification of Diseases (ICD9). For the purpose of these analyses, the hospital admissions data were grouped into two general classifications: total and respiratory hospital admissions. The total daily hospital admissions category includes all diagnoses, excluding accidents. The respiratory daily admissions category includes diagnoses of acute bronchitis/bronchiolitis, pneumonia, COPD and asthma (i.e., ICD9 codes 466, 480-486, 490-493).

Mortality Data

Daily mortality counts for residents of the Buffalo metropolitan area were obtained from the National Center for Health Statistics (NCHS) mortality tapes for the years 1988 through 1990. Cause-specific categories included total deaths and respiratory related deaths related deaths. These data were classified according to the ICD9 codes listed for the hospital admissions category. Accidental deaths and deaths occurring outside of the designated area were excluded from this dataset.

Environmental Data

Environmental data used in this analysis consists of previously collected daily measurements of air pollution and weather variables. Daily aerosol acidity and sulfate ion measurements were made by the New York University Medical Center (NYUMC) from May 19, 1988 to October 10, 1990 in the Buffalo metropolitan area. These measurements were made using the sequential acid aerosol system developed at the NYUMC (Thurston et al., 1992b). The Buffalo NYUMC monitor was located in the residential neighborhood of Amherst, NY, within the Buffalo metropolitan area. Direct comparisons with daily H^+ measurements made for 2 months (August-September 1990) in Rochester, NY confirmed that the site was regionally representative for this pollutant (R^2=0.91). Other daily environmental measurements were obtained for this period from the EPA's Aerometric Information Retrieval System (AIRS), these include SO_2, NO_2, O_3, CO, PM_{10} and coefficient of haze (CoH). Due to the fact that PM_{10} was monitored only every 6th day by the NYDEC, missing days were estimated using a regression model which predicted daily PM_{10} concentrations from available daily measurements of $SO_4^=$ and CoH, as well as a winter/summer indicator variable. These two measures provide excellent indices of each of the two major components of fine particulate matter: CoH is an indicator of primary particulate matter emissions; and, $SO_4^=$ is an indicator of secondary particulate matter formed in the atmosphere. Buffalo PM_{10} values were well predicted by this model, as shown in Figure 1. Sites selected from the AIRS network were located throughout the Buffalo-Rochester metropolitan area. Correlations between sites were calculated to assess inter-site variability and mean daily values were obtained by averaging individual site values. Daily weather data were obtained from the Buffalo International Airport.

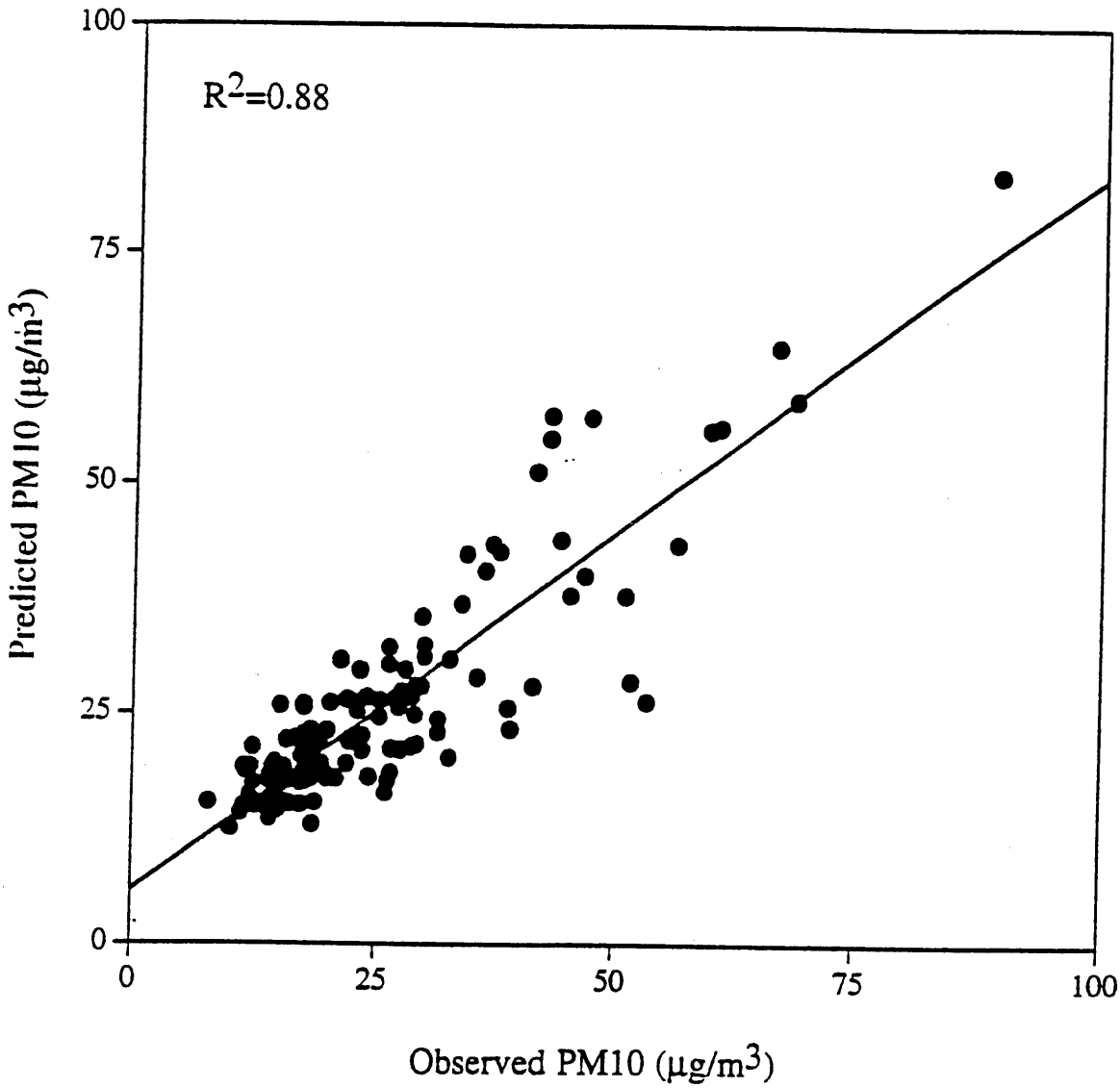


Figure 1. Predicted versus Observed PM10 Concentations for the Buffalo & Rochester, NY Area 1988-1990.

Statistical Methods

Health Effect Analysis

Due to the count nature of the mortality and hospital admissions data (in which the distribution of the observations is skewed and observations are non-negative whole numbers), Poisson regression was used to model the effects of the individual air pollutants on the different human health endpoints. The basic model, which controlled for major potentially confounding variations in the data, was:

$$\begin{aligned} log(health\ index) &= \beta_0 + \beta_1(seasonal\ adjustment) + \beta_{2\text{-}7}(day-of-week) \\ &+ \beta_8(linear\ time\ trend) + \beta_{9,10}(quadratic\ hot\ \&\ cold\ terms) \\ &+ \beta_{11}(heat/humidity\ interaction) + \beta_{12}(individual\ pollutant) \end{aligned}$$

Time series plots of the data (see Figure 2), show that different seasonal cycles exist in the various environmental variables considered. Pollutants such as PM_{10}, H^+ and O_3 tended to be higher in the summer and lower in the winter, while primary pollutants like SO_2 were higher in the winter and lower in the summer. Additionally, the health outcome variables were also dominated by seasonal influences, and are generally higher in winter and lower in summer. To avoid confounding of the acute health effect associations by these long-wave cycles, a seasonal adjustment variable was included in the model. In addition to the seasonal adjustment, six indicator variables were included to adjust for day-of-week variations in hospital admissions and mortality. This type of variation is most apparent in the hospital admissions data, which are generally lower during the weekend and higher during the week, especially on Monday. A linear time trend was also included to control for general increases or decreases in the data over time. Dual quadratic hot and cold terms were included in the model to account for the separate effects of hot and cold temperatures on human health, as demonstrated in Figure 3. In addition to the hot/cold quadratic terms, a heat/humidity interaction term was included to control for increases in hospital admissions or mortality due to the potentially additive effects of high temperatures and high relative humidity. Lastly, linear pollutant terms were added individually to the model to estimate the relationship between each pollutant and human health. Figure 4 demonstrates this approximate linear association between H^+ and the various health endpoints.

Relative Risks (RR's) were estimated from the Poisson regression results by applying the model's coefficients to increments equivalent to the difference between the maximum and mean concentrations for each pollutant. The significance of each pollutant's RR was evaluated using the t-statistic of that coefficient. One-way 95 % confidence intervals were also computed for each pollutant, based on the standard error of its coefficient.

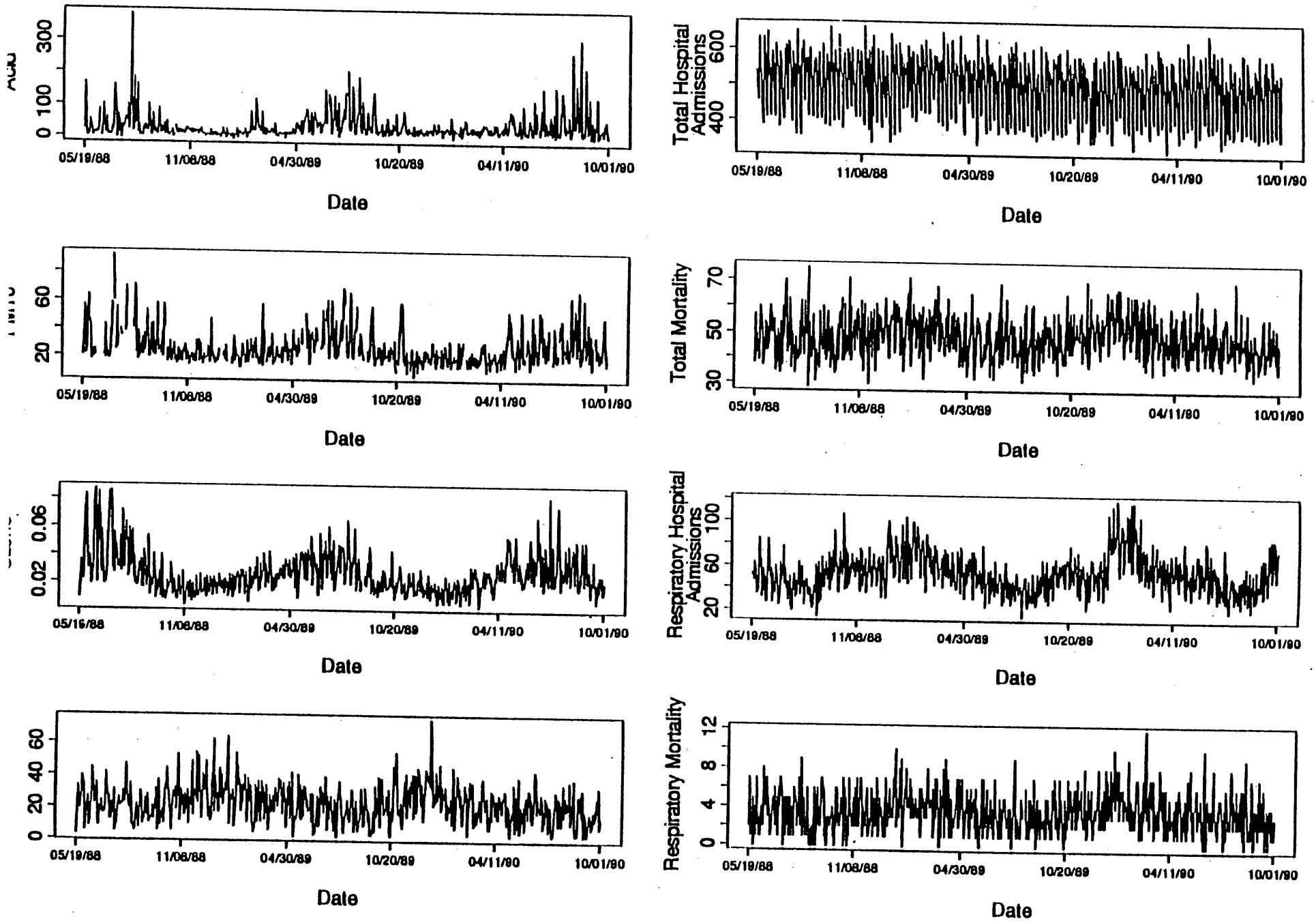


Figure 2. Time-Series Plots of Environmental and Health Outcome Variables for Buffalo, NY.

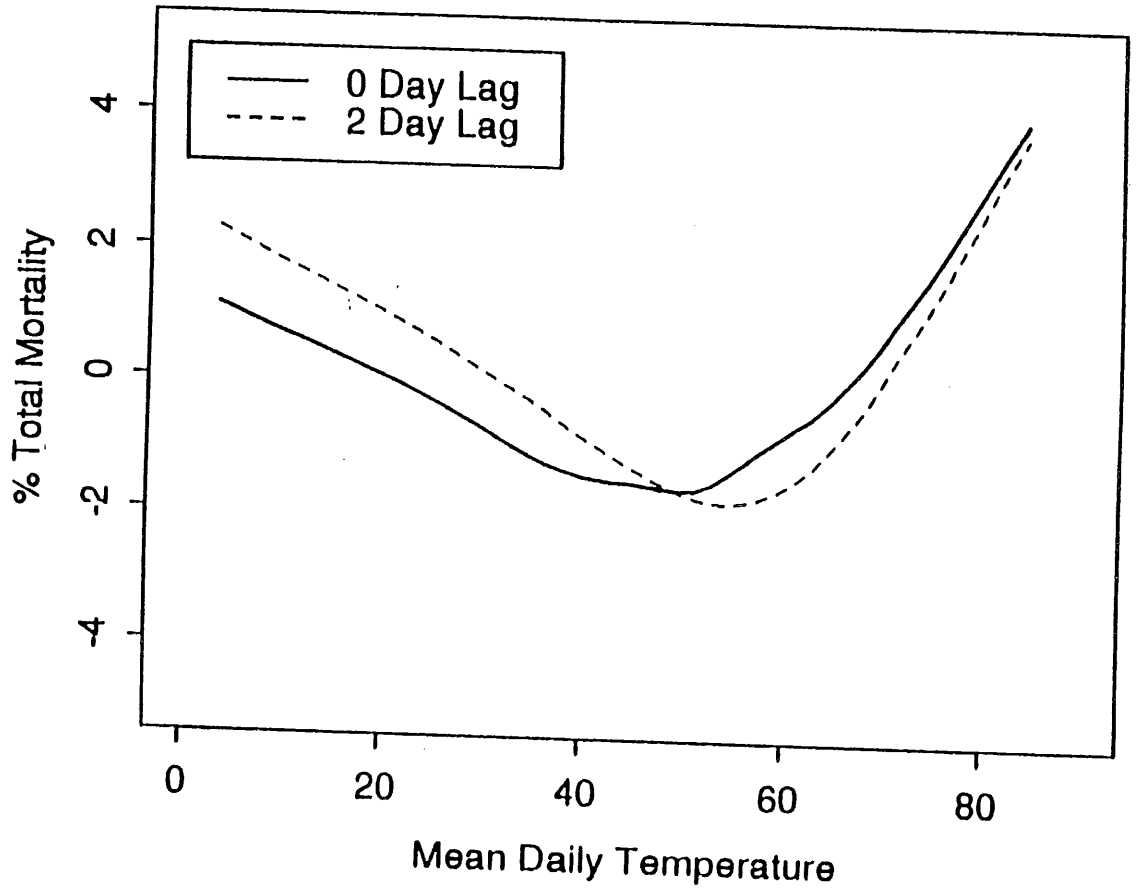


Figure 3. Percent Total Mortality vs. 0 & 2 Days Temperature Lags in Buffalo, NY.

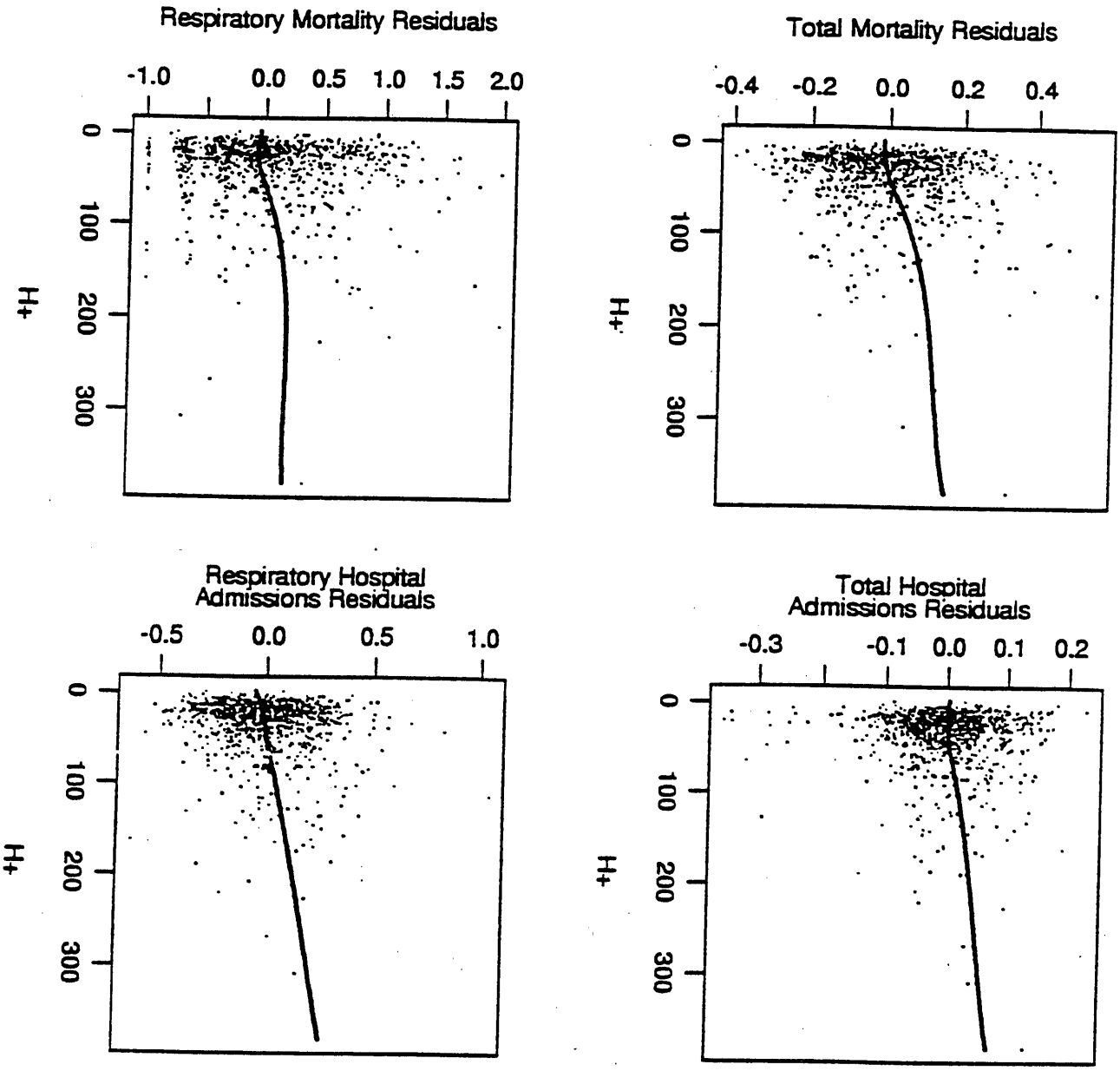


Figure 4. Filtered Health Outcomes vs. Acid for Buffalo, NY.

Sensitivity Analyses

To assess the sensitivity of the pollutant coefficients to various model specifications, two modeling approaches were used. First, three different techniques were individually incorporated into the basic model to control for seasonal variations: five sine and cosine series (2yr, 1yr, 6mo, 3mo & 1mo) ; a weighted 31-day moving average filter; and, a locally weighted regression estimated smoothed (LOESS) fit. Second, multiple pollutants were simultaneously included in the basic regression model for comparison with the single pollutant model results.

RESULTS

Figure 5 shows the cause-specific RR estimates for each pollutant. These RR's were estimated using coefficients from the basic model with the weighted 31-day moving average filter to adjust for seasonal influences. Overall, most of the pollutants demonstrated positive associations across all health endpoints, many of which are statistically significant, indicating there is a general air pollution effect. Determining exactly which pollutant was responsible for what degree of this general effect was complicated by the high collinearities of the various pollutants. However, for the maximum minus mean increments, H^+ produced higher relative risks than all of the other pollutants. Specifically, H^+ produces relative risks for respiratory hospital admissions which are larger and more significant than that of the PM_{10} surrogate (H^+ RR and 95% C.I. : 1.38, 1.27-1.51; PM_{10} RR and 95% C.I.: 1.18, 1.12-1.24) .

Figure 6 presents the statistical significance of each pollutant/health endpoint association, as indicated by the regression coefficient's t-statistic. Several of the pollutants exhibited individual associations with specific health endpoints which were more significant than that of H^+, for example $SO_4^=$ and SO_2 with respiratory hospital admissions. However, H^+ yielded the most consistent strongly significant associations across all health indices. For example, $SO_4^=$ always had less significant associations than H^+, with the sole exception of respiratory hospital admissions, and SO_2 was non-significant for respiratory mortality.

In general, the pollutant/health endpoint associations exhibit coherence across the hospital admissions and mortality categories, with the exception of SO_2, for which the RR's for respiratory mortality were somewhat lower than that of respiratory hospital admissions (and not statistically significant). The total admissions and mortality categories had lower RR's than the respiratory categories for H^+, PM_{10}, $SO_4^=$ and O_3. Both respiratory hospital admissions and respiratory mortality showed similar increases in relative risk (resp. hosp. adm. : RR=1.38, C.I.=1.27-1.51; resp. mort.: RR=1.48, C.I.=1.11-1.99, see figure 5), while total hospital admissions and total mortality showed smaller, but also significant increases (total hosp. adm.: RR=1.04, C.I.=1.02-1.07; total mort: RR=1.24, C.I.=1.14-1.34).

Figure 7, demonstrates the influence of using different types of seasonal adjustment variables on the H^+ and PM_{10} RR's for the various health endpoints. The sine/cosine model does seem to give slightly (though not significantly) larger RR estimates for the hospital admissions categories. This was apparently due to the

inability of the sine/cosine approach to account for long wave fluctuations in human health which differ from year to year, such as influenza epidemics. Overall, the changes in RR's for each outcome due to the choice of seasonal adjustment variables are minimal.

The results of the simultaneous inclusion of multiple pollutants are shown in Figures 8a and 8b. Figure 8a demonstrates the effect of adding the gaseous pollutants to the model on the H^+ RR for respiratory hospital admissions. While there are slight decreases in the H^+ RR, it was relatively unaffected by the simultaneous inclusion of gaseous pollutants in the model. Figure 8b shows the effect adding H^+ to the model with the individual gaseous pollutants on the gaseous pollutant RR's for respiratory hospital admissions. The simultaneous regression with H^+ generally lowers the relative risk of the other pollutants, but these decreases are not significantly different. Thus, H^+ associations with morbidity and mortality were found to be relatively independent of the association shown by other gaseous pollutant metrics.

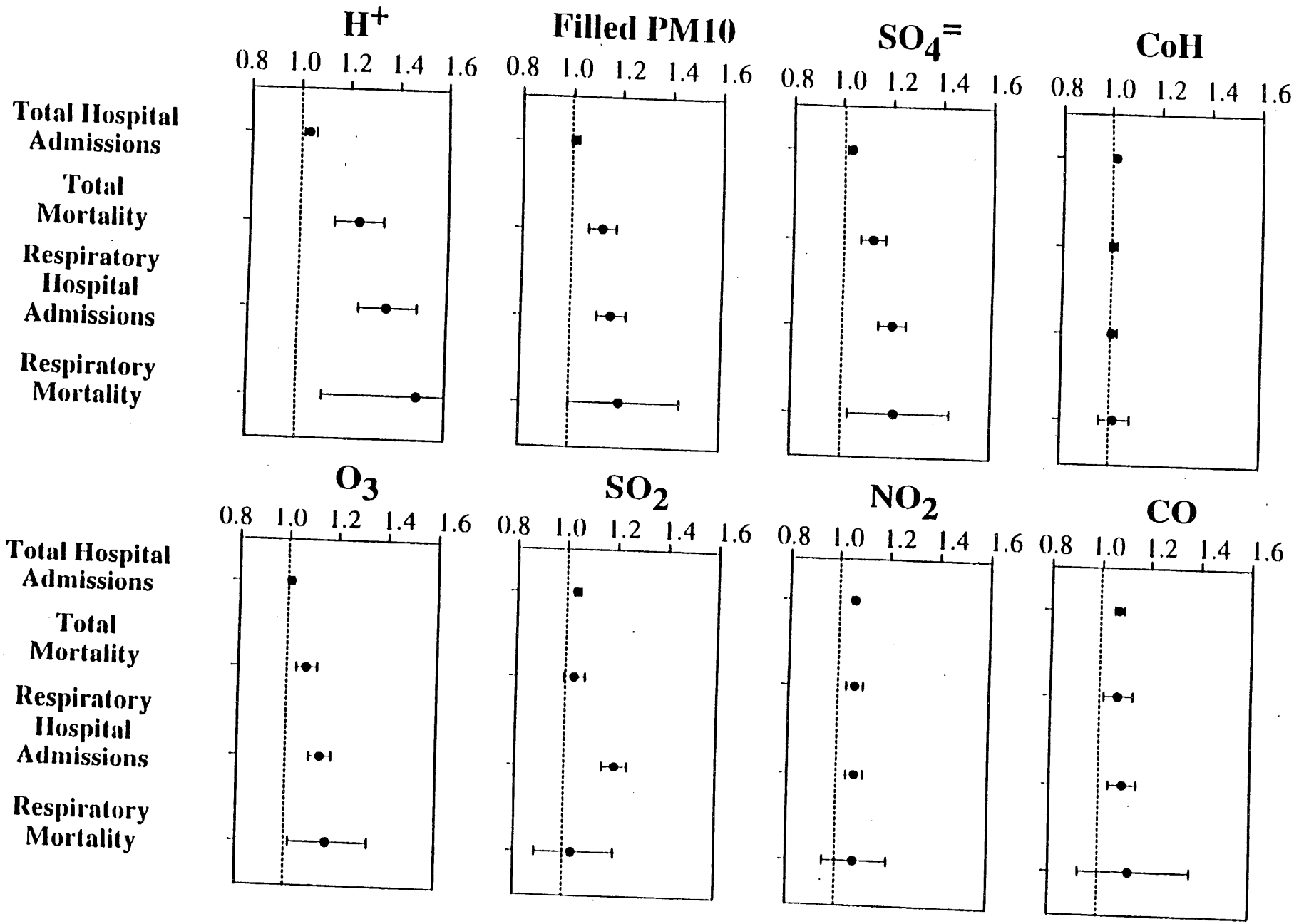


Figure 5. Pollutant Cause-Specific Relative Risks (One-way 95% C.I.) Based on the Difference between the Maximum and Mean Concentrations.

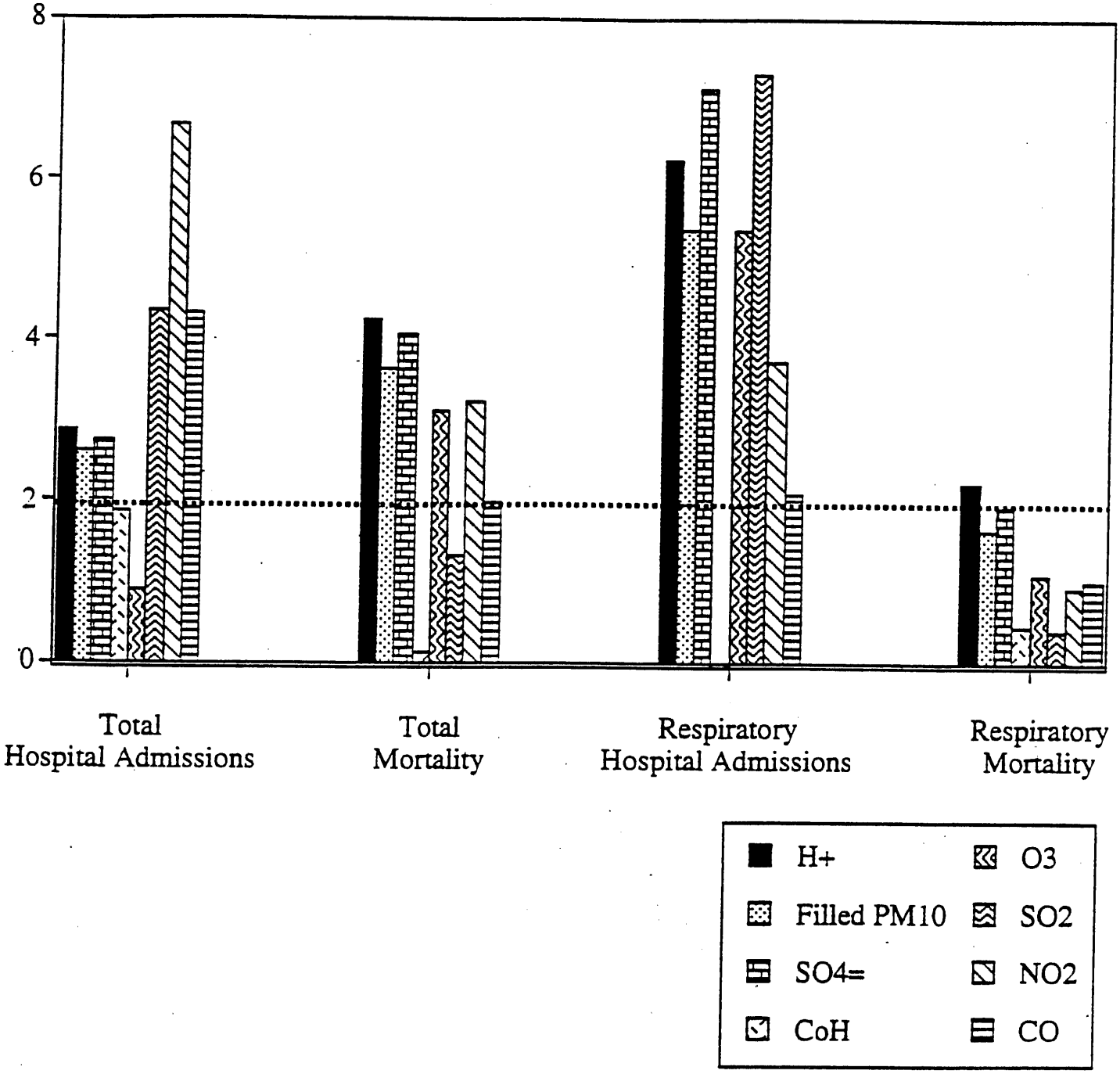


Figure 6. Significance of Pollutant Coefficients for All Outcomes as Shown by the t-statisitic.

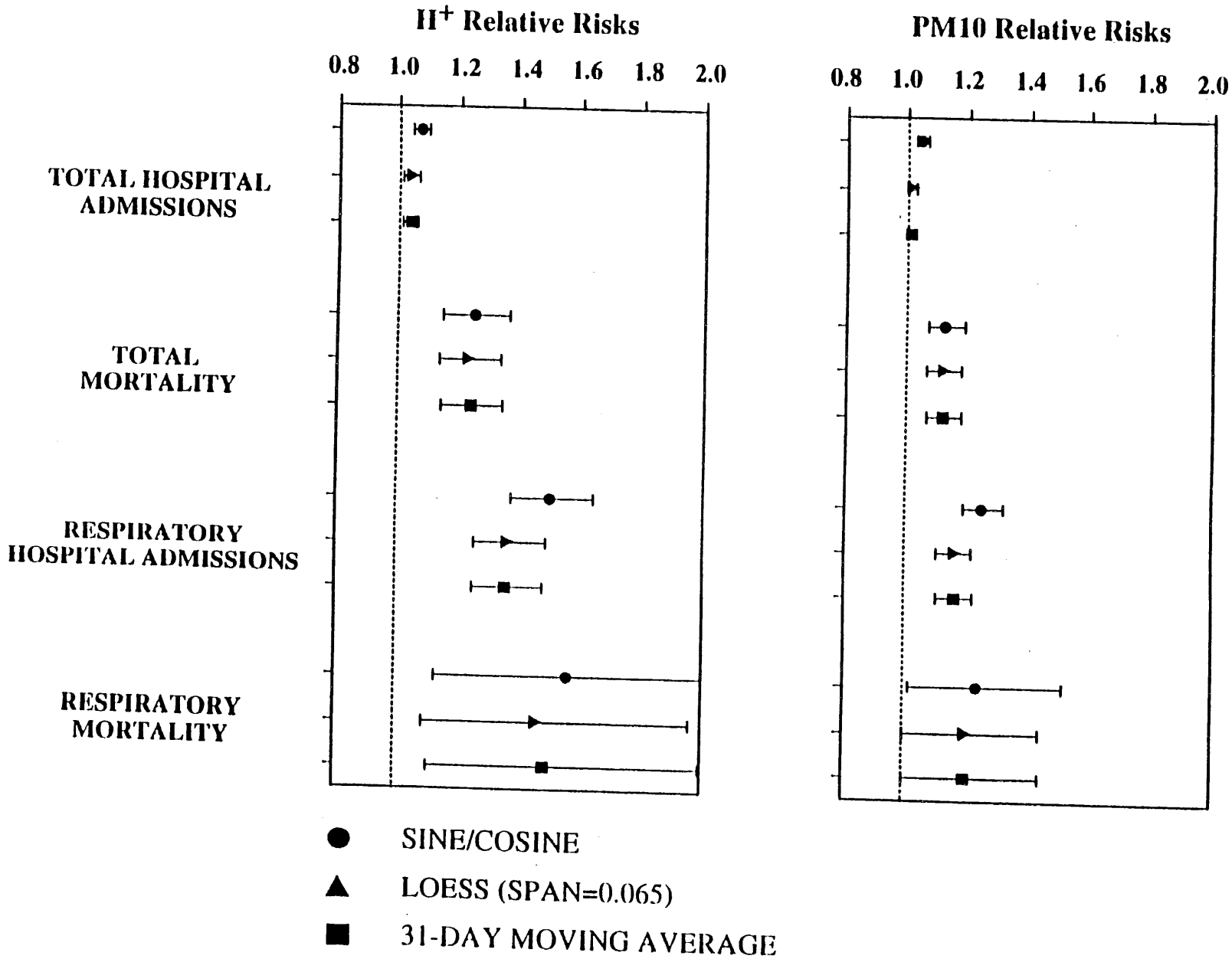
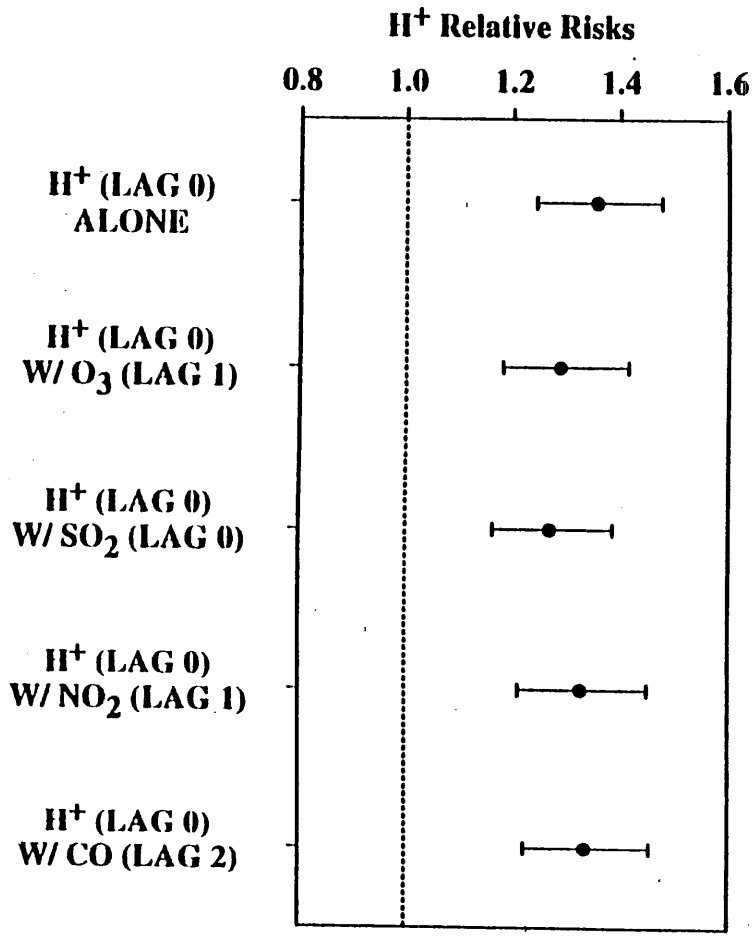
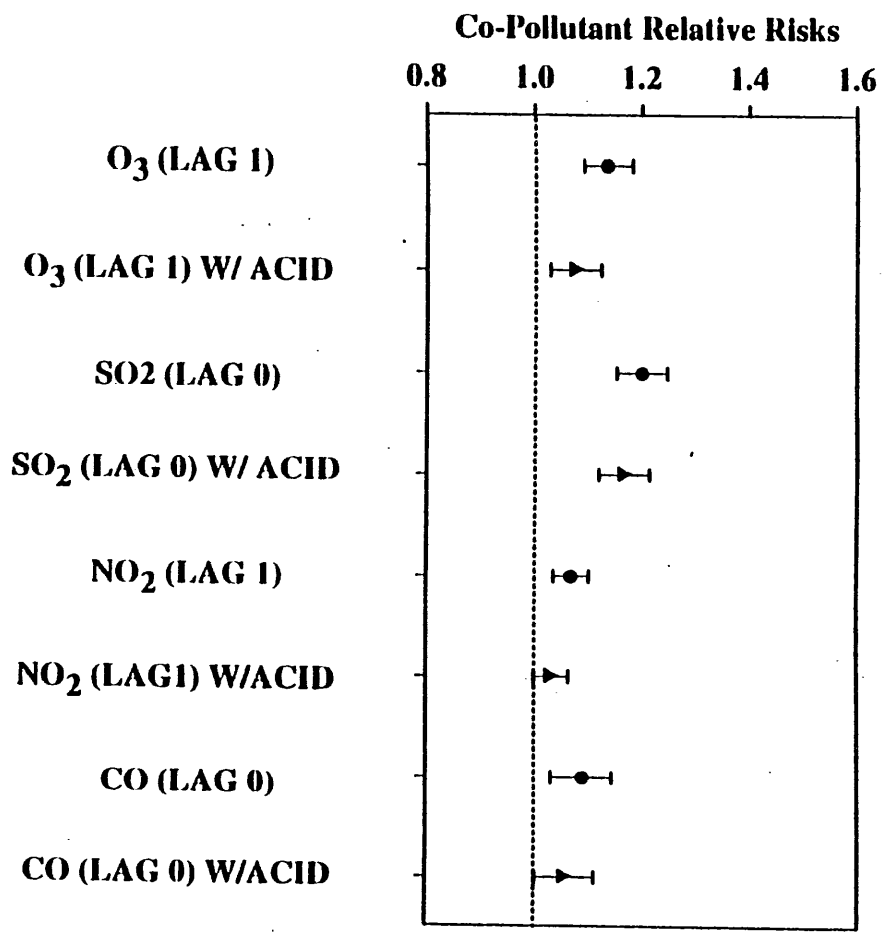


Figure 7. Effect of Various Seasonal Adjustments on Cause-Specific Relative Risks for H^+ and PM10



● RR Associated with H^+ Increase

(a)



● Pollutant Alone

▲ Pollutant Simultaneously Included with H^+

(b)

Figure 8. Respiratory Hospital Admissions Relative Risks For Simultaneously Included Pollutants.

DISCUSSION & CONCLUSIONS

To evaluate the hypothesis that H^+ is an especially harmful component of PM_{10}, it is necessary to compare the H^+ RR for the various health outcomes with those of PM_{10}. From Figure 5, it is clear that the H^+ RR's are larger and more significant than those of the PM10 surrogate across all health outcomes. The size of the relative risk is a function of both the pollutant coefficient and a specific pollutant increment. In this analysis, the difference between the maximum and the mean pollutant concentrations were used to estimate the relative risk of each pollutant. This was selected to represent the risk a population might experience on a high pollution day versus that of an average pollution day. The significance of the RR estimate, however, is not based on the specific increment choice, rather it is solely a function of the size of the coefficient and its standard error.

Comparisons of the H^+ and PM_{10} RR's may be effected by the fact that more than 80% of the PM_{10} values are estimated from daily $SO_4^=$ and CoH values. However, it is reasonable to use the filled PM_{10} values as a surrogate for PM_{10} in these analyses, as the correlation between the observed and predicted values is quite high (R=0.88). The remaining variation is most likely dominated by day to day variations in the coarse particle fraction, which is not represented in the PM10 prediction equation, except by the intercept (both $SO_4^=$ and CoH are dominated by the sub-micron fraction). Thus, the PM_{10} surrogate used in this analysis may be more representative of ambient fine particle concentrations which are thought to have a greater health relevance than coarse particles (EPA Draft Particulate Matter Criteria Document, 1995). Despite the high correlation, 12% of the variation in PM_{10} is not being accounted for by the predictor variables. Maximum estimates of the PM_{10} associations can be calculated by attributing this remaining 12% variation directly to increases in human health effect outcomes due to PM_{10}. This is done by dividing the estimated PM_{10} surrogate coefficient by the correlation coefficient of the PM_{10} prediction equation. This increases the estimated PM_{10} coefficients, causing them to become more significant. However, as shown in Figure 9, despite adjusting for error introduced by filling in missing PM_{10} values, H^+ remains most significant across the health outcome indices, with the sole exception of the total hospital admissions category.

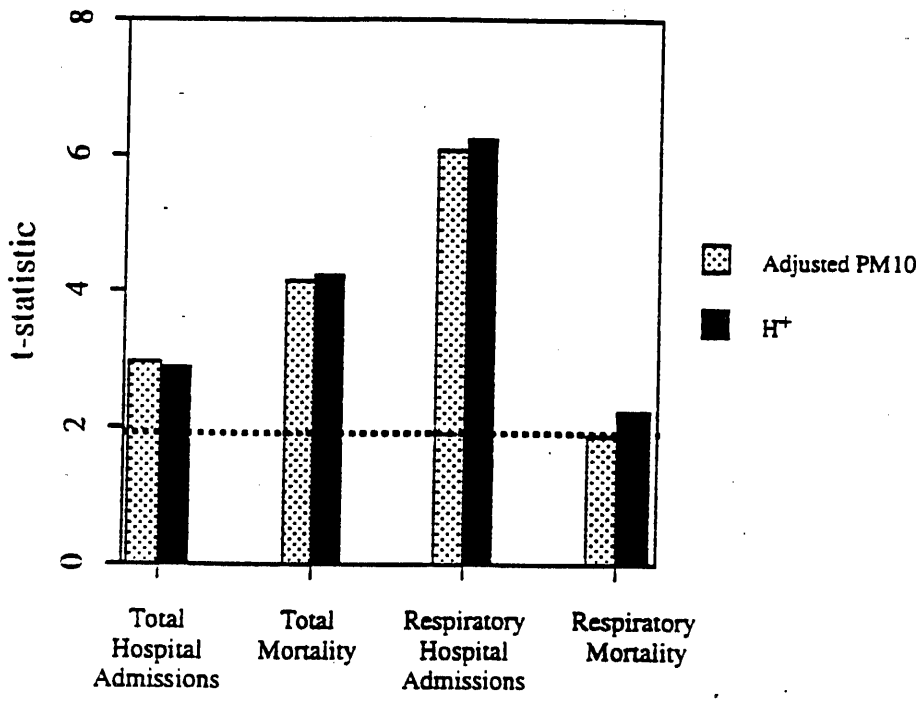


Figure 9. t-statistics for Adjusted PM10 Compared with that of H^+.

The second objective of this research was to evaluate the coherence of the associations across health outcome groups. With the exception of SO_2, all of the pollutants exhibited coherence across similar mortality and hospital admission categories. Based on the route of exposure, it would be expected that pollutant associations would be larger for the respiratory categories that the total categories. This is true for most of the pollutants, supporting the biological plausibility of these pollution/health effect associations.

The sensitivity of the associations to various model specifications was evaluated first, by using different techniques to control for seasonal variations and second by including simultaneous pollutants in the model. The type of seasonal control technique used minimally affected the PM_{10} and H^+ coefficients. While the sine/cosine techniques seem to be the least conservative, its estimates were not significantly different from that of the 31-day moving average filter and the LOESS fitted curve (which were very similar to one another). Significant decreases in pollutant risk estimates are possible with the simultaneous inclusion of other correlated pollutant measures. However, this was not the case for H^+ in these analyses. This suggests that H^+ may not be a surrogate for other air pollutant effects, but rather it may independently contribute to the observed increases in health effects.

The results from these analyses indicate a need to further define the role of acidic aerosols in the observed PM_{10}/health effect associations. As demonstrated above, H^+ is one of the many pollutants which are significantly associated with increases in adverse health effects. However, the fact that H^+ is the most consistent of all the pollutants considered, including PM_{10}, with respect to its strength of association and its statistical significance, indicates that it may be a significant contributor to the increases in adverse health effects which have been attributed to PM as a whole. The H^+ associations are reinforced by the coherence observed across health endpoints and the minimal effect of different model specifications. While, H^+/mortality associations have not been observed elsewhere in the U.S., this analysis is based on a two and a half year series of daily H^+ observations, which is the longest considered to date at a single site. Therefore, evaluation of H^+/health effect associations in other cities with multiple years of daily H^+ data is necessary to investigate the consistency of these results.

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