Table 1a.

Cellular Parameters in Bronchoalveolar Lavage Fluid from Young Rats following Exposure to Combinations of Endotoxin, Carbon, and Ozone

	Total Cells	%AM	%PMN	%Lymph.	%Viable	Protein (mg/ml)	LDH	β-Glucuronidase (mg/ml/min)
Young Rats: **Untreated**	0.93 ± 0.10 [a]	98.87 ± 0.95	0.31 ± 0.38	0.82 ± 0.64	94.84 ± 1.85	0.12 ± 0.01 [a]	33.30 ± 9.15	0.24 ± 0.01
Carbon Only	1.50 ± 0.23	98.35 ± 0.68	0.48 ± 0.27	1.18 ± 0.41	95.02 ± 1.80	0.14 ± 0.01	42.62 ± 3.66	0.39 ± 0.08
Ozone Only	1.21 ± 0.05	95.39 ± 0.15	2.47 ± 0.44	2.14 ± 0.31	94.42 ± 1.86	0.40 ± 0.03	60.25 ± 14.79	0.35 ± 0.00
Carbon/Ozone	1.17 ± 0.04	93.10 ± 1.72	4.05 ± 1.15	2.85 ± 0.57	93.33 ± 1.57	0.35 ± 0.04	60.04 ± 26.98	0.33 ± 0.02
LPS Only	1.36 ± 0.04	88.25 ± 2.43	9.47 ± 2.06	2.28 ± 0.37	94.85 ± 0.61	0.11 ± 0.01	35.72 ± 2.96	0.21 ± 0.02
LPS/Carbon	1.45 ± 0.04	83.84 ± 2.79	14.52 ± 2.70	1.64 ± 0.38	94.25 ± 0.76	0.16 ± 0.00	41.21 ± 6.52	0.24 ± 0.04
LPS/Ozone	1.36 ± 0.11	83.41 ± 3.78	13.51 ± 3.29	3.08 ± 0.60	94.96 ± 0.57	0.37 ± 0.05	43.33 ± 12.12	0.33 ± 0.05
LPS/Carbon/ Ozone	1.57 ± 0.23	80.04 ± 2.33	17.43 ± 1.98	2.53 ± 0.56	93.16 ± 1.77	0.33 ± 0.04	95.09 ± 13.74	0.35 ± 0.02

Table 1b.

Cellular Parameters in Bronchoalveolar Lavage Fluid from Old Rats following Exposure to Combinations of Endotoxin, Carbon, and Ozone

	Total Cells	%AM	%PMN	%Lymph.	%Viable	Protein (mg/ml)	LDH	β-Glucuronidase (mg/ml/min)
Old Rats: **Untreated**	2.18 ± 0.53 [a]	98.70 ± 0.83	0.47 ± 0.30	0.83 ± 0.54	91.06 ± 2.66	0.17 ± 0.01	49.05 ± 18.60	0.35 ± 0.08
Carbon Only	1.65 ± 0.24	98.26 ± 0.44	0.32 ± 0.09	1.42 ± 0.41	91.59 ± 2.21	0.17 ± 0.02	26.15 ± 19.38	0.25 ± 0.05
Ozone Only	2.27 ± 0.64	97.82 ± 0.26	0.78 ± 0.30	1.40 ± 0.04	90.99 ± 0.42	0.45 ± 0.04	65.43 ± 9.58	0.43 ± 0.09
Carbon/Ozone	2.29 ± 0.58	97.78 ± 0.77	1.59 ± 0.40	0.97 ± 0.84	89.88 ± 3.75	0.49 ± 0.06	69.44 ± 5.07	0.41 ± 0.03
LPS Only	1.88 ± 0.16	89.91 ± 2.71	7.82 ± 2.18	2.27 ± 0.77	88.76 ± 4.58	0.21 ± 0.01	43.09 ± 19.55	0.30 ± 0.02
LPS/Carbon	1.90 ± 0.58	95.33 ± 0.19	2.41 ± 0.61	2.25 ± 0.75	92.32 ± 1.18	0.21 ± 0.06	46.92 ± 20.51	0.24 ± 0.03
LPS/Ozone	2.20 ± 0.21	88.77 ± 3.84	8.81 ± 3.52	2.41 ± 0.60	91.83 ± 0.49	0.45 ± 0.09	55.94 ± 14.50	0.30 ± 0.07
LPS/Carbon/ Ozone	2.15 ± 0.63	75.62 ± 7.61	20.71 ± 7.61	3.67 ± 1.28	88.70 ± 1.63	0.47 ± 0.17	60.83 ± 4.03	0.49 ± 0.24

[a] Values are reported as means +/- SD; $n = 3$ per group.

Table 2a.

Three-Way ANOVAs for Main Effects and Interactions between Factors in Young Rats [a]

Source	BAL PMNs (p)	Resting ROS (p)	PMA-Stimulated ROS (p)
Carbon	0.0015	0.0015	0.0519
Ozone	<0.0001	0.0013	0.0001
Endotoxin	<0.0001	0.0003	<0.0001
Carbon + Ozone	0.9679	0.0148	<0.0001
Endotoxin + Carbon	0.2650	0.0008	0.8071
Endotoxin + Ozone	0.0085	0.0053	0.0066
Endotoxin + Carbon + Ozone	0.4189	0.0087	0.0696
R^2	0.966	0.855	0.970

Table 2b.

Three-Way ANOVAs for Main Effects and Interactions between Factors in Old Rats [a]

Source	BAL PMNs (p)	Resting ROS (p)	PMA-Stimulated ROS (p)
Carbon	0.7470	0.4065	0.3398
Ozone	<0.0001	0.0008	<0.0001
Endotoxin	<0.0001	<0.0001	<0.0001
Carbon + Ozone	0.0014	0.0086	0.0062
Endotoxin + Carbon	0.2938	0.0017	0.2017
Endotoxin + Ozone	0.9961	0.0742	0.4111
Endotoxin + Carbon + Ozone	0.2110	0.0999	0.4895
R^2	0.929	0.874	0.933

[a] The three factors were the presence or absence of carbon, endotoxin, and ozone. Analysis of residuals was used to check the assumptions of normality and equal variance.

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Are Males More Susceptible to Ambient PM than Females?

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Abstract

Recent epidemiologic studies of modern air pollution show statistically significant relationships between fluctuations of daily mortality and fluctuations of daily ambient particulate matter (PM) levels at low concentrations. A review of historic smoke-fog (smog) episodes (Meuse Valley, Donora, London) was conducted to seek common characteristics of victims of very high concentrations of ambient PM air pollution to help identify a susceptible cohort for an effect at lower ambient PM concentrations. The Meuse Valley episode had been investigated in depth, but the published report (Firket, 1936) did not detail the age and sex of the victims. We obtained the complete set of death certificates from all the Belgian villages in the area extending over the period of the smog (December 1 - 7, 1930). These data on age and sex from the Meuse Valley are provided here for the first time. The results show a ~50% excess male death rate (36♂, 25♀) consistent with the finding of excess male deaths in both the Donora Fog (15♂, 5♀) and the London Fog (autopsied cases; 419♂, 287♀). In all three episodes, mortality was predominantly amongst the aged, and most of those autopsied presented with pre-existing cardiopulmonary lesions.

Because the ages, genders and smoking habits of the total populations at risk are unrecorded, we are unable to adjust for an expectation of a larger number of aged females than aged males and an expected higher male smoking rate and time outdoors compared to females of similar ages. However, recent epidemiologic studies of PM and mortality have found no statistically significant different risk of mortality for smokers and non-smokers (Pope et al., 1995). We present data on other causes of respiratory failure that also have a 50% male excess death rate, which have been found previously to be suggestive of a genetic X-linkage that may also be responsible for the ~50% excess male death rate associated with fluctuations of PM concentrations. We therefore hypothesize, with adjustments for these potential confounders of population-by-age and smoking rates not withstanding, that males may indeed be more susceptible to the effects of ambient PM exposures than females.

The 1930 Meuse Valley Smog Episode. The highly industrialized Meuse River valley of Belgium, between Huy and Liege, experienced an atmospheric stagnation period from December 1 to December 5, 1930. A highly toxic smog (smoke + fog) covered the area of the valley during this period. No air quality measurements were taken during this period, and the death count did not begin to rise precipitously until the fourth day of the smog. We collected all 83 death certificates for the area from December 1 through December 7, 1930, and the death counts, by gender, are

[1] This manuscript has been reviewed for technical accuracy. It does not represent policy of the U.S. EPA or VITO.

presented in Table 1. The youngest male dying on the two days when the smog-related deaths occurred was 37 years old and the youngest female dying was 42 years old. We estimate that if the smog did not occur, 5 males and 5 females would have died (equal in number to those dying on December 2 and 3), so the smog related deaths are estimated as 36 - 5 = 31 males and 25 - 5 = 20 females, for a male : female ratio of 1.55 : 1, or a 55% male excess.

Table 1. Summary of Daily Deaths During the December 1930 Meuse Valley Smog Episode

Date in December	Male Mortality	Female Mortality	Comments
December 1	0	3	First day of smog. Three adults die.
December 2	2 1 stillborn	2 1 stillborn	Second day of smog. Four adults die. Two infants are stillborn in the same village at the same time.
December 3	3	3	Third day of smog. No deaths due to the fog were reported but two male deaths occurred at 9 p.m.
December 4	19	18	Fourth day of smog. Deaths suddenly occur simultaneously throughout the region.
December 5	17	7	Fifth day of smog. Many deaths still occur but at a lower rate.
December 6	2	3	Fog clears and no immediate deaths are reported from the smog.
December 7	1	1	Back to normal, although some people may have died later from complications of morbid conditions initiated during the smog period.
Totals December 4 - 5	36 male	25 female	Note the 5 male and 5 female deaths two days before the recorded deaths from the smog.

The 1948 Donora, PA Smog Episode. The highly industrialized Monongahela River valley of Pennsylvania experienced an atmospheric stagnation period from October 27, through October 31, 1948. Particulate and gaseous emissions from basic steel industries and other chemical plants produced a toxic smog that covered the area of Donora, PA. As in the Meuse episode, no air quality measurements of any kind were made, and people did not start to die of the smog until the fourth day (October 30, 1948) when 10 people died between 2 a.m. and 8:30 a.m. The U.S. Public Health Service conducted a thorough investigation (Schrenk et al., 1949) and identified 20 possibly smog related deaths (15 male and 5 female). The youngest male dying was 52 years old and the youngest female dying was 55 years old. The numbers by gender are shown in Table 2. The population data for Donora were reported and Donora had a ~10% higher number of males than females above 50 years old (i.e., retirees from the heavy industry there) so we estimate that for an equal number of males to the actual number of females, that the number of males dying would be reduced by 2, so the ratio of 13 male : 5 female is 2.6 : 1, for an estimated male excess of 160%.

Table 2 Summary of Daily Deaths During the October 1948 Smog Episode in Donora, PA

Date in October	Male Deaths	Female Deaths	Comments
October 27 - 29	0	0	No deaths occurred
October 30 a.m.	8	4	
October 30 p.m.	4	1	
October 31 a.m.	2	0	One subject went into a coma and died on November 8, 1948
October 30 p.m.	1	0	

The 1952 London, UK Smog Episode. The highly urbanized area of the Thames River valley experienced an atmospheric stagnation period from December 5 through December 8, 1952, when a toxic smog covered the London Metropolitan area. As opposed to the Meuse and Donora smogs, which were mainly from heavy industry including steel production, the main source of the PM and gaseous pollutants in London was the combustion of coal in local power plants and in the vast majority of domestic heating units. These emissions into a surface inversion layer caused a precipitous rise in mortality on the very first day of the smog, and the measurements of PM as British Smoke (reflectance) and sulfur dioxide are reported by the UK Ministry of Health (MOH, 1954). Although MOH (1954) reported total numbers of deaths by day during the period, they did not report the gender breakout of that cohort. The only published data on gender during the smog period is for those deaths which were unattended by a physician, or suspicious, which were required by law to have an autopsy, so no record is available for the ~2500 deaths which were in hospital or attended by a physician who could sign the death certificate. Because the reporting of mortality by

ages was by the week, and the first two smog days were in a different weekly total than the last two days, it is not possible to know the age distribution during the smog with any certainty, so it is not clear if any of the young were affected. The autopsied deaths by cardiac and respiratory causes, broken out by sudden death and death after the first day of onset of the condition are shown in Tables 3a and 3b respectively. The period December 1 - 4 represents the pre-smog conditions, December 5 - 8 represents the high smog conditions, and December 9 - 12 represents the milder post-smog conditions with smog concentrations still higher than the pre-smog concentration levels. In terms of raw counts, there were 419 male deaths and 287 female deaths, a 46% male excess. Subtracting twice the number of deaths in the 4-day pre-smog period to represent the expectation of 8-days of non-smog related mortality, the values are 291 male and 187 female, a 56% excess of male mortality. The results for all three smogs are summarized in Table 4, showing a consistent male excess mortality of order 50% overall. The common finding of autopsied cases in all three smogs was that "they were chronic bronchitic and emphysematous (COPD) people with consequent commencing myocardial damage" (MOH, 1954). The absence of youths in the mortality totals of the Meuse Valley and Donora smogs, and the absence of their mention in London (MOH, 1954) may be either an indication that the young are more resistant to smog or an artifact of a relatively small number of young people at risk.

Table 3a. Manner of Death in Coroners' Cases of those Dying Suddenly on First Day of Symptoms During the London Smog of December 1952.

Dates of the 1952 London Smog	Number of Sudden Male Respiratory Deaths	Number of Sudden Female Respiratory Deaths	Number of Sudden Male Cardiac Deaths	Number of Sudden Female Cardiac Deaths
Dec. 1 - 4 Pre-Smog	5	1	23	18
Dec. 5 - 8 Smog	39	30	99	66
Dec. 9 - 12 Post-Smog	36	19	49	31
Dec 5 - 12 Total Deaths	75	49	148	97

Table 3b. Manner of Death in Coroners' Cases of those Dying Later, After the First Day of Symptoms During the London Smog of December 1952.

Dates of the 1952 London Smog	Number of Latent Male Respiratory Deaths	Number of Latent Female Respiratory Deaths	Number of Latent Male Cardiac Deaths	Number of Latent Female Cardiac Deaths
Dec. 1 - 4 Pre-Smog	21	13	15	18
Dec. 5 - 8 Smog	69	43	38	42
Dec. 9 - 12 Post-Smog	68	36	21	20
Dec 5 - 12 Total Deaths	137	79	59	62

Table 4. Summary of Male and Female Deaths in the Meuse, Donora and London Smogs

Location	Male - Expected*	Female - Expected*	Excess Male Percent
Meuse Valley, Belg.	36 - 5 = 31	25 - 5 = 20	55%
Donora, PA	13 - 0 = 13	5 - 0 = 5	160%
London, UK	419 - 138 = 281	287 - 100 = 187	56%
Total all cases	470 - 143 = 327	317 - 110 = 212	55%

*Expected = Deaths occurring in the preceding equal number of days before the smogs.

Deaths by Gas (CO + methane) Suffocation in Paris, 1949 - 1962. In the period between 1949 and 1962, *Gaz du France* and the Paris police investigated all fatal cases and non-fatal cases of heating/cooking gas exposure requiring emergency response, to determine whether the cases were suicidal, homicidal or accidental due to malfunction of equipment (Grémy et al., 1968). If males and females have the same susceptibility to hypoxia, then all circumstances being equal (closed room and all gas jets open wide until discovery), the percentages of successful suicide attempts should be the same for males and females. However, the data reported in Table 5 show that males, on average, were 45% more successful at committing suicide by gas than females. Note the consistency of this excess from year to year and its similarity to the 55% excess adult male fraction of mortality from smog-related deaths.

Table 5. Male Excess of Successful Suicide Attempts by Main Gas Inhalation in Paris, 1949 - 1962.

Year	Male Suicide Success %	Female Suicide Success %	Male Excess Rate %
1949	54	39	38
1950	57	40	43
1951	58	35	66
1952	59	36	64
1953	60	41	46
1954	59	42	40
1955	63	45	40
1956	60	42	43
1957	54	45	20
1958	62	48	29
1959	59	40	48
1960	61	40	53
1961	48	34	41
1962	50	32	56
Totals	58 %	40 %	45 %

The U.S. Male Excess in Infant Deaths from Respiratory Causes. There is also an approximate 50% excess in male infant deaths from respiratory causes in the U.S. and throughout the world. Mage and Donner (1997) compiled data on Sudden Infant Death Syndrome (SIDS) from 36 published papers on autopsied cases from world-wide SIDS studies. They found 41,238 male and 26,140 female SIDS cases, corresponding to a male fraction of 0.612, which represents a 50% excess male susceptibility when corrected for the 5% global excess male birth rate (0.612/1.05) / 0.388 = 1.50. Table 6 shows U.S. male excess infant mortality from respiratory causes as reported by WHO (1998) and from public records at http://wonder.cdc.gov.

Table 6. Male Excess Infant (0 - 1 year) Mortality from Respiratory Causes in the U.S.

Respiratory Cause	Years of Data	Males	Females	Male Excess Mortality % (male/female/1.05)* - 1
Post-Neonatal SIDS	1993-1994	5,018	3,179	50.3 %
Pneumonia	1993-1994	639	434	40.2
Other Respiratory Diseases	1993-1994	592	375	50.3
Birth Trauma	1993-1994	269	175	46.4
Drowning and Submersion	1979-1993	513	150	39.9
Inhalation of Food or Other Object	1979-1993	781	499	49.0
Totals of Above	----------	7,812	5,012	48.4 %

* The 1.05 factor corrects for the 5% excess in the U.S. male birth rate.

Hypothesized Cause of the Consistency of Excess Male Respiratory Mortality. Mage and Donner (1997) hypothesized that the 50% excess in male mortality from respiratory causes, shown to present in infancy as well as adulthood by the above analysis, may be X-linked. A gene locus on the X-chromosome with a dominant allele, that occurs with a frequency of 1/3, protective against cerebral anoxia and respiratory failure, may be required to survive a respiratory crisis. The probability of an XY male not having that protection is 2/3 and the probability of an XX female not having that protection is 4/9. The ratio of 2/3 to 4/9 is 1.5, perhaps representing the 50% excess male susceptibility that is found in the mortality data described above in this paper.

Mage and Donner later noted that such a genetic mechanism could explain the male fraction of all infant mortality p(m) by the following equation: $p(m) = p(m/R)\,p(R) + p(m/C)\,p(C)$ (1) where $p(m/R)$ = probability of a male given a respiratory failure

$p(R)$ = probability of respiratory failure
$p(m/C)$ = probability of a male given a cardiac failure
$p(C)$ = probability of a cardiac failure.

Given a population of M males and F females, the probability $p(m/C) = M/(M+F)$; the probability $p(m/R) = (2/3)\,M / [(2/3)\,M + (4/9)\,F]$; The probability of respiratory failure given a respiratory crisis $p(R) = [(2/3)\,M + (4/9)\,F] / (M + F)$; The probability of cardiac failure $p(C) = 1 - p(R)$. Equation (1) was tested using the CDC public data tapes for U.S. Mortality 1979 - 1996 found at http://wonder.cdc.gov, using the data for all races, all years, and for all ICD codes 001 to 999.9.

Table 7. Total of Infant Mortality in the U.S. from 1979 to 1996 reported by CDC

Age	Gender (all Races)	Death Count	Population at Risk
Under 1 Year	Male	388,754 (m)	35,382,187 (M)
Under 1 Year	Female	297,235 (f)	33,697,511 (F)
1 - 4 years	Male	74,763 (m)	133,336,137 (M)
1 - 4 years	Female	56,790 (f)	127,294,685 (F)
Total Under 5 years	Male	463,517 (m)	168,718,324 (M)
Total Under 5 years	Female	354,025 (f)	160,992,196 (F)

Equation (1) predicts the male fraction p(m) of all infant mortality, m/(m + f), only from the total M and F data, and the results are shown in Table 8. None of the predictions are rejected.

Table 8. Chi-square test statistics (with Yates' correction) for one degree of freedom (1 d.f.)

CDC Data 1979 -1996	Predicted p(m)	Observed m /(m + f)	Chi-square 1d.f.
Age Under 1 year	0.56772	0.56671	2.85
Age 1 to 4 Years	0.56712	0.56831	0.76
Age Under 5 years	0.56724	0.56696	0.26

Conclusions

Males do appear to be more susceptible to respiratory failures than females in air pollution episodes involving high PM concentrations and in other conditions in infancy and adulthood that are of respiratory origin. The aged with COPD appear to be more at risk than the young. An X-linkage model has been hypothesized to be the cause of this male excess susceptibility and it is not rejected by testing with U.S. mortality data. We recommend that this model be subjected to further testing.

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Activation of the autonomic nervous system and blood coagulation in association with an air pollution episode

(30.06.99)

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Air pollution has been associated with increases in hospital admissions for cardiovascular diseases. This paper aims to identify subgroups of individuals who exhibit early biological responses consistent with the health effects of ambient air pollution.

Resting heart rates have been recorded in a cohort of 2681 men and women aged 25-64 years who participated in the MONICA Augsburg survey during the winter 1984/85 and in a re-examination during the winter 1987/88.

Increases in heart rate of 1.8 beats per minute (bpm) (95% confidence interval: 0.7 to 2.8 bpm) were observed during the air pollution episode compared to non-episode days adjusted for potential cardiovascular risk factors and meteorological parameters. Among persons whose plasma viscosity was above the 90th percentile, heart rates increased 5.1 bpm (95% confidence interval: 2.1 to 8.2 bpm) during the air pollution episode. Persons with normal values of plasma viscosity only had an increase of 1.4 bpm (95% confidence interval: 0.3 to 2.5 bpm) during the air pollution episode.

Subjects with increased plasma viscosity showed a more pronounced acceleration in heart rate at rest pointing towards a modification of the autonomic control of the heart during an air pollution episode.

Keywords: Air pollution, epidemiology, heart rate, plasma viscosity.

Introduction

Increases in total mortality have been consistently observed during episodes with elevated concentrations of ambient air pollutants attributable to respiratory as well as cardiovascular disease (Dockery, Pope, 1994; Schwartz, 1994; Bascom et al., 1996). The involvement of the cardiovascular system was strongly supported by recent analyses showing an association between particulate matter and hospital admissions for ischemic heart disease and congestive heart failure (Schwartz, Morris, 1995; Burnett et al., 1995). In January 1985, an air pollution episode occurred throughout Central Europe resulting in elevated levels of sulfur dioxide (SO_2) and total suspended particles (TSP) (de Leeuw, van Rheineck Leyssius, 1989). Increased mortality, hospital admissions, and ambulance requests had been reported during this episode compared to a control period in the Ruhr area, Germany (Wichmann et al., 1989). Evidence was also found that plasma viscosity levels were elevated during the air pollution episode in a random sample of the first MONICA-survey (**mo**nitoring trends and determinants of cardiovascular disease) carried out in Augsburg (southern Germany) (Keil et al., 1988). Elevated levels of plasma viscosity have been identified as a risk factor for cardiac death and myocardial infarctions in population based cohort studies. Elevated levels of plasma viscosity might be a marker for inflammation accompanying atherosclerosis and is also thought to be atherogenic.

Increases in heart rate have been reported earlier (Peters et al., 1999) in association with both the air pollution episode and concentrations of sulfur dioxide and total suspended particles. The increase in heart rates was observed in all persons, whose heart rates were determined on high air pollution conditions. However, the epidemiological evidence obtained for hospital admissions and mortality, suggest that only a subgroup of person experiences severe events in association with air pollution. The current paper is to

characterize a subgroup of individuals who might be most susceptible to ambient air pollution.

Methods

The first MONICA-survey in Augsburg (southern Germany) was carried out in 1984-85. 4022 of the 5069 randomly sampled eligible subjects, aged 25-64 years, took part (response 79.3%).(Keil et al., 1988) All survey methods were as in the MONICA protocol (World Health Organisation, 1990). A twelve lead resting electrocardiogram (ECG) was derived from subjects in supine position. The mean heart rate was determined from ECG records with a duration of 20 seconds using computerized ECG analysis systems which provided consistently operating measurement algorithms for all ECG-records analyzed. Non-fasting blood samples were drawn with only short-term venous occlusion and minimal suction. Blood was taken into EDTA (1.5 mg/ml), centrifuged at 1500g for 15 min, and stored at 4°C for a maximum of 4 days. Plasma viscosity was measured at 37°C in a Coulter-Harkness capillary viscometer (Coulter Electronics, Luton, UK).(Koenig et al., 1994) Measurement procedures and sample preparations met the criteria of the International Committee for Standardization in Hematology (International Committee for Standardization in Haemotology, 1982). In 1987/88 a re-examination of the participants of the first MONICA survey was conducted using the same methodology and according to the same protocol as three years earlier. This report is based on a sub-sample of 2681 men and women, in whom valid ECG-readings were obtained both in 1984/85 and 1987/88 and plasma viscosity was measured in 1984/85 (Koenig et al., 1994; Peters et al., 1997). Patients were excluded from the analyses in case there was evidence for an acute infection.

Sulfur dioxide (SO_2), carbon monoxide (CO) and total suspended particulates (TSP) were measured as part of the automated Bavarian air quality network operated by „Bayerisches Landesamt für Umweltschutz„. The monitoring station was located in the center of the

city. Twenty-four hour mean concentrations (midnight to midnight) were provided. Twenty-four hour mean temperature, relative humidity and air pressure (midnight to midnight) were measured on the outskirts of the city.

Multivariate linear regression models were used to estimate the association between ambient air pollution and increased heart rate as described earlier (Peters et al., 1999). The analyses were conducted separately for men and women to allow different associations of the heart rate with the covariates in both genders (Koenig et al., 1994). Summary estimates are presented to provide an estimate of the overall effect taking into account the differences in risk profiles between men and women. The summary estimate was calculated as the mean of the estimates for men and women weighed by the inverse of their variances. Air pollution was considered as an indicator for the 1985 episode. Categorical variables were constructed to control for known or potential risk factors such as age (10 year categories) and current smoking. Body-mass index, systolic and diastolic blood pressure, total and HDL cholesterol entered the models as continuous variables. Temperature, relative humidity and air pressure were therefore considered as possible confounders in the regression analyses. Temperature was considered below freezing, 0 to 10°C and above 10°C, an indicator for relative humidity was one if the relative humidity exceeded 85%, and an indicator for high air pressure was one if the air pressure exceeded 1020 mbar. Additional logistic regression analyses restricted to the first examination were conducted as described earlier (Peters et al., 1997) to evaluate the impact of the air pollution episode on plasma viscosity and heart rate jointly during the first MONICA survey. Plasma viscosity was turned into a binary variable, which was one in the case that it exceeded the 90^{th} percentile (1.35 mPa·s for men (N=133) and 1.33 mPa·s for women (N=105)) of the respective sex group. Heart rate was turned into a binary variable which was one in case

that the heart rate exceeded 80 beats per minute, which corresponded to the 90^{th} . percentile in men (N=148) and women (N=134).

Results

Between January, 7 and January, 19 1985 SO_2 concentrations above 150 $\mu g\ m^{-3}$ were recorded (Peters et al., 1997). During this air pollution episode average SO_2 concentrations of 200 $\mu g\ m^{-3}$ were recorded while on all other days of the MONICA survey the concentrations were below 100 $\mu g\ m^{-3}$ (table 1) (Peters et al., 1999). TSP was elevated during this episode, and particularly accumulated towards the end of the episode. The episode was characterized by low temperatures, stable relative humidity and easterly winds (Peters et al., 1997). Three years later, mean concentrations of SO_2 concurrent with the examinations had more than halved, while TSP was reduced by 10% and CO was reduced by 10% on average (table).

During the air pollution episode increases in heart rate have been observed as reported earlier (Peters et al., 1999). A shift in the distribution of the heart rates was observed comparing episode days to non-episode days (figure 1) which is consistent with an increase in heart rates comparing episode to non-episode days (table 1). Table 2 summarizes the results after adjustment for cardiovascular risk factors and meterological parameters. Subgroup analyses were conducted in order to evaluate whether the observed associations depended on participants with certain risk factors or pre-existing diseases. Effects of comparable size and stability were observed for participants without a history of myocardial infarction, those who were not taking medication to treat cardiovascular diseases and non-smokers (table 1). Participants, who lived within the city limits of Augsburg in contrast, showed slightly larger effect estimates consistent with higher exposures in the urban environment. Participants who had elevated levels of plasma viscosity during the first examination were considered as a subgroup (table 2). Even

though only 133 men and 105 women had plasma viscosity levels above 1.35 mPa s and 1.33 mPa s respectively, a statistically significant increase in heart rate of 5 bpm was observed for this subgroup. However, the effect estimates in the total cohort were not entirely due to this subgroup, because the mean heart rate was 1.4 bpm higher during the episode compared to non-episode days for persons with plasma viscosity below the 90th percentile.

Analyses restricted to the first examination showed that the odds of observing plasma viscosity levels above the 90th percentile was around two during the episode (figure 2) as reported earlier (Peters et al., 1997). However, the odds of observing heart rates above 80 bpm was not different from one, emphasizing that the whole distribution shifted during the episode (figure 1). Nevertheless, the odds of observing both an increase in plasma viscosity and an increase in heart rate was clearly elevated comparing episode to non-episode days. Only univariate analyses were possible because the combined indicator was too rare to provide enough information for the multivariate analyses.

Subjects whose plasma viscosity and heart rates were elevated during the air pollution episode had a higher prevalence of chronic cardiopulmonary diseases than subjects who had elevated levels of both risk factors (table 3). This indicated that persons with cardiopulmonary disease might be more responsive to air pollution than those without. Subjects in whom only high plasma viscosity levels were noted during the episode, but heart rates below 80 beats per minute, had a lower prevalence of cardiovascular disease but a higher prevalence of chronic bronchitis compared to the subjects who were examined outside the episode. This might indicate that the elevated levels of plasma viscosity are attributable to the air pollution rather than underlying atherosclerosis.

During the 1985 air pollution episode, increases in heart rate were present in a random sample of the population of Augsburg after adjusting for cardiovascular risk factors and meteorological parameters. Increases in heart rate were consistently observed in patients without treatment for cardiovascular diseases or patients without MI or plasma viscosity levels within the normal range. A three times higher effect estimate was observed in persons with elevated levels of plasma viscosity compared to persons with normal plasma viscosity levels. During the 1985 air pollution episode an increased risk for extreme values of plasma viscosity was observed both in men and women (Peters et al., 1997). However, the odds for having plasma viscosity levels and heart rates above the 90th percentile was three to four times higher during the episode than outside the episode.

The overall result is consistent with increases in heart rate observed by Pope et al. (Pope et al., 1999) in Utah Valley. In association with 100 $\mu g/m^3$ PM_{10} increases of 0.8 bpm in heart rate were observed in Utah Valley, and therefore smaller than in Augsburg (Peters et al., 1999). Subjects with both elevated heart rates and increased levels of plasma viscosity had a higher prevalence of chronic diseases. Nevertheless, the air pollution episode by itself seemed to be able to induce changes in cardiovascular risk factors because subjects who either had elevated levels of plasma viscosity or heart rate during the episode had a lower prevalence of cardiovascular diseases than patients who had one risk factor elevated outside the episode.

Elevated resting heart rate has been recognized as a risk factor for all cause mortality as well as cardiovascular mortality independent of other major risk factors (Wannamethee et al., 1995; Shaper et al., 1993; Dyer et al., 1980). Data from the Framingham Study suggest that during a longer follow-up among subjects with hypertension, elevated heart rate is a

strong predictor of death and fatal heart disease both in men and women (Kannel et al., 1987). Elevated heart rates can serve as a marker for altered autonomic activity (Bootsma et al., 1994) and thereby identify patients at higher risk of sudden deaths during ischemic events (Gilman et al., 1994). An increased risk of sudden death in association with elevated heart rates was observed in survivors of an myocardial infarction.(Hjalmarson et al., 1990) Transient increases in heart rate before transient ischemic episodes have been noted in patients with unstable angina pectoris or non-Q-wave infarction undergoing holter monitoring (Patel et al., 1997). A review of 72 sudden deaths occurring during holter monitoring detected tachyarrhythmias preceding ventricular fibrillation in 90% of the cases (Panidis, Morganroth, 1984). In this scenario, acute increases of plasma viscosity and heart rate together during air pollution episodes might put a person at a considerable higher risk for more severe ischemic events or congestive heart failure. In elderly persons with a compromised cardiopulmonary health, these observed changes in the resting heart rate might be clinically relevant. Responders reported underlying chronic cardiopulmonary diseases such as myocardial infarction, angina pectoris or chronic bronchitis more often than subjects without elevated levels of plasma viscosity and heart rates.

The epidemiological evidence presented in this paper points towards an increase in sympathetic nerve activity which might be responsible for the adverse health effects seen in association with air pollution episodes. Subjects with increased plasma viscosity showed a more pronounced acceleration in heart rate at rest pointing towards a modification of the autonomic control of the heart during an air pollution episode.

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Table 1: Air pollution[a], meteorological parameters[a] and heart rates and plasma viscosity on the days of the Augsburg MONICA[b] cohort.

	Winter 84/85[c]						Winter 87/88[d]		
	Outside the air pollution episode			Air pollution episode[e]					
	N	Mean +/- Std	range	N	Mean +/- Std	range	N	Mean +/- Std	range
Sulfur dioxide [µg m^{-3}]	116	48.1 +/- 23.1	13 - 103	10	200.3 +/- 26.6	160 - 238	158	23.6 +/- 12.2	6 -71
TSP [µg/m^3]	112	47.4 +/- 28.7	7 - 135	11	97.7 +/- 31.7	62 - 176	142	48.3 +/- 22.1	12 - 134
Carbon monoxide [mg/m^3]	116	4.51 +/- 0.18	0.91 – 11.51	10	4.54 +/- 0.47	2.39 – 6.85	158	4.10 +/- 1.25	1.72 - 8.19
Temperature	133	3.4 +/- 5.9	-18.0 – 14.5	11	-15.5 +/- 6.1	-24.8 - -5.1	153	6.0 +/- 6.5	-11.3 - 18.7
Heart rate									
Men [beats per minute]	1235	66.0 +/- 10.9	40 - 111	148	66.8 +/- 10.5	46 - 101	1383	64.9 +/- 10.2	40 - 107
Women [beats per minute]	1180	66.9 +/- 9.9	42 - 115	118	69.4 +/- 9.9	46 - 106	1298	65.8 +/- 9.7	42 - 108
Plasma viscosity									
Men [mPa s]	1235	1.257 +/- 0.064	1.09 - 1.53	148	1.267 +/- 0.071	1.11 - 1.46			
Women [mPa s]	1180	1.244 +/- 0.064	1.10 - 1.53	118	1.258 +/- 0.079	1.12 - 1.53			

[a] 24 hour averages (midnight to midnight)
[b] monitoring trends and determinants of cardiovascular disease
[c] Examinations on 144 days between October, 9 1984 and May, 24 1985
[d] Examinations on 158 days between October, 12 1987 to June, 24 1988
[e] January, 7 to January, 19 1985

Table 2: Associations between the air pollution episode and heart rate 1383 men and 1298 women from the MONICA survey 1984/85 and an re-examination 1987/88. Analyses for different subgroups.

	Men			women			men and women		
Mean heart rate	Beta [bpm]	95% CI		Mean heart rate	Beta [bpm]	95% CI	Beta [bpm]	95% CI	
All subjects									
65.5 bpm (100%)	1.38	-0.08	2.83	66.4 bpm (100%)	2.29	0.71	3.88	1.79	0.72	2.87
Persons without a self-reported history of MI									
65.5 bpm (95%)	1.19	-0.28	2.67	66.5 bpm (98%)	2.33	0.75	3.92	1.72	0.64	2.80
Non-medicated subjects									
65.3 bpm (80%)	1.60	0.06	3.14	66.5 bpm (74%)	1.75	0.01	3.50	1.67	0.51	2.82
Non-smokers									
64.4 bpm (61%)	1.75	-0.11	3.60	66.2 bpm (76%)	2.18	0.33	4.04	1.96	0.65	3.28
Persons living within the city-limits of Augsburg									
66.5 bpm (44%)	3.15	0.84	5.46	66.9 bpm (43%)	3.34	0.83	5.85	3.24	1.54	4.94
Persons with plasma viscosity below the 90th percentile									
65.0 bpm (90%)	1.01	-0.50	2.52	66.2 bpm (92%)	1.78	0.13	3.42	1.36	0.25	2.47
Persons with plasma viscosity above the 90th percentile									
70.3 bpm (10%)	4.75	0.50	8.99	69.3 bpm (8%)	5.55	1.24	9.87	5.14	2.12	8.17

Table 3: Prevalence of self-reported history of chronic diseases and EKG changes according to the Minnesota code. MONICA Project Augsburg: Survey 1984/85.

	Low heart rates and plasma viscosity		elevated heart rates only[a]		Elevated plasma viscosity only[b]		Elevated heart rates and plasma viscosity[a,b]	
	non-episode	episode	non-episode	episode	non-episode	episode	non-episode	episode
N	2000	207	215	20	165	26	34	13
Coronary artery disease	5.0%	3.9%	4.2%	0%	9.1%	0%	2.9%	15.4%
Any cardiovascular disease	6.9%	6.3%	7.9%	0%	14.5%	3.8%	5.9%	15.4%
Diabetes	1.8%	1.0%	4.2%	5.0%	6.7%	3.8%	8.8%	15.4%
Chronic bronchitis	3.5%	2.9%	4.2%	10.0%	6.7%	15.4%	14.7%	15.4%
Any of the diseases above	10.7%	9.7%	14.0%	15.0%	22.4%	19.2%	23.5%	38.5%
ST-Segment changes	5.2%	7.2%	7.4%	5.0%	12.7%	19.2%	5.9%	7.7%
Arrhythmia	7.4%	4.8%	6.5%	10.0%	4.2%	7.7%	8.8%	0%

[a] heart rates > 80 beats per minute.
[b] 90th percentile: plasma viscosity > 1.35 mPa·s in men and > 1.33 mPa·s in women.

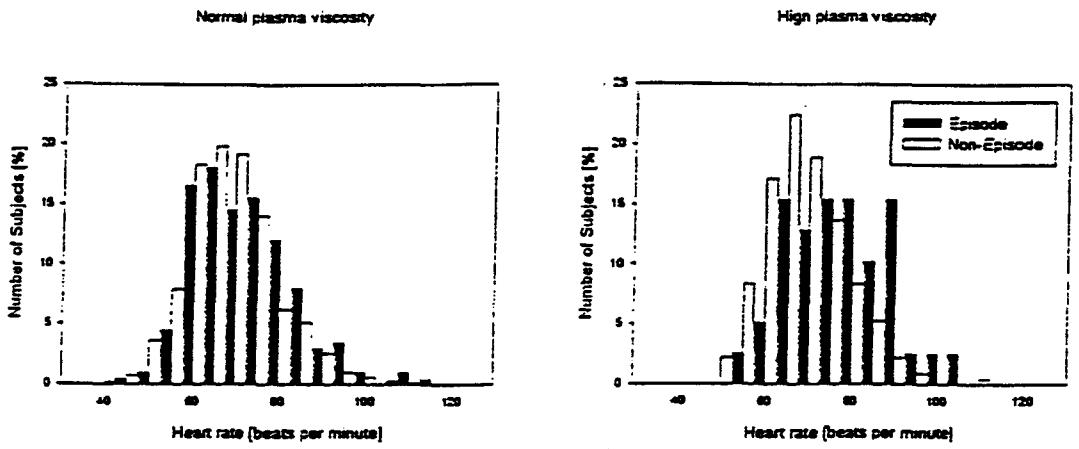

Figure 1: Distribution of heart rates in beats per minute for patients with normal and elevated levels of plasma viscosity for episode and non-episode days during the first MONICA survey Augsburg, 1984/85.

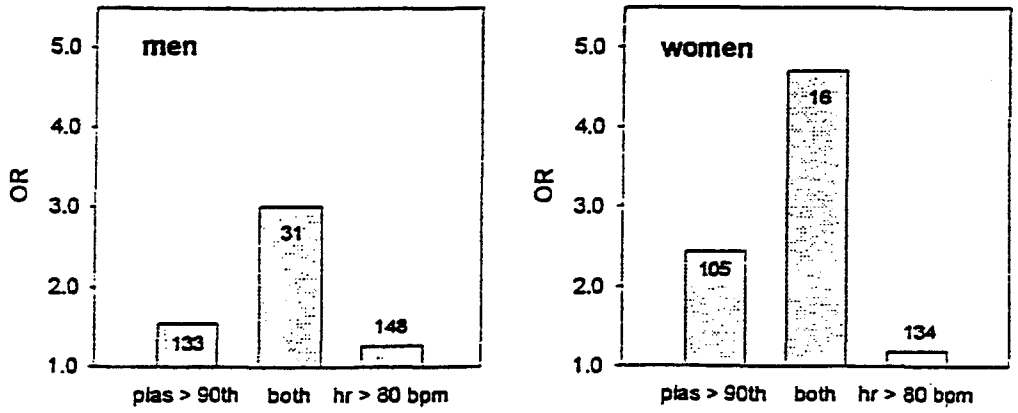

Figure 2: Crude odds ratios for the episode for having elevated plasma viscosity levels, elevated heart rates and both together in men and women.

Daily Respiratory Mortality And Air Pollution In Mexico City: The Importance Of Considering Place Of Death

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Abstract

We studied the association between particulate matter equal or less than 10μm (PM_{10}) and ozone ambient concentrations and the daily number of death from respiratory causes among elderly residents of Mexico City. Ambient air pollution data were provided by the monitoring network of the metropolitan area of Mexico City that is composed of 33 stations. During the study period (1994), PM_{10} daily average levels ranged from 23.4 to 175.3 $\mu g/m^3$, and ozone daily 1-hour maximums ranged from 39.4 to 216.7 ppb. We compiled information on primary causes of death as well as on secondary causes that may have led to death. The analyses were conducted separately according to the place of death (within or out of a hospital unit) using time-series methodology including Poisson regression and the iteratively weighted filtered least squares (IWFLS method). The total number of deaths from all respiratory causes and the mortality for chronic obstructive pulmonary diseases (COPD) were significantly related to PM_{10} over different lags. An increase of 30 $\mu g/m^3$ was related to a 8.7% (95% CI 2.7% to 15.1%) increase in the total number of deaths from all respiratory causes and to a 12.6% (95% CI 3.9% to 22.1%) increase in mortality for COPD with a 3-day lag when death occurred out of medical units. For deaths occurring in medical units, we observed a longer lag (5 days) and a smaller risk estimate. Ozone ambient levels were also related to deaths from respiratory causes, but results were inconsistent. Results also suggest an interactive effect between PM_{10} and ozone for deaths from respiratory causes. Focusing on sensitive populations, accounting for both primary and secondary causes of death, and considering place of death may reduce misclassification in epidemiological studies and provide more accurate estimates of the adverse impact of PM_{10} on mortality.

Introduction

A series of recent studies have reported significant associations between daily number of deaths and levels of ambient air pollutants, especially levels of particulate matter. These associations have been found across a wide range of air pollutant levels and weather patterns in western countries (1-6) as well as in some developing countries (7). However many factors, such as particulate composition, exposure pattern, simultaneous exposure to other pollutants, and underlying health status may affect the association and explain variations in the effects observed (1,5).

Although these reports indicate that exposure to particulate air pollution is related to short-term increases in mortality and morbidity, they provide limited information on mortality among more sensitive subgroups for specific causes of death (4). Furthermore, these reports consider only the primary cause of death and not the underlying associated causes; and such an omission could lead to underestimating the effect of air pollution on mortality. In addition, none of these reports considers the place of death which ultimately may help us understand the lag structure of the association between exposure to air pollution and human health.

In this study, we report the results of a time-series study conducted in Mexico City, Mexico, in which the association between mortality due to respiratory causes and air pollution was evaluated in an elderly population (65 years and older). We obtained information on primary and secondary causes of death as well as on the place of death. Because Mexico City experiences high levels of both PM_{10} and ozone, we were able to study the interactive effect of these pollutants on death from respiratory causes.

Material and Methods

Study Area

This study was carried out in Mexico City, a city of approximately 8,800,00 people from January 1 through December 31, 1994 (365 days). The study population comprised elderly people who died during the study period and who had lived in the Distrito Federal (DF) at the time of death.

Air Pollution

The air pollution and meteorological data were provided by the automatic network of ambient monitoring of Mexico City which comprised 33 stations; 22 are located within the DF, and 11 are located in surrounding areas of the city. These concentrations are measured in adherence with the US Environmental Protection Agency (EPA) standard methods.

On the basis of statistical criteria, as well as on the units in which norms are stated, we decided to use the maximum daily 1-hour levels for ozone and the 24–hour average for PM_{10}.

We estimated the mean population exposure by averaging the measurements provided by all monitors that recorded the corresponding pollutants. The mean correlation coefficients assessed between monitoring stations were 0.43 for PM_{10} and O.48 for O_3.

Mortality Data

The Directorate of Statistics and Computer Sciences, Ministry of Health in Mexico, provided the death certificates, which included individual information such as age, sex, county of residence, place of dead (in or out of a medical unit), and primary and secondary causes of death.
Incorporating the information on secondary causes of dea, may help us to understand better the relationship between air pollution and death due to respiratory causes.

The causes of death were classified in two groups according to the International Classification of Diseases, Ninth Revision (ICD-9): 1) total respiratory diseases, which includes chronic obstructive pulmonary disease (ICD-9 490-496), and lower respiratory infections (ICD-9 466, 480-487) and 2) the specific group for chronic obstructive pulmonary diseases (COPD). The outcome variables were defined as the daily number of deaths for each of these groups.

Statistical Analysis

We modeled the daily number of total respiratory deaths as well as the number of deaths due to COPD and their relationship with the daily levels of O_3 and PM_{10} using a time series analysis with Poisson

regression. To account for potential serial autocorrelation due to longitudinal data and overdispersion, we re-estimated the final models using the iteratively weighted and filtered least-squares method (IWFLS). Since the results we obtained with these models were very similar to those found using Poisson regression, we decided to present those obtained with the standard technique.
We adjusted for long-term trends by controlling for cold or warm months (October-January/February-September); we controlled for short-term trends by including in the model the minimum temperature on the day before and the day of death; in addition, we adjusted for place of death (in or out of a medical unit). Variables such as month of the study, relative humidity, day of the week, or holidays, were not statistically significant, and we therefore did not included them in the models.

Results

Of the 20, 669 deaths that occurred during the study period, 4,919 (23.8%) were attributed to respiratory causes; of these 2,294 (11.1%) were due to COPD. The average number of daily respiratory deaths was 13.5 and was similarly distributed in and out of medical facilities (7.2, 6.3 respectively). For COPD, the daily average number was 6.3 and was also similarly distributed in and out of medial units (3.1 and 3.2, respectively).

During the study period, O_3 levels exceeded the Mexican standard (110 ppb 1-h maximum) on 287 days (78.6%) and the Mexican standards for PM_{10} (150 $\mu g/m^3$ 24-h average) on 3 days. The 24-hour average PM_{10} levels exceeded 50 $\mu g/m^3$ on 85.2% of the days. The correlation between PM_{10} and O_3 ambient levels was 0.46. The highest concentrations of particulate matter, ranging from 35.9 to 175.3 $\mu g/m^3$, with a mean of 84.5 $\mu g/m^3$ were registered during the cold months; during the warm months, these levels ranged from 23.4 to 130.9 $\mu g/m^3$, with a mean of 70.3 $\mu g/m^3$. Ozone levels were relatively homogenous over the year. During the cold months, the levels ranged from 39.4 to 216.7 ppb with a mean of 139.5 ppb, while during the warm months, the levels ranged from 48.9 to 212.3 ppb with a mean of 131.9 ppb.

We observed an important impact of PM_{10} on respiratory mortality among elderly subjects residents of Mexico City. For deaths occurring out of medical units, we observed a 4.1% (95% CI: 1.3% to 6.9%) increase in mortality due to COPD that are associated with a 3-day lag increase of 10 $\mu g/m^3$ in the daily average level of PM_{10}. This estimate reached 6.1% (95% CI 2.4% to 9.9%) when we considered cumulative exposure over 5 days was considered. The effect was smaller and less consistent for deaths occurring within medical units.

The mortality pattern occurring out of a medical unit differed with that occurring in a medical unit in that we observed effects of lower magnitude and longer lag structures. We also observed a small increase in deaths due to respiratory causes associated with O_3 exposure. However, this effect was not consistent and was mostly present when exposure over several days was considered.

Because of the high correlation between PM_{10} and O_3 ambient levels, we studied the effect of PM_{10}, stratifying for days with high and low O_3 levels; the cut-off point used was the observed median (116.7 ppb). We observed no significant effect of PM_{10} during the days when O_3 levels were low; however, on those days when O_3 levels exceeded the cut-off point, we observed a significant effect of PM_{10} on the total number of respiratory deaths and death due to COPD that occurred both in and out of medical

units. This finding suggests that a synergic effect of PM_{10} and O_3 on respiratory mortality exists on days when ambient O_3 levels are high.

Another important factor to account for in estimating the impact of air pollution on health is the underlying cause of death. In our data, the impact of 30 μg/m3 (interquartile range) of PM_{10} on deaths for respiratory causes increased from 2% to 8.7% when secondary as well as primary causes of death were considered. For deaths due to COPD these estimates raised from 6.2% to 12.6%.

The lack of stratification by place of death may lead to an underestimation of the impact of air pollution on mortality and may also obscure the lag structure.

One of the major limitations of time-series studies is that exposure is estimated at the population level and is based on ambient monitoring networks. Because of the large potential for misclassifying exposure and outcomes, it has been argued that the effects observed in this type of study underestimate the true effect. Although in our study the estimation of exposure was based on data we obtained from ambient monitoring stations, focusing on a sensitive group, considering underlying as well as primary causes of death and accounting for place of death are likely to have decrease the possibility of misclassification and to have provided a more accurate estimate of the impact of air pollutants on deaths from respiratory causes.

Table 1. Regression coefficients and relative risk (RR) for total number of deaths from respiratory causes adjusted for minimum temperature and season, Mexico, DF, 1994.

			Outside medical unit			Inside medical unit		
Variables		Q3 – Q1*	RR+	95 % CI		RR+	95 % CI	
PM_{10} ($\mu g/m^3$)								
	lag 1	29.7	1.0742	1.0129	1.1392	1.0474	0.9913	1.1067
	lag 2	29.7	1.0817	1.0220	1.1448	1.0338	0.9801	1.0904
	lag 3	29.7	1.0874	1.0270	1.1514	1.0472	0.9924	1.1049
	lag 4	29.7	1.0777	1.0177	1.1412	1.0272	0.9735	1.0840
	lag 5	29.7	1.0609	1.0019	1.1235	1.0717	1.0160	1.1305
	avg. 3	27.4	1.1068	1.0391	1.1788	1.0557	0.9952	1.1198
	avg. 5	27.4	1.1198	1.0460	1.1989	1.0685	1.0028	1.1386
	avg. 7	27.4	1.1115	1.0348	1.1939	1.0780	1.0086	1.1522

			Outside medical unit			Inside medical unit		
Variables		Q3 – Q1*	RR+	95 % CI		RR+	95 % CI	
O3 (ppb)								
	lag 1	39.9	1.0173	0.9673	1.0698	1.0377	0.9900	1.0878
	lag 2	39.9	1.0557	1.0048	1.1092	1.0164	0.9708	1.0641
	lag 3	39.9	1.0358	0.9863	1.0879	0.9978	0.9533	1.0443
	lag 4	39.9	1.0296	0.9803	1.0814	1.0315	0.9851	1.0800
	lag 5	39.9	1.0403	0.9899	1.0932	1.0227	0.9766	1.0711
	avg. 3	31.1	1.0612	1.0028	1.1229	1.0293	0.9766	1.0849
	avg. 5	31.1	1.1075	1.0317	1.1887	1.0530	0.9861	1.1244
	avg. 7	31.1	1.0779	0.9924	1.1708	1.0686	0.9896	1.1538

* Q3 – Q1: Interquartile range

+ Relative risk was computed for an increase equivalent to the interquartile range: RR = exp(beta*)q3-q1)

Table 2. Regression coefficients and relative risk (RR) for mortality from chronic obstructive lung diseases adjusted for minimum temperature and season, Mexico, DF, 1994.

Variables		Q3 – Q1*	Outside medical unit RR+	95 % CI		Inside medical unit RR+	95 % CI	
PM_{10} ($\mu g/m^3$)								
	lag 1	29.7	1.0901	1.0036	1.1841	1.0572	0.9726	1.1492
	lag 2	29.7	1.1247	1.0384	1.2181	1.0577	0.9755	1.1468
	lag 3	29.7	1.1259	1.0385	1.2205	1.0834	0.9987	1.1754
	lag 4	29.7	1.1078	1.0218	1.2010	1.0758	0.9915	1.1673
	lag 5	29.7	1.1043	1.0188	1.1970	1.1010	1.0153	1.1939
	avg. 3	27.4	1.1504	1.0526	1.2573	1.0855	0.9925	1.1873
	avg. 5	27.4	1.1757	1.0677	1.2946	1.1156	1.0128	1.2289
	avg. 7	27.4	1.1598	1.0485	1.2829	1.1199	1.0120	1.2392

Variables		Q3 – Q1*	Outside medical unit RR+	95 % CI		Inside medical unit RR+	95 % CI	
O3 (ppb)								
	lag 1	39.9	1.0418	0.9703	1.1186	1.0457	0.9736	1.1232
	lag 2	39.9	1.0451	0.9749	1.1203	0.9903	0.9239	1.0615
	lag 3	39.9	1.0824	1.0095	1.1604	1.0176	0.9493	1.0907
	lag 4	39.9	1.0017	0.9350	1.0731	1.0557	0.9842	1.1324
	lag 5	39.9	1.0160	0.9475	1.0894	1.0743	1.0009	1.1532
	avg. 3	31.1	1.0981	1.0136	1.1897	1.0313	0.9521	1.1172
	avg. 5	31.1	1.1190	1.0311	1.2144	1.1039	0.9985	1.2203
	avg. 7	31.1	1.0914	0.9712	1.2265	1.1244	1.0003	1.2640

* Q3 – Q1: Interquartile range

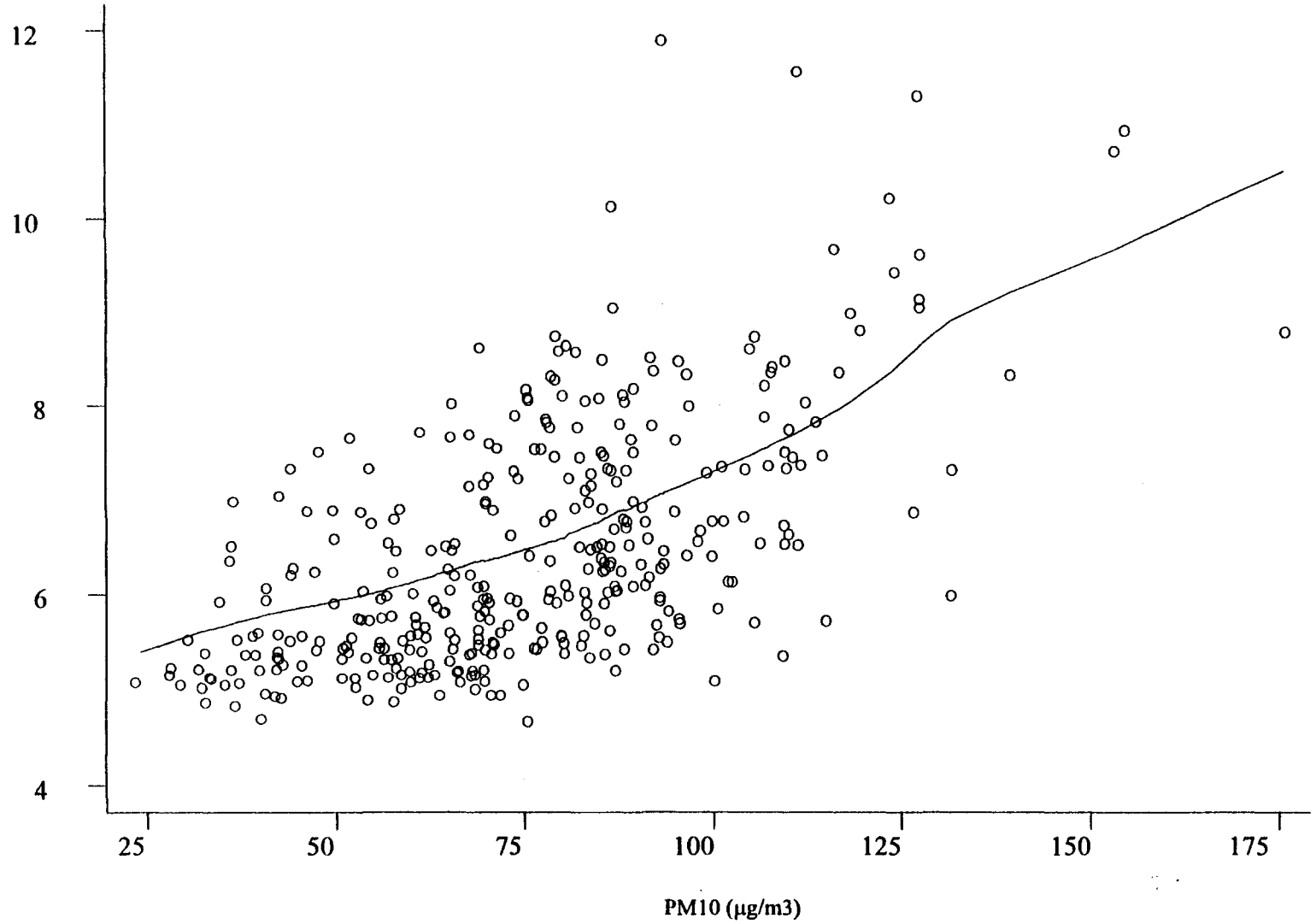
+ Relative risk was computed for an increase equivalent to the interquartile range: RR = exp(beta*)q3-q1)

Table 3. PM_{10} effect on daily mortality stratified for ozone levels. México, DF, 1994

Total respiratory mortality							
		Upper ozone levels *			Lower ozone levels *		
		RR +	95 % CI		RR +	95% CI	
Outside medical unit	PM_{10} (avg. 5 days)	1.164	1.066	1.271	1.034	0.892	1.198
Inside medical unit	PM_{10} (avg. 7 days)	1.095	1.005	1.193	1.032	0.893	1.191
Chronic obstructive lung diseases (COPD)							
		Upper ozone levels *			Lower ozone levels *		
		RR +	95 % CI		RR +	95% CI	
Outside medical unit	PM_{10} (avg. 5 day)	1.219	1.078	1.379	1.084	0.875	1.342
Inside medical unit	PM_{10} (avg. 7 days)	1.179	1.035	1.343	0.935	0.744	1.175

+ using as cut-off point the median ozone level (116.7 ppb)
* Relative risk was computed for a 30 μg/m3 increase in PM_{10}

Figure 1. Smooth function of the number of daily deads by total respiratory cuases outside a medical unit and PM10 levels with a 3 days lag. Mexico, D.F., 1994.



Short-Term, Low-Dose Inhalation Of Ambient Particulate Matter Exacerbates Ongoing Pneumococcal Infections In *Streptococcus Pneumoniae*-Infected Rats

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Introduction

Infection, specifically pneumonia, contributes substantially to the mortality among elderly individuals exposed to airborne particulate matter (PM) and disproportionate increases in deaths due to pneumonia have been observed immediately or just following even moderate episodes of particulate air pollution (Ware et al., 1986; Pope, 1991; Schwartz, 1994). These epidemiological findings suggest that PM may act as an immunosuppressive factor that can undermine the normal pulmonary immune response. Thus, given that older individuals with chronic respiratory disease are not only at increased risk of pneumonia but also are less likely to recover from infections (Vial et al., 1984; Wilson, 1996), alterations in the pulmonary immune system may well play a role in the observed increase in mortality following PM episodes.

Toxicological studies demonstrating the immunosuppressive potential of particles in the lungs strengthen the epidemiological observations and support our proposed hypothesis that compromised pulmonary host immunocompetence and immune defense mechanisms important for resistance against *Streptococcus pneumoniae* infections contribute to the observed increase in particle-induced mortality in elderly individuals. Studies using rodent models have clearly demonstrated that exposure to inhaled particles (alone or in combination with gaseous air pollutants) can compromise pulmonary host resistance against microbial infections and/or alter specific immune mechanisms important for anti-bacterial defense. For example, Aranyi et al. (1983) demonstrated that intratracheal-instillation of mice with either quartz, ferric oxide (a constituent of PM), calcium carbonate, or sodium feldspar particles at 10, 33, or 100 mg/mouse increased mortality from subsequent *S. pneumoniae* infection. In other bacterial infectivity studies, instillation of aged urban air particles (0.4 μm, MMAD) and/or coal fly ash particles (0.9 μm, MMAD) reduced the resistance of mice to bacterial infection (Hatch et al., 1985). Jakab (1992) demonstrated that mice co-exposed to carbon black (10 mg/m^3, 4 hr/d for 4 d) and acrolein (2.5 ppm), followed by a bacterial challenge, had suppressed intrapulmonary killing of *Staphylococcus aureus*, impaired elimination of *Listeria monocytogenes* and influenza A virus, and altered intrapulmonary killing of *Proteus mirabilis*.

While it has been demonstrated that the elderly with pre-existing cardiopulmonary disease and/or pneumonia appear to be at higher risk from the adverse effects of PM than healthy individuals, considerable uncertainty still remains about specific biological mechanisms that might underlie this effect. The current studies in which previously-infected *S. pneumoniae*-infected rats were

exposed to concentrated PM 2.5 (at a level at or just above the $PM_{2.5}$ National Ambient Air Quality Standard [NAAQS]) from New York City air were undertaken to determine whether acute exposure (i.e., 5 hr) to PM induces immunological alterations within the lungs that could exacerbate ongoing *S. pneumoniae* infections and, thus, contribute to the epidemiologic observations of PM-related increases in host mortality in the elderly. Investigations demonstrated that while a single 3 hr PM exposure of normal healthy rats (at concentrations as high as 750 $\mu g/m^3$) had only slight effects upon host immunocompetence (Li et al., 1997), a slightly-longer exposure of rats previously-infected with *S. pneumoniae*: increased pulmonary bacterial burdens and the incidence of bacteremia; enhanced the extent and severity of pneumoniae-associated lung lesions; decreased levels of lavageable neutrophils and pro-inflammatory cytokines; and reduced the amounts of bronchus associated lymphoid tissue (BALT) compared to those in bacterially-infected filtered air-exposed control animals (Zelikoff et al. 1999). While all of the previously-cited PM-induced effects were reported at the Colloquium, only those data demonstrating PM-induced changes in pulmonary bacterial burdens, lavageable lung cell values, and BALT are presented in these Proceedings.

Methods

Experimental Design. Rats were infected by intratracheal (IT)-instillation with approximately 2-4 x 10^7 encapsulated *S. pneumoniae* (Type 3). Forty-eight hr following infection, rats were exposed by inhalation (nose-only) for 5 hr to either clean-filtered (unconcentrated) New York City (NYC) air or to particulate matter (PM < 2.5) concentrated ~10-fold with a modified Gerber Centrifugal Concentrator (Gordon et al., 1999). Following PM exposure, rats (3-4 per group) were sacrificed at different time-points post-exposure (depending upon the specific endpoint examined) and the lungs either: homogenized for determination of the effects of PM exposure on pulmonary bacterial burdens; lavaged for cytokine measurements identification of cell profiles; or, formalin-fixed for histologic examination.

Animals. Seven-mo-old male Fisher 344 rats (Harlan Sprague Dawley, Indianapolis, IN) were quarantined for 1 wk prior to infection. Rats were housed individually in stainless steel cages in temperature (20°C)- and humidity (50% RH)-controlled rooms, and provided food and water *ad libitum*.

Generation and Characterization of PM. The methodology for the concentration and delivery of particles from the ambient Manhattan atmosphere, as well as the inhalation system, is the same as that described previously by Gordon et al. (1999). Animals were exposed on the 8th floor of the Public Health Building in NYC. The 10 L/min output of the concentrator passed through sulfur dioxide (SO_2) and ozone (O_3) denuders before delivery to the nose-only exposure port; control animals were exposed to ambient NYC air which was conditioned to remove particles (HEPA filter), as well as SO_2 and O_3. Ground level ambient PM was sampled previously for comparison with the ambient PM entrained at the 8th floor level and no differences in particle composition were noted (other than differences in non-respirable road dust). The exposure atmospheres and the ambient atmosphere were continuously monitored using a condensation particle counter (TS) and a Real-time Aerosol Monitor (GCA). In addition, filter samples were collected on Teflon membrane filters to measure integrated exposure mass concentrations

(gravimetric), trace metals (XRF analyses), strong acidity, and sulfate and nitrate concentrations (ion chromatography).

Bronchopulmonary Lavage and Cell Collection. Rats, euthanized by injection of sodium pentobarbital, were lavaged by washing the lungs *in situ* twice with Ca^{2+}-Mg^{2+}-free phosphate-buffered saline (PBS, pH 7.2; 7 ml/instillation) according to the method described by Cohen et al. (1996). Aliquots of acellular lavage fluid were used to evaluate lactate dehydrogenase, total protein, and cytokine levels. Cell numbers and viability were determined by hemocytometer counting and trypan blue exclusion, respectively; recovered cell types were characterized by differential counting.

Bacterial Culture and Pulmonary Clearance. Viable cultures of encapsulated *S. pneumoniae* (type 3) were maintained on blood agar plates. Six days prior to instillation, cultures were initiated by inoculation into Todd-Hewitt broth (THB) and maintained at 37°C in a 5% CO_2 atmosphere. The cultures were passaged at 12 hr intervals thereafter; this protocol has been shown in our laboratory to maintain the virulence and capsulated nature of the organism (capsulation confirmed by the Quelling reaction). On the day of instillation, the concentration of *S. pneumoniae* was spectrophometrically determined using an absorbance calibration curve previously prepared at 540 nm (Cohen et al., 1986) and a suspension diluted with PBS to a suitable concentration for delivery of 2-4 x 10^7 organisms per instillate.

To monitor bacterial burdens in the lungs of control and PM-exposed infected rats at 6, 9, 13.5, 18, 24, and 48 hr post-exposure, the lungs were removed after sacrifice, weighed, and homogenized (Cohen et al., 1989). To obtain estimates of total remaining viable organisms, aliquots of the homogenate were serially-diluted and plated onto triplicate sheep blood agar plates for a 24 hr incubation at 37°C (5% CO_2). Both the absolute levels of bacteria and the levels of bacteria/gram lung (as compared with the initial burden) served as indicators of bacterial survival in the host lung, and bacterial clearance kinetics were calculated from the changes in total bacteria per unit time post-infection. The initial (t_0) burden was assessed in 3 naive rats sacrificed immediately after bacterial instillation; an estimate of actual lung bacterial burdens at the initiation of each exposure was determined in 3 infected rats sacrificed immediately prior to PM exposure.

Histopathology. At the time of sacrifice, a single lung lobe was fixed with neutral buffered-formalin instilled via the trachea. The left upper lobe was then sliced along the axis of the main airway, embedded in paraffin, and 5 μm thick lung sections prepared and stained with Hematoxylin and Eosin. The area of BALT (per unit length of airway) was determined from 5 - 8 slides per animal using computer-assisted image analysis; a total of 10 low magnification fields were sampled per section. An Olympus microscope equipped with a Dage MTI camera was used to transmit the microscope image to a Macintosh II computer. Cross-sectional area of BALT and airway length were determined using National Institute of Health Image software. From these measurements, the cross-sectional area of BALT per airway (in pixels) for each slide was calculated using the following formula: area of BALT per airway/area of BALT per total length of airway.

Statistical Analyses. The effects of exposure to PM (exposed vs. air control) and post-exposure duration were analyzed using 2-way ANOVA. A statistically-significant main effect for exposure indicated that the exposure influenced the outcome being tested. For outcomes such as bacterial clearance which was measured on a percent scale, arcsine transformation was considered prior to analysis. Differences were considered significant when probability (p) values were < 0.05.

Results

Effects of Inhaled PM on Lavage Cell Profile. Results in Table 1 demonstrate that instillation of unexposed control animals with 2-4 x 10^7 *S. pneumoniae* evokes a dramatic inflammatory response (as marked by polymorphonuclear leukocyte [PMN] influx) that is notable even 3 d after infection (i.e., 24 hr post-exposure). Lavaged PMN values waned with increasing post-infection time, but still remained well-above those levels found in uninfected rats (data not shown; 0.5 - 1.5% of the lavaged cell population are PMN) even 7 d after instillation. A single 5 hr exposure of *S. pneumoniae*-infected rats to $PM_{2.5}$ (48 hr following infection) at a dose equivalent to the NAAQS for $PM_{2.5}$ (i.e., 65 $\mu g/m^3$) significantly reduced (compared to time-matched infected, filtered-air-exposed control) the percentages of lavageable PMN in the absence of any changes in lymphocyte or monocyte values. PMN levels were reduced by 63% as early as 24 hr post-exposure and remained well-below their time-matched air control counterpart values for up to 3 d. Even at 5 d post-PM exposure, PMN levels in the lungs were still below those of the filtered air control, although the differences failed to reach statistical significance ($p < 0.06$). In these same studies, macrophage values significantly increased compared to control values.

Table 1. Effects of Inhaled PM Upon the Percentage of Lavaged Cells Recovered From Hosts Previously-Infected with *S. pneumoniae*

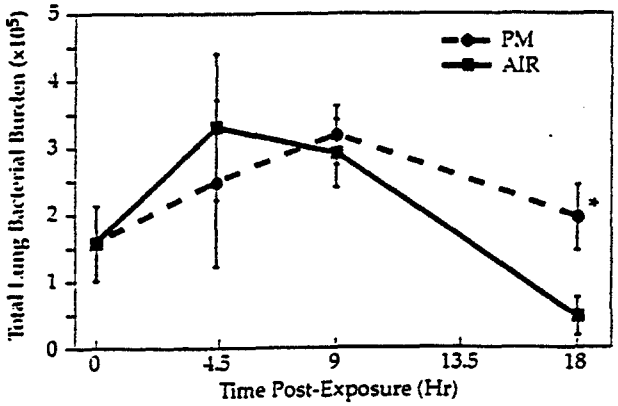
Treatment	Time Post-Exposure (hr)	Cell Type (%)[a]			
		PMN	Lymphocyte	Monocyte	Ma
Filtered Air	24	37.0 ± 2.0	11.0 ± 0.6	1.0 ± 0.9	49.0 ± 1.0
	48	20.0 ± 7.0	17.0 ± 4.0	0.7 ± 0.4	71.0 ± 2.0
	72	19.0 ± 2.0	8.0 ± 0.4	1.0 ± 0.2	72.0 ± 2.0
	120	9.0 ± 2.0	8.0 ± 0.7	0.5 ± 0.3	83.0 ± 2.0
PM	24	14.0 ± 1.0*	12.0 ± 1.0	0.3 ± 0.1	69.0 ± 4.0**
	48	9.0 ± 2.0*	11.0 ± 1.0	0.5 ± 0.3	78.0 ± 1.0**
	72	10.0 ± 0.3*	8.0 ± 2.0	0.7 ± 0.2	82.0 ± 2.0**
	120	3.0 ± 0.8	7.0 ± 0.7	0.1 ± 0.1	89.0 ± 0.7***

Significantly different from time-matched air control group ($p < 0.01$, **$p < 0.03$, or ***$p < 0.05$)
[a]Mean ± SEM (n = 3-4 rats/exposure group)

Effects of Inhaled PM on Pulmonary Bacterial Burdens. In these studies, lung burdens of *S. pneumoniae* were determined from two studies (exposure concentrations between 90 and 150 $\mu g/m^3$) using already-infected rats sacrificed either immediately, 4.5, 9, 18, 24, 72, or 120 hr following PM exposure. In the first study in which rats were sacrificed either 24, 72, or 120 hr post-PM exposure, results demonstrated that while bacterial burdens decreased in both the air

and PM-exposed animals with increasing time post-infection, rats exposed to PM demonstrated substantially greater (i.e., 70%) bacterial burdens at 24 hr post-exposure compared to that measured in the filtered-air control rats; at the later post-exposure timepoints (i.e., 72 and 120 hr), the total number of bacteria/g lung were still above that measured in the control lungs, but the results were less dramatic than that observed at 24 hr (data not shown). Therefore, to determine that particular timepoint at which post-exposure clearance kinetic patterns first became divergent and to examine whether 24 hr represented the time of maximum difference between the groups, infected animals were sacrificed at post-exposure timepoints earlier than 24 hr (i.e., 4.5, 9, and 18 hr) and effects upon pulmonary bacterial burdens determined (Figure 1). Results of these latter studies demonstrated that while numbers of pulmonary bacteria were approximately equal in the two exposure groups at the earliest post-exposure timepoint (at 4.5 hr post-exposure burdens were ~25% lower in the PM-exposed group compared to control values), bacterial burdens in the PM-exposed animals were ~10% above those measured in the filtered-air control animals by 9 hr post-exposure, and, by 18 hr, bacterial burdens were elevated by >300%.

Figure 1



*Significantly different from filtered-air control (p<0.01)

Effects of Inhaled PM on Bronchus Associated Lymphoid Tissue (BALT). Histological analyses of *S. pneumoniae*-infected animals exposed for 5 hr to PM at ~65 $\mu g/m^3$ 48 hr following infection revealed a dramatic decrease (compared to its time-matched filtered-air control) in the amount of BALT in rats examined 24 and 48 hr post-exposure (Figures 2A and B). While infection of unexposed control animals with *S. pneumoniae* resulted in increased amounts of BALT 24 and 48 hr following exposure (72 and 96 hr post-infection), exposure to PM reduced the amount of BALT (compared to infected unexposed controls) by 52 and 73%, respectively (Figure 2B). Moreover, while the amount of BALT in filtered-air exposed rats increased 1.7-fold over time, BALT levels in PM-exposed animals remained unchanged over the same 24 hr time period.

Figure 2A

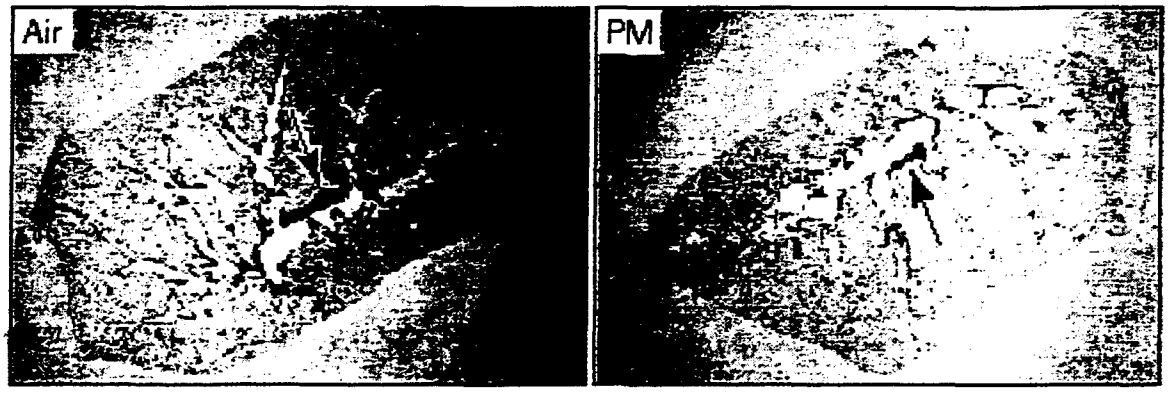
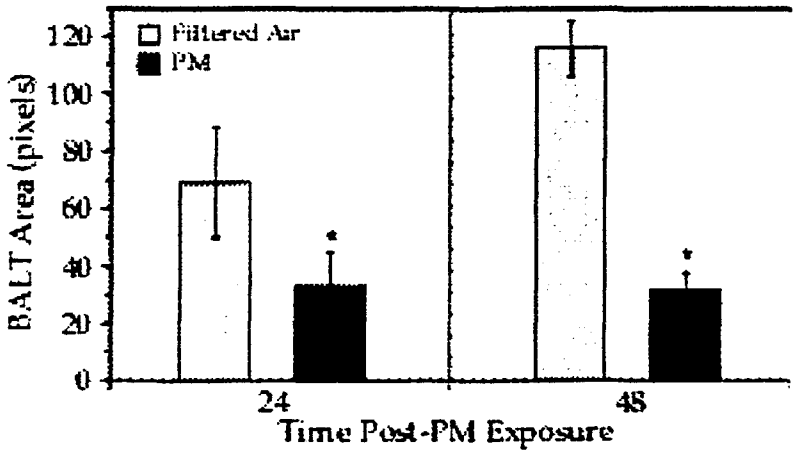


Figure 2B



*Significantly different from filtered-air controls ($p<0.01$)

Discussion

Results of this study have demonstrated that exposure of *S. pneumoniae*-infected rats to concentrated NYC PM, at a concentration equal to or somewhat greater than the 24 hr NAAQS of 65 $\mu g/m^3$, reduces lavageable numbers of PMN, increases pulmonary bacterial burdens, and decreases the amount of BALT (compared to those values in infected, filtered-air exposed controls).

While no definitive conclusions can be reached at this time regarding the mechanism(s) by which PM may be producing the aforementioned effects, compromised pulmonary immune defense mechanisms important for the removal/killing of *S. pneumoniae* (i.e., PMN, extracellular factors) or increased bacterial survival as a result of PM-induced changes in the lung mileu are two plausible hypotheses by which PM exposure might have acted to increase pulmonary bacterial burdens. Given that the PMN, in the presence of opsonins, is the principal immune cell type responsible for killing/removing *S. pneumoniae* from the lungs of infected rodents and humans (Coonrod and Yoneda, 1981; Lister et al., 1993), a decrease in this particular cell type, as seen in these studies could prolong/exacerbate an ongoing pneumococcal infection which could, ultimately, contribute to the observed increase in mortality associated with PM exposure. Of further interest is that the time course in which the observed effects occurred in this study "fits" with the epidemiological data which indicates that death of exposed individuals occurs relatively quickly following a PM episode and that older individuals with chronic respiratory disease are less likely to recover from pulmonary infections. Whatever the mechanism, if, as shown in these experimental studies, inhalation of PM reduces the rate at which *S. pneumoniae* is cleared from the lungs of exposed elderly individuals, a situation would be created, particularly in individuals with pre-existing disease, that could readily contribute to the previously-documented increase in pneumonia-related deaths among PM-exposed individuals.

These studies also noted a reduction in the amount of BALT formed in response to *S. pneumoniae* infection in PM-exposed animals. Because of the importance of this lymphoid tissue in the production of opsonizing- and anti-capsular antibodies essential for the killing of *S. pneumoniae* by PMN, a decrease in the amount of BALT might suggest an inability of the host to adequately "handle" pulmonary infections. While the importance of BALT in the human disease/infection process is still somewhat controversial, results from these studies are worth pursuing given the: increasing evidence which supports the presence of BALT in humans under conditions of disease and microbial stimuli (Koss, 1995; Pabst and Tschernig, 1995; Tschernig et al., 1995); the ability of low-dose PM exposure to reduce its formation in *S. pneumoniae*-infected hosts; and, the fact that changes observed in BALT may help define the mechanism(s) by which PM-induced effects upon bacterial burdens may have occurred.

Taken together, findings from these studies support the notion that a single low-dose exposure to PM can exacerbate pre-existing *S. pneumonia* infections possibly by suppressing immune functions important for host resistance against infectious pneumonia-producing bacteria. These changes, in turn, could potentially contribute to the increased incidence of pneumonia-related deaths observed in PM-exposed elderly individuals. However, while the aforementioned findings in 7-mo-old rats are compelling, many questions still remain unanswered including: whether elderly rats (and, possibly, elderly individuals) are at greater risk than younger animals for the immunosuppressive effects of PM; or if the stage of infection at which exposure to PM occurs influences the extent to which changes in the disease process arise. These and related questions must be answered in order to better understand the mechanisms by which PM may act to increase mortality in exposed-elderly individuals.

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IX. SATELLITE MEETING REPORT

Risk Assessment and Risk Management of Ambient Air PM

Summary of a pre-colloquium workshop on scientific considerations for standard setting and targeted control policy

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Short-term and long-term exposures to respirable ambient air particles (*particulate matter*, PM_{10}) have been associated in numerous studies with a serious threat to human health in populations throughout the world. Though the relative risk may be perceived as relatively small at current exposure levels in most developed countries, current PM exposures appear to present a serious public health problem because of the large number of people exposed, the severity of the health outcomes (including mortality and morbidity), and the existence of specific subpopulations who are at increased risk. Despite the strength of the epidemiological evidence linking health risks with PM exposures, important uncertainties remain. The characteristics of PM, including particle size and chemical or biological components, and their respective sources, that might be most responsible for the health effects are marginally known and plausible biological mechanisms underlying these adverse health effects are just beginning to be identified and evaluated. In addition, there are substantial knowledge gaps in source-receptor relationships and characterization of actual human exposures to PM, the role of co-pollutants in producing toxicity, and the elucidation of factors enhancing susceptibility to PM toxicity.

Recently, National Ambient Air Quality Standards (USA) and Air Quality Limit Values (EU) for particulate air pollution have been established. These values apply to the mass concentrations of particles with aerodynamic diameters smaller than or equal to 2.5 μm ($PM_{2.5}$, USA only) and 10 μm (PM_{10}). These new primary standards and limit values are intended to provide increased protection against a wide range of PM-associated health effects. The Table shown below summarises the various new annual and daily mean air quality standards and limit values and the statistical form for determining compliance.

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[1]**Disclaimer: This manuscript has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency.**

Particulate Matter National Ambient Air Quality Standards (USA) and Air Quality Limit Values (EU) (simplified)

		Annual mean		Daily mean	
USA	PM_{10}	50	3-y average of annual mean concentrations (2012*)	150	3-y average of the 99th-percentile of 24-h mean concentrations (2012)
	$PM_{2.5}$	15	3-y average of annual mean concentrations (2012)	65	3-y average of the 98th-percentile of 24-h mean concentrations (2012)
EU	PM_{10}	40 20	(2005) (2010)	50	exceedance -- 35/year (2005) exceedance -- 7/year (2010)

* Years in which standards and limit values have to be effective.
Mean values expressed as mass concentrations in $\mu g/m^3$.

The form of the 24-hour EU limit values and USA primary standards differ (i.e., number of exceedances - versus concentration-based percentile form), precluding direct comparison. It is difficult to compare the relative stringency of the US and EU standards, especially in 2005, because of the difference in form and indicator. By comparison, 35 exceedances of 50 $\mu g/m^3$ (the EU form of daily mean values) is close to the 90th percentile of 24-hour mean concentrations in the US, and 7 exceedances is close to the 98th percentile. The relative stringency depends in part on the fraction of PM_{10} that is made up of $PM_{2.5}$; in the USA with perhaps 60-90% of PM_{10} comprised of $PM_{2.5}$ the $PM_{2.5}$ primary standard is anticipated in most areas to be the controlling standard. These PM standards will be reviewed in 2002 (USA) and 2003 (EU) following a critical review of data from new studies on exposure, air quality, emission and source apportionment, PM toxicity and adverse health effects. In particular, in 2003 the EU will also consider whether the EU PM Daughter Directive should also be adjusted to control for the fine fraction of PM_{10}, i.e. $PM_{2.5}$, or a source-related PM fraction, such as traffic. In addition, the current PM_{10} EU limit values may result in the need in the EU for reductions in emissions from various sources to a much greater extent than formerly anticipated.

The scientific and technical uncertainties complicate health risk assessment and standard setting as well as make difficult identifying the most cost-effective emission and risk control strategies, because such strategies should be ideally targeted on responsible components related to the most important sources and emissions. In reality, policy makers cannot wait interminably for all information to be available, but move toward the ideal as new information is obtained. For a targeted control policy focused on critical sources and effective risk reduction, new data should be extracted from studies on PM health effects, toxicity, exposure, air quality, emissions and sources. Source-effect chains should be carefully analyzed and modeled to quantitatively link emissions and effects to evaluate alternative control strategies.

The aim of this workshop on "**Risk Assessment and Risk Management of Ambient Air PM - *Scientific considerations for standard setting and targeted control policy*"**, held in Durham at June 5, 1999 and organized preceding the "Third Colloquium on Particulate Air Pollution and Human Health", had the aim to bring individuals together who work in the science-policy interface to:

- discuss the scientific and regulatory uncertainties in health risk assessment and standard setting of ambient air PM
- provide a forum for regulators, local and national authorities, scientists, representatives from industry, NGO's, parliaments, and media
- provide a conceptual framework to quantitatively model source-effect chains that are most relevant to PM regulatory needs
- gain perspective on priorities for reducing uncertainties in risk assessment and management from risk assessors and risk managers

The programme was as follows:

Keynote speakers:

John Bachmann (US EPA)

"The long and winding road - science, policy, and particles"

Eltjo Buringh (RIVM)

"What does a PM risk manager want to know about PM risk assessment?"

Perspective speakers:

Ron White (American Lung Association)
Mark Saperstein (ARCO)
Daniel Krewski (University of Ottawa)
Marco Martuzzi (WHO EU Center for Environment and Health)

Open Discussion among speakers and participants

During the workshop, attended by 60 participants, issues related to PM science, policy, and their interface were presented and discussed. The type of relevant issues included:

1. How much certainty can scientists offer (or can regulators accept) for a targeted but "no-regrets" control policy and standard setting of ambient PM?

2. PM health effects appear associated with a number of exposure variables (NO_2, SO_2, CO, O_3). Are some more important than others related to health effects?

3. Should PM risk management be served with options for science-based, cost-effective health risk reduction, based on quantitative source-risk chain analyses and on exposure to critical fractions and components?

4. Should regulation and control of ambient PM be targeted on the impact of short-term or long-term health effects and will a preference for one of these set guidance to a specific control policy?

5. Are scientific and/or regulatory aspects involved in the differences in PM standard setting between USA and EU?

6. Are health effects expected to occur at PM levels below the new standards and if so, are they acceptable from the public health point of view? How shall we deal with serious health effects which seem to have no threshold?

7. PM appears to make us change from a "one-pollutant-at-the-time" strategy to control of mixtures. What are the impediments to creating more comprehensive, multipollutant air quality management approaches? Should barriers to more coherent management be eliminated, and if so, is there a role for science?

8. With decades of control program history behind us, what do we have to show as benefits that outweigh the cost to society of emissions controls? Can we prove that science and policy are effective?

The keynote and perspective speakers provided a stimulating range of views, with the challenges presented to all communities by the science/policy interface well recognized by the participants. The foremost workshop objective, to stimulate discussion focused on the science and policy interface and interplay with a large number of highly interested and qualified people, was met. Further, the opportunity for forthright communication that this workshop provided can serve as a model for future interface among diverse stakeholders on issues of great public health importance.

The organizers of this workshop gratefully acknowledge the financial support from the US EPA and the RIVM.

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X. EVALUATIONS

Participants were encouraged to evaluate the Colloquium and to suggest how future meetings might be improved. Seventy-eight forms were turned in and analyzed. The numerical rating scale used was: 1 = "poor;" 2= "fair;" 3 = " average;" 4 = "good;" and 5 = "excellent." The responses were as follows (half scores were rounded down).

- Adequacy of Facility.

Score	Number	
1	0	
2	1	
3	12	average = 4.2
4	34	
5	30	

- Completeness of Program.

Score	Number	
1	0	
2	3	
3	12	average = 4.0
4	46	
5	16	

- Quality of Program.

Score	Number	
1	0	
2	1	
3	10	average = 4.2
4	39	
5	28	

- Accommodations

Score	Number	
1	2	
2	5	
3	10	average = 3.8
4	28	
5	19	

- Staff
  (Note: The only negative comments related to hotel, not Colloquium staff.)

Score	Number	
1	1	
2	1	
3	8	average = 4.3
4	30	
5	33	

- Discussions

Score	Number	
1	2	
2	3	
3	18	average = 3.9
4	35	
5	19	

- Overall Evaluation

Score	Number	
1	0	
2	1	
3	7	average = 4.2
4	49	
5	21	

Questions: Would you like to attend a fourth conference?

Yes	77
No	0

If so, when?

	Number	
in 1 year	11	
in 2 years	42	average = 2.2 years
in 3 years	26	
in 4 years	2	

And where? (Locations with 2 or more responses are listed exclusively.)

Where	Number
West Coast	9
Not Durham	6
Durham	5
A big city with	5
direct flights	5
Anywhere	4
USA	4
Europe	4
Midwest	4
Boston	3
Seattle	3
Washington, DC	3
Northeast	2
California	2
Colorado	2
a resort	2
Chicago	2
Los Angeles	2
San Diego	2

Other suggestions and comments were solicited. Those with 3 or more similar ideas were as follows:

1.	Have more time/emphasis on the posters	n = 18
2.	Excellent/Good/Fine as is, don't change	n = 18
3.	Add breakout groups/some parallel sessions	n = 6
4.	Add a policy issues session	n = 5
5.	Find a more convenient location	n = 5
6.	Involve additional disciplines	n = 3
7.	Emphasize new research findings more	n = 3
8.	Have more invited talks	n = 3
9.	Have more integrative/big picture talks	n = 3
10.	Mail the abstracts out earlier	n = 3
11	Improve the audiovisual aspects	n = 3

Many suggestions involved more emphasis on a particular discipline or topic (e.g. statistical methodology, air chemistry, risk assessment and cardiovascular disease). There were numerous miscellaneous suggestions such as including exhibits, tutorials, and a more diverse demographic mix (women especially). Some suggestions conflicted (e.g. "have more speakers"; "have fewer speakers"). As in the past, all of the comments and suggestions are examined in order to improve future colloquia, should they occur.

XI. ABSTRACTS (By Session Number)

Session 1. Exposure/Characterization Related

001 DEVELOMENT OF A CONTINUOUS MONITORING SYSTEM FOR PM_{10} AND COMPONENTS OF $PM_{2.5}$.

M Lippmann, J Q Xiong and W Lei. Dept. of Environmental Medicine, New York University, Tuxedo, NY.

We have assembled, tested, and will be validating an ambient particulate matter (PM) monitoring package. It will provide records of concentration data as a function of time for thoracic particles (PM_{10}) and eight of its components, including coarse mode particles (PM_{10}-$PM_{2.5}$), fine particles ($PM_{2.5}$), ultrafine particles ($PM_{0.15}$) and the constituents of the accumulation mode aerosol ($PM_{2.5}$-$PM_{0.15}$) of primary interest, including sulfate ($SO_4^=$), nitrate (NO_3^-), ammonium (NH_4^+), hydrogen (H^+), and particle associated water (H_2O). For aerosol sulfur, we use a flame photometric detector. For NH_4^+ and NO_3^-, we use a two-channel chemiluminescent NO detector, catalytically converting NH_4^+ to NO in one channel while converting NO_3^- to NO in the second. These systems have been installed in an instrument cart housing all of the other components. Lab evaluations of detectors for particle associated water are underway. A mass monitor for the ultrafine particles has been developed in prototype, and its technical feasibility was demonstrated for measuring very low mass concentration of ultrafine particles in ambient air over one to several hours. Since particle number concentration and mass concentration are highly correlated for ultrafine particles (but not for larger particles), it will be possible, to conveniently examine the correlations between the ambient $PM_{0.15}$ and health effects measures such as daily mortality, hospital admissions, emergency room admissions, and respiratory function in time-series analyses. The monitoring system has been designed to support: 1) studies of particulate matter (PM) exposures and their health effects; and 2) studies of PM source attribution and control efficacy. Such studies are needed for the periodic review of the National Ambient Air Quality Standard (NAAQS) for PM.

007 A REAL-TIME SAMPLER, RAMS, FOR THE DETERMINATION OF $PM_{2.5}$, INCLUDING SEMI-VOLATILE SPECIES.

DJ Eatough, F Obeidi, Y Pang and NL Eatough, Department of Chemistry and Biochemistry, Brigham Young University, Provo UT

The RAMS is a real-time ambient monitor for the determination of fine particulate mass, including the volatile components. The RAMS has a particle concentrator, followed by a Nafion diffusion dryer to remove water, diffusion denuders to remove gas phase compounds which can be absorbed by charcoal, a second Nafion dryer to remove water, and a "sandwich filter" containing a Teflon coated filter to collect particles and a charcoal impregnated filter to retain volatile components which can be lost from the particles during sample collection. Semi-volatile fine particulate material retained on the "sandwich filter" includes ammonium nitrate and semi-volatile organic compounds. The "sandwich filter" is located at the tip of the tapered oscillating element of a TEOM monitor and mass retained on the "sandwich filter" is measured as a function of time. The various dryers and denuders of the RAMS are designed to remove gas phase organic compounds, nitric acid, sulfur dioxide, nitrogen dioxide, ammonia, ozone and water, each of which can be absorbed by the charcoal portion of the "sandwich filter". The concentrations of these species are reduced to acceptable (>20 g/m^3), but not zero concentrations prior to the collection of particles on the TEOM probe. Therefore, a second, parallel system preceded by a filter is used to obtain an active blank for the correction of the RAMS monitor data. The results obtained for the continuous determination of $PM_{2.5}$ with the RAMS have been validated by comparison with results obtained from diffusion denuder integrated samples to determine the mass of fine particulate material retained on a filter and the semi-volatile organic material and ammonium nitrate lost from the filter during sampling. This has included comparisons with sampling periods for the denuder samplers as short as 2 hour. Results obtained with RAMS and denuder samplers show that semi-volatile fine particulate species can be continuously and accurately monitored with the RAMS. The research reported here was supported by the U.S. Environmental Protection Agency STAR Program, the Electric Power Research Institute, and Rupprecht and Patashnick, Inc.

008 SEMI-VOLATILE SPECIES IN $PM_{2.5}$: CONCENTRATIONS IN URBAN ATMOSPHERES AND SAMPLING ERRORS USING THE $PM_{2.5}$ FRM.

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Fine particles in urban atmospheres contain substantial quantities of semi-volatile material (e.g. ammonium nitrate and semi-volatile organic compounds) which can be lost from particles during collection on a filter. The extent to which these species will be lost during sampling with the $PM_{2.5}$ FRM is not well known. However, it can be expected that the FRM will substantially under-measure $PM_{2.5}$ in environments where significant concentrations of semi-volatile particulate material is present. These semi-volatile fine particulate components can be more accurately sampled using diffusion denuder techniques. We have compared the concentrations of $PM_{2.5}$ determined using $PM_{2.5}$ FRM samplers, Harvard $PM_{2.5}$ filter packs and HEADS, and annular denuder and PC-BOSS denuder samplers. Results obtained with these various samplers are compared for six-week sampling programs in Riverside CA in the summer, Bakersfield, CA in the winter and Provo UT in the winter. The results indicate that $PM_{2.5}$ mass measured by a single Teflon filter sampler is often less than 3/4 of the $PM_{2.5}$ actually present in these urban atmospheres, averaging 61%, 76% and 78% of the total for samples collected in Riverside, Bakersfield and Provo, respectively. The PC-BOSS results indicate that about one-half of the fine particulate organic material is semi-volatile and is lost during collection on a filter. There is a clear need for independent validation of these results. The fraction of ammonium nitrate lost varies from above 50% to negligible and is a function of the ambient RH and temperature. The variations in both ammonium nitrate and fine particulate semi-volatile organic material lost from particles during sampling with single Teflon filter samplers will be presented. The research reported here was supported by the Electric Power Research Institute, the U.S. Environmental Protection Agency STAR Program, and Rupprecht and Patashnick, Inc.

009 DESIGN AND EXPERIMENTAL CHARACTERIZATION OF A PM_1 AND A $PM_{2.5}$ PERSONAL SAMPLER.

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This paper presents the development, laboratory and field evaluation of two personal particle samplers (PPS). Both samplers operate at a flow rate of 4 l min^{-1}, and collect particles smaller than 1.0 and 2.5 μm in aerodynamic diameter, respectively, on 3.7 cm Teflon filters. In each sampler, particles larger than 2.5 or 1.0 μm are retained by impaction onto a coated porous metal disk, which minimizes particle bounce. Using the substrates without any coating results in a substantial

reduction of the collection efficiency for particles larger than the 50% cutpoint of the sampler. Particle losses in each sampler are quite low (e.g., on the order of 10% or less) and do not depend significantly on aerodynamic particle diameter. Both samplers display sharp particle cut characteristics, with the ratio of the aerodynamic particle diameter corresponding to 84% collection efficiency to the 50% cutpoint being approximately 1.18 and 1.27 for the PM_1 and the $PM_{2.5}$ samplers, respectively. Field tests showed that the mass, sulfate and nitrate concentrations measured by the $PM_{2.5}$ PPS and a collocated $PM_{2.5}$ Personal Exposure Monitor (PEM) agreed within 10% or less. Such agreement, however, was not observed between the $PM_{2.5}$ PPS and the Harvard/EPA Annular Denuder System (HEADS), with the HEADS nitrate concentrations being on the average higher by a factor of 2.1. The particle mass, sulfate and nitrate concentrations obtained with a modified MOUDI sampler collecting all particles smaller than 1 μm in aerodynamic diameter on a filter and the PM_1PPS were also in very good agreement (e.g., within 7% or less). The development of these samplers is part of our ongoing efforts to improve technologies for human exposure assessment to PM. These personal samplers will be used in subsequent field studies in different locations of the US. Differences in the mass concentration and chemical composition between PM_1 and $PM_{2.5}$ obtained with the two different personal samplers at different locations (including indoor sites) will provide useful data in assessing the adequacy andappropriateness of a single particle standard for the accumulation mode of PM.

010 DEVELOPMENT AND EVALUATION OF A PROTOTYPE AMBIENT ULTRAFINE PARTICLE CONCENTRATOR FOR INHALATION EXPOSURES

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This paper presents the development and experimental characterization of a prototype ultrafine particle concentrator. In this system, ultrafine particles pass over a pool of warm water where they become saturated with vapor, and subsequently drawn through a condenser, kept at a lower temperature, that allows the ultrafine particles to grow to super-micrometer size by vapor condensation on their surface. In order to increase particle concentration, the grown particles are drawn through a virtual impactor with an approximate 50% cutpoint at 1.5μm. The concentrated particles from the minor flow of the virtual impactor finally pass through a diffusion dryer that removes the excess water on the ultrafine particles and returns them back to their original size and relative humidity. In its optimum configuration, the ultrafine concentrator operates at a sampling flow rate of 106.5 or 110 l min^{-1} and concentrates the ultrafine particles to 3.5 or 7 l min^{-1} by an enrichment factor of approximately 15 and 25.5, respectively. Our experimental results identified saturation of the ultrafine aerosols at 35°C and cooling to 25 °C as the optimum temperatures for operation of the ultrafine particle concentrator. Lower temperatures either do not concentrate, or concentrate less efficiently the ultrafine particles. Increasing the saturation temperature to 40 °C and cooling to 31 °C does not improve the concentration enrichment achieved by the optimum configuration. Our results also indicated that the concentration enrichment does not depend on the chemical composition of the ultrafine aerosol. Hygroscopic ammonium sulfate, volatile ammonium nitrate, hydrophobic polystyrene latex and actual "real-life" indoor air ultrafine particles were all concentrated by practically the same factor. More importantly, the experimental results show that particle concentration occurs without any coagulation, which would have distorted the size distribution of the original ultrafine aerosols. The Ultrafine Particle Concentrator is being currently used to conduct animal and human exposures to real-life ultrafine particulate matter.

016 RECONSTRUCTING EXPOSURE TO PM10 FOR EPIDEMIOLOGICAL PANEL STUDIES.

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As part of a study of the relationship between particulate air pollution and blood coagulation factors we have developed a model which uses information from time-activity diaries to reconstruct 24-hour average exposure to PM_{10}. Twenty four hour personal exposure (E) was defined as being the sum of an individual's exposures in four different microenvironments: outside (E_{out}), inside their own home (E_{in_home}), on transport (E_{transp}) and indoors other than in their own home(E_{other_in}), all expressed as $\mu g/m^3$ averaged over 24 hours. The model is defined as follows:

$$E = E_{out} + E_{in\_home} + E_{transp} + E_{other\_in}$$

Each of these exposures was based on the average microenvironment PM_{10} concentration and the proportion of time spent in that particular microenvironment. The model contains terms for the average daily concentration measured at the local urban monitoring site, the location of a subject's home within the city, the relative volume of traffic outside the home, the penetration of PM_{10} into the home, the estimated room air-exchange rate, the likely aerosol deposition rate, plus the concentrations associated with various activities and microenvironments such as cooking, cleaning, other dusty activities, exposure to cigarette smoke and pets. These concentrations were modified by whether or not the activity was judged to take place in a subject's near-field, i.e. within 1m, or in their far-field, i.e. elsewhere in the microenvironment. Personal PM_{10} exposure was measured on 111 occasions in 109 elderly individuals. Using the model, estimates of an individual's exposure to PM_{10} were made from the diary and the AUN data over the same period. The Spearman correlation coefficient between the estimated and measured PM_{10} was 0.4 ($p<0.001$) while the correlation between the central monitoring site and the personal exposures was 0.04 ($p=0.72$).

017 PM EXPOSURE ASSESSMENT IN HIGH-RISK SUBPOPULATIONS.

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This study was designed to provide sufficient information to resolve the crucial issue: whether or not the actual personal exposes among the high-risk subpopulations to particles of ambient origin correlates with PM concentrations measured at a centrally located site. This study are being conducted in Seattle and will continue through 2001. Personal, biological, indoor, and outdoor PM_{10}, $PM_{2.5}$, CO, SO_2, semi-volatile organic compounds (SVOCs), and NO_2 measurements will be taken throughout the year using the Harvard personal exposure monitors for PM, passive monitors for CO, SO_2, and NO_2, the URG personal sampler for SVOCs, and nephelometers for continuous PM_1 The urine biomarkers for wood smoke, including a group of 11 methoxylated phenols and related oxygenated aromatics, will serve as a unique tracer for ambient PM exposures. Eight subjects will be monitored simultaneously and 48 subjects will be monitored in each season. Subjects include 144 elderly adults with chronic obstructive pulmonary disease and/or cardiovascular complications. We will also collect individual time-location activity-symptoms, building characteristics, continuous indoor CO_2 concentrations, temperature, and relative humidity. Furthermore, the exposure data will be supplemented with peak expiratory flow rate, pulse rate, and oxygen saturation measurements from all subjects. This poster presents the study design issues and the preliminary monitoring results. The results are analyzed to: 1) Determine the strength of the relationship of the particle exposures of high-risk subpopulations to the concentrations measured by a central monitoring station; 2) Characterize the key factors

influencing this relationship; and 3) Link exposure data with health outcomes to focus exposure modeling on unbiased estimation of health effects.

033 LABORATORY AND FIELD TESTING OF A PASSIVE AEROSOL SAMPLER.

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A miniature, passive aerosol sampler has been developed to estimate long-term average concentrations and size distributions. The passive sampler is designed to monitor ambient, indoor, or occupational aerosols over a period of weeks, and has potential utility as a personal sampler. The sampler is inexpensive and easy to operate, and its long sampling times are useful for investigating chronic health effects. Particles are collected by gravity, convective diffusion, and inertia in a small cavity. Microscopy and automated image analysis are used to count and size the collected particles. The measured particle flux and a particle-size dependent deposition velocity model are then used to estimate the average concentration and size distribution over the sampling period. To determine the empirical portion of the deposition velocity model, a special wind tunnel was developed. The small-scale tunnel incorporates a non-volatile, polydisperse, high-output aerosol generator. An eight-stage impactor is connected to the tunnel with an isoaxial, isokinetic probe, and is equipped with oleic acid-saturated, polycarbonate-membrane substrates to minimize particle bounce. Aerosol concentrations were determined to have a CV = 5.6% across the tunnel's test section. The friction velocity, an index of turbulence, was determined in the test section as a function of tunnel wind speed. Duplicate experiments were performed at wind speeds of 1.5, 3, 4, and 5 m/s. Using the wind tunnel data, the deposition velocity model was optimized with respect to PM2.5, PM10, mass median diameter, and geometric standard deviation. Three passive samplers were used in each experiment to test precision. Average CVs for PM2.5 and PM10 were 16 and 34% respectively. The model's accuracy and precision were found to be independent of wind speed, relative humidity, and aerosol concentration level. The most sensitive parameter in the calculations was the assumed volume shape factor for the collected particles. Results are also presented for field tests of the passive sampler in an occupational environment.

041 A SYSTEM FOR REAL-TIME EXPOSURE OF SMALL ANIMALS TO CONCENTRATED AMBIENT PM2.5.

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An important problem in environmental toxicology today is the health effect of real world $PM_{2.5}$. To date, most "fine" particle research has been conducted using field collected, processed, and resuspended ambient aerosols, or artificial aerosols, generated as test atmospheres to challenge existing animal models. Research reported here involves real-time use of virgin, uncompromised, ambient $PM_{2.5}$ aerosols. These particulates are captured, concentrated, and presented to our animal models in real-time. The exposure system described is operational at the US EPA's ERC facility at Research Triangle Park, NC. A multi-stage virtual impactor co-developed by EPA's NHEERL and the HSPH Environmental Sciences Dept. was adapted to a custom built 130 L stainless steel and glass exposure chamber. Ambient air from outside the laboratory is drawn into the concentrating assembly through a size selective inlet, eliminating particles larger than 2.5 µm. This air stream then passes through a series of 4 virtual slit impactors and a diffusion drier before entering a whole body exposure chamber. Most "ultrafine" particles exit the concentrator with the impactor major flow streams resulting in a final size range for the concentrator of 0.15-2.5µm. Exposures of up to 6 hours are conducted using either rats or mice. Both "set duration" and "concentration X time" protocols are available. Measurements indicate a typical chamber $PM_{2.5}$ concentration of around 50 times ambient levels. With R.T.P. ambient $PM_{2.5}$ varying from 5-50 µg/m³, concentrations achieved in the chamber have ranged from 350 µg/m³ to over 3000 µg/m³. Chamber particle size ranged from 0.5-1.1 µm. Likewise, chemical analysis of particles before and after entry to the system is in progress. Gaseous pollutants including O_3, NO_2, SO_2, CO, and THC have been monitored according to NAAQS guidelines to assess whether ambient gas concentrations are altered during aerosol concentration and delivery to the chamber. Both SO_2 and O_3 concentrations are reduced from ambient levels, with O_3 being almost entirely removed. Particle size appears to be unaltered. Typical exposures include monitoring of particle concentration, size, and chemical composition as well as ambient gas concentrations to address questions of multiple causation or mixture effects. (This abstract does not reflect EPA policy).

045 SOURCE APPORTIONMENT AND CHARACTERIZATION OF PARTICULATE MATTER FROM PHOENIX, ARIZONA USING BOTH ENERGY DISPERSIVE X-RAY FLUORESCENCE AND SCANNING ELECTRON MICROSCOPY WITH ENERGY DISPERSIVE X-RAY FLUORESCENCE.

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Numerous epidemiological studies have found associations between airborne particulate matter measured at community monitors and increased mortality and morbidity. Characteristics of particulate matter such as the trace element composition and sources are required to help understand the observed health associations and to provide information for particulate matter toxicology studies. The sources and composition of particulate matter in Phoenix, Arizona were investigated by analyzing $PM_{2.5}$ and $PM_{10-2.5}$ filters with energy dispersive x-ray fluorescence (XRF) and scanning electron microscopy with energy dispersive x-ray fluorescence (SEM/EDX). Samples used in this analysis were from the EPA National Exposure Research Platform in central Phoenix, which collected dichotomous samples every third day and $PM_{2.5}$ samples every day for 3 years. This study analyzed a subset of the $PM_{10-2.5}$ samples from a modified dichotomous sampler (VAPS) which has approximately 10 percent of the $PM_{2.5}$ fraction on the $PM_{10-2.5}$ sample. SEM/EDX samples were evaluated in the manual mode and the lightly loaded samples were classified using the computer-controlled mode. Factor analyses of the $PM_{2.5}$ and $PM_{10-2.5}$ XRF elemental concentrations were compared to SEM/EDX results to show the benefit of evaluating samples with SEM/EDX. In addition, back trajectory analysis was used to evaluate the SEM/EDX and XRF results. This is a proposed abstract and does not necessarily reflect U.S. EPA policy.

048 RELATIONSHIP BETWEEN A COMMUNITY MONITOR AND PERSONAL EXPOSURES TO $PM_{2.5}$ MASS AND TRACE ELEMENTS IN BALTIMORE, MARYLAND.

M S Landis, G A Norris, R Zweidinger, R. Williams, J Suggs. US EPA. National Exposure Research Laboratory, Research Triangle Park, NC. USA

The relationship between a community monitor and personal exposures to $PM_{2.5}$ mass and trace elements was investigated for 28 days during the summer of 1998 in a retirement home in Baltimore, Maryland. Daily community, outdoor, and indoor $PM_{2.5}$ was measured with a URG Versatile Air Pollutant Sampler (VAPS). In addition, daily personal and apartment $PM_{2.5}$ samples were collected with a Marple Personal Exposure Monitor (PEM) for 10 subjects. Both the VAPS and PEM samples were analyzed with energy dispersive x-ray fluorescence spectrometer (XRF). The spatial correlation coefficients between the

community and outdoor monitor, and the penetration factors were calculated for elemental species typically above the XRF detection for the VAPS. Penetration for $PM_{2.5}$ mass, and sulfur were similar when determined with linear regression with factors of 0.35 (r^2= 0.94) and 0.39 (r^2 = 0.94), respectively. Indoor sampler, apartment, and personal exposures were also evaluated. These results are compared to previous particulate matter exposure studies. This is a proposed abstract and does not necessarily reflect U.S. EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

049 EVALUATION OF PERSONAL EXPOSURE MONITOR SAMPLES FOR TRACE ELEMENT ANALYSIS: WHAT ELEMENTS CAN BE QUANTIFIED USING ENERGY DISPERSIVE X-RAY FLUORESCENCE, NEUTRON ACTIVATION, AND INDUCTIVELY COUPLED PLASMA-MASS SPECTROSCOPY.

M S Landis, G A Norris, R Zweidinger, R Williams. US EPA. National Exposure Research Laboratory, Research Triangle Park, NC. USA.

Recent toxicological research has indicated that exposure to certain trace elements is associated with adverse health effects. Since personal exposure monitors (PEM) have relatively low flow rates (2 - 4 LPM), trace element analysis of the collected particulate mass is typically quite difficult. The ability of energy dispersive x-ray fluorescence spectrometry, instrumental neutron activation, and inductively coupled plasma mass spectrometry analysis to resolve trace element concentrations from PEMs was evaluated. Marple $PM_{2.5}$ PEM samples were collected from a retirement home for 28 days during the summer of 1998 in Baltimore, Maryland. The PEM samples were collected on 37 mm Teflo filters at 2 LPM for 24 hours. The results from each of the analytical techniques and associated detection limits will be discussed. The PEM gravimetric mass and elemental concentrations were also compared to a collocated indoor versatile air pollutant sampler that had a flow rate of 15 LPM on 47 mm Teflo filters. This investigation provides a list of trace elements that can be quantified from low flow PEMs in an East Coast city using three analytical techniques. The results of this investigation should be considered when designing future exposure studies focusing on $PM_{2.5}$ trace element characterization. This is a proposed abstract and does not necessarily reflect U.S. EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

050 SIZE SPECTRA AND ATMOSPHERIC BEHAVIOR OF RESPIRABLE URBAN AEROSOL PARTICLES: AN IMPROVED PARADIGM.

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Discussions of excess mortality and morbidity from exposure to urban aerosol particles typically invoke the now 20-year-old trimodal aerosol paradigm proposed by Whitby to explain the structure and behavior of ambient aerosol volume and its major constituent, sulfate. However, this paradigm largely ignores the primary high-temperature-combustion (HTC) components, which contribute minor amounts of the aerosol mass, but carry the bulk of the particulate toxins and numbers of aerosol particles. Herein, studies encompassing >100 size distributions of many important intrinsic tracers of primary particles from HTC sources collected over the last decade in rural, urban, and industrial environments are reviewed. These show that ambient urban aerosol contains a complex mixture of physically-discrete fresh and aged, primary particle populations from a variety of sources, which can be revealed by observing the size spectra of intrinsic marker species. Furthermore, whereas the behavior of fine-particulate aerosol mass and sulfate was described in terms of coagulation and accumulation aerosol scavenging of new secondary sulfate nuclei, newer studies suggest that the behavior of primary aerosol is mediated more by hygroscopic growth and cloud processing, accompanied by oxidation of SO2 on wet particles and droplets. Clearly, the distribution of airborne particulate toxins and their atmospheric behavior is far more complex than commonly conceptualized on the basis of the classical trimodal model. Size distributions of source marker elements in aerosol of the MidAtlantic region will be presented as will an improved aerosol paradigm in which the focus is on the primary accumulation aerosol.

052 USE OF MOTION DETECTORS AND TIME/ACTIVITY DIARIES TO ASSESS THE VALIDITY OF PERSONAL EXPOSURE MEASUREMENTS.

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Speculations exist as to whether the subjects participating in personal exposure studies tend to over-report the time they wear their personal samplers. Anecdotal evidence suggests that personal samplers might be left unworn by the participants for a prolonged period of time, thus reducing the accuracy of the personal exposure measurements. To assess subject compliance with the personal monitoring protocol, a motion sensor (Onset Corp.) was placed inside the monitoring pack of each child (9-13 years old) participating in our multi-pollutant exposure study conducted in Baltimore during the summer of 1998. Personal particulate ($PM_{2.5}$) and gaseous (O_3, SO_2, NO_2) exposures were measured for ten children, with each child monitored for eight 24-hr periods. In addition, children recorded each of their activities (time, duration, and description) throughout each monitoring period in a diary. This paper presents preliminary results from our analyses of the motion sensor and time/activity data and discusses the use of motion sensors to assess subject compliance in personal monitoring studies. The compliance of each child with monitoring protocols was assessed by comparing the motion sensor data with time/activity data to determine their agreement. Results from this analysis showed that the average percent compliance of each child was over 90%. However, daily percent compliances varied, at times substantially, where five subject-days fell below 85% percent compliance, indicating that personal exposures for these five subject-days should be excluded from subsequent data analyses. Percent compliance did not decrease over time for any of the monitored children, indicating that an eight-day personal monitoring protocol is appropriate.

055 FINE PARTICLE MASS AND COMPOSITION IN THE TENNESSEE VALLEY

William J. Parkhurst, Roger L. Tanner, and Frances P. Weatherford

Historical dichotomous sampler $PM_{2.5}$ data collected at eight predominantly rural monitoring stations from 1983 through 1992 suggested that the Tennessee Valley region might have difficulty meeting the recently adopted annual $PM_{2.5}$ metric of 15 $\mu g/m^3$. In order to establish current $PM_{2.5}$ mass and composition levels, the Tennessee Valley Authority (TVA) and Tennessee Valley state and local regulatory agencies began operation of an urban-oriented, prototype FRM $PM_{2.5}$ monitoring network on Earth Day (April 22) 1997. The prototype-FRM network consists of eight monitoring stations in Nashville, Knoxville, Chattanooga, Memphis, and Lawrence County TN, Decatur and Huntsville AL, and Paducah KY. The ongoing assessment of these data suggest the following conclusions:

1. Annual average $PM_{2.5}$ concentrations range from 14.7 to 20.5 $\mu g/m^3$. Only the station in rural/background Lawrence County TN, was below the level of the annual $PM_{2.5}$ metric while all seven urban monitoring stations have or will likely exceed this metric. No station exceeded the level of the 65 $\mu g/m^3$ 24-hour $PM_{2.5}$ metric. The mean ratio of FRM $PM_{2.5}$ mass to collocated FRM PM_{10} and TSP mass is 0.65 and 0.38, respectively. Summer-high, winter-low seasonality was evident and the summer of 1998

proved a banner year for secondary air pollution in the Tennessee Valley.

2. Composition measurements (which include elemental and organic carbon measurements from quartz fiber filters) suggest that Tennessee Valley $PM_{2.5}$ consists of organic/elemental carbon compounds and inorganic sulfate (primarily ammonium sulfate) which, on average, make up about two-thirds of $PM_{2.5}$ mass in approximately equal proportions. Ammonium sulfate provides the largest fraction (≈50%) in background air (Lawrence County) with organic/elemental carbon making up≈33%. For urban stations the situation is reversed with the organic/elemental carbon fraction being dominant (≈50%) followed by sulfate at ≈30%.

058 EXPOSURE ASSIGNMENT IN TIME-SERIES STUDIES: USE OF A SINGLE DAILY VALUE VERSUS A DISTRIBUTED APPROACH.

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To date, in a majority of air pollution time-series studies involve a single geographic area and have used a single exposure value and single outcome count for each day. Air pollution metrics in these studies are typically derived from a single central monitor or an average of two or more monitors. However, in many cities, and particularly in the coastal areas such as the South Coast Air Basin (SoCAB), significant daily differences can occur within a geographic area. We sought to document the level of disagreement between a single daily value and a distributed exposure assignment approach using data from two studies in the SoCAB. In the distributed exposure approach using data from local monitoring stations, the SoCAB was overlaid with 89 ten kilometer (km) by ten km grids, which were further aggregated into 57 groups based on joining contiguous grids with high daily correlations and absolute levels for ozone and PM_{10}. In addition, a single daily mean value to represent the entire SoCAB was obtained by calculating the arithmetic average for all study groups, similar to studies that use an average of multiple monitors. This single daily pollutant value was compared to the daily value for each group for each day of 1995. Substantial variation was noted between the more refined exposure assignment and the single value. The monthly average of the daily absolute difference for all groups varied the most for ozone in the summer months, from an average of 14.6 to 18.5 ppb difference, whereas PM_{10} varied most in the summer, fall and winter months, from an average of 10.4 to 17.1 g/m^3. Daily differences up to 95 ppb for ozone and 116 g/m^3 for PM_{10} were observed during the study period. These data suggest that time-series studies that can exploit both the temporal and spatial variation in ambient exposure will be able to improve the exposure assignment over that of studies using a single value.

061 EXPOSURE AND RISK ASSESSMENT FOR FINE AND ULTRAFINE PARTICLES IN AMBIENT URBAN AEROSOLS.

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Background: Health effects of fine particles have been consistently characterized while the significance of ultrafine particles in quantifying exposures to ambient air pollution and in eliciting health effects remains unclear. Three studies specifically addressing this issue have been started in 1996.

Studies: 1) intercomparisons of the aerosol spectrometers to compare particle size and number distributions in ambient air 2) air pollution monitoring and aerosol spectrometry study in winter 1996/1997 in Germany, Finland and the Netherlands 3) an ongoing epidemiological panel study in Amsterdam, Helsinki, and Erfurt during winter 1998/1999 together with urban air monitoring.

Results: 1) In the ambient side-by-side comparisons, the three aerosol spectrometers were very well comparable in total number concentrations and concentrations ultrafine (0.01 - 0.1 μm) and accumulation mode (0.1 - 0.5 μm) particles. Number concentration of the coarse fraction (0.5 - 2.5 μm) were less comparable, which, however, added less than 2% to the total number concentration. 2) In the 3-cities winter study 1996/97, there were clear differences in the concentrations of accumulation particles, PM2.5 and the blackness of the PM2.5 filters between the cities, but not in the concentrations of ultrafine particles. According to principal component analysis airborne particulate pollutants seem to be divided into two source categories, one related to number concentrations and the other to mass-based information.

Conclusions: Ultrafine particles can be measured reliably in ambient air. Exposures to ultrafine and fine particles are not identical within European cities during a winter season and the ongoing epidemiological study aims at quantifying effects of ultrafine and fine particles on cardiopulmonary endpoints in patients with coronary artery disease. (funded by the European Union, ENV-CT96-0205; ENV-CT97-0568).

062 EXPOSURE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) PATIENTS TO PM2.5: RELATIONSHIP BETWEEN PERSONAL AND AMBIENT AIR CONCENTRATIONS.

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Most time series studies of particulate air pollution and acute health outcomes assess exposure of the study population using fixed site outdoor measurements. Here we evaluate the relationship between ambient $PM_{2.5}$ concentrations and personal exposures of a population expected to be at risk of particle health effects. Sixteen subjects (non-smoking, ages 54 - 86) with physician-diagnosed COPD, wore personal $PM_{2.5}$ monitors for seven 24-hour periods, randomly spaced approximately 1.5 weeks apart. Sampling was conducted within the Vancouver metropolitan area during April-September 1998. Time-activity logs for each personal sampling period and dwelling characteristics data were also obtained for each subject. Daily 24-hour ambient $PM_{2.5}$ concentrations were measured at five fixed sites spaced throughout the study region. Sulfate, a marker of ambient combustion-source particulate, was also measured in all samples. The mean personal and ambient $PM_{2.5}$concentrations were 18μg/m^3 and 11μg/m^3, respectively. Ambient concentrations were expressed either as an average of the five values obtained for each day of personal sampling, or the concentration obtained at the site closest to each subject's home. Regression analyses were conducted for each subject separately. The median Pearson's r between personal and average ambient $PM_{2.5}$ concentrations was 0.48 (range: -0.68 to 0.83). Using sulfate as the exposure metric, the median Pearson's r between personal and average ambient concentrations was 0.96 (range: 0.66 to 1.0). Use of the closest ambient site did not improve the median correlation of the group for either PM2.5 or sulfate. The mean personal to average ambient concentration ratio was 1.79 (range: 0.64 to 4.26) for $PM_{2.5}$, and 0.75 (range: 0.39 to 1.05) for sulfate. These results indicate a relatively low correlation between personal exposure and ambient $PM_{2.5}$ that is not improved by assigning exposure to the closest ambient monitor. The correlation between personal exposure and ambient concentration is high, however, when using sulfate as a marker of outdoor combustion-source particulate.

072 **EXPOSURE ANALYSIS FROM PERSONAL AND AMBIENT AIR SAMPLING: RESULTS OF THE 1998 BALTIMORE STUDY.**

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An integrated epidemiological-exposure panel study was conducted during July-August 1998 which focused upon establishing relationships between potential human exposures to particulate matter (PM) and related co-pollutants with detectable health effects. The study design incorporated repeated 24-hour integrated $PM_{2.5}$ personal exposure measures as well as a variety of both integrated and continuous $PM_{2.5}$ and PM_{10} monitoring. A total of 305 $PM_{2.5}$ personal exposure samples were obtained over the four week study period using a subject pool of twenty-one elderly residents of an eighteen story retirement facility near Baltimore, Maryland. Each sample represented a unique 24-hour breathing-zone measurement of $PM_{2.5}$ mass concentration. Matched $PM_{2.5}$ and PM_{10} micro-environmental measures were obtained on at least an every-other-day schedule in the apartments of those participating in personal monitoring. Likewise, daily residential indoor, residential outdoor, and community platform measures were taken to investigate spatial variation of PM mass concentration. Results indicated that the $PM_{2.5}$ size fraction was responsible for >86 % of collected indoor PM_{10} mass. Daily $PM_{2.5}$ personal exposure concentrations ranged from 2.4 to 47.8 $\mu g/m^3$ with an overall individual study mean of 12.9 $\mu g/m^3$. The mean values from 15 days of apartment monitoring was 10.1 $\mu g/m^3$, with mean residential indoor and outdoor concentrations of 9.5 and 22.1 $\mu g/m^3$, respectively. Individual correlation (r^2) of personal exposures to matched residential indoor $PM_{2.5}$ measures ranged from 0.31 to 0.96 with a mean of 0.74. The mean correlation of personal exposures to residential outdoor$PM_{2.5}$ concentration was 0.78. This is a proposed abstract and does not necessarily reflect U.S. Environmental Protection Agency policy.

073 RESULTS FROM EXPOSURE MONITORING **PERFORMED DURING THE 1997 BALTIMORE PM PILOT STUDY.**

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An eighteen day winter-time ambient and personal exposure monitoring study of particulate matter (PM) was conducted as part of an integrated epidemiological-exposure pilot study of an aged population. Goals of the study were to determine the feasibility of performing active personal exposure monitoring upon subjects over 65 years old living in a common retirement facility and to investigate activity patterns that might affect individual exposures. Daily measures of $PM_{2.5}$ and PM_{10} were performed outside of the retirement center and at a community platform. Repeated $PM_{2.5}$ measurements were conducted inside of the facility as a means to compare indoor/outdoor mass concentrations. Five elderly residents of a 3-story retirement facility were successfully recruited to wear $PM_{1.5}$ active samplers on a Monday-Saturday basis for three weeks (10 samples/subject) during the study period. Each sampling period represented a continuous 24-hour collection of potential breathing zone PM exposure. Collection of daily individual activity logs and survey questionnaires were utilized to develop activity profiles of all participants in the study. Results indicated that the outdoor PM_{10}size fraction was 82.7 % $PM_{2.5}$ by mass and that little spatial variation existed between indoor and outdoor concentrations of $PM_{2.5}$ (ratio of 0.80). $PM_{1.5}$ personal exposure monitoring of an aged population was determined to be feasible with some individuals measured at levels approaching 60 $\mu g/m^3$ under certain activity profiles. Correlation of the average $PM_{1.5}$ personal exposure to the outdoor $PM_{2.5}$ measure was r^2=0.4521 (P=0.0472,n=9). This is a proposed abstract and does not necessarily reflect U.S. Environmental Protection Agency policy.

074 **EPIDEMIOLOGIC RESEARCH TO ESTABLISH AN UNDERSTANDING OF THE PM-HEALTH RELATIONSHIP**

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An integrated research program has been implemented to understand the above relationship. These include detailed efforts to characterize the ambient environments and particulate matter in urban areas, to understand how these ambient environments are related to personal exposures, and to determine if health responses are related to alternative PM fractions or other environmental agents. Data which characterize the levels and composition of particulate matter will be presented for several urban areas in the US; research programs to relate the ambient concentrations to personal levels will be described as well. Controlled human studies to determine whether there are health responses to specific particulate fractions are underway. A series of epidemiology studies are also underway to determine the relationship between alternative fractions of PM to health endpoints. These studies include both acute and chronic studies. Interim results will be presented for these studies.

085 **REINVENTING PERSONAL EXPOSURE TO PARTICULATE MATTER.**

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The concept of total personal exposure as exposure through all routes and in all micro-environments has been very useful in understanding exposure to specific agents in which exposure may occur by a variety of mechanisms. However, when applied uncritically to exposure to particulate matter (PM), this concept has led to confusion and misinterpretation, as evidenced by published papers and formal comments to EPA. PM is not a distinct substance nor does it have a specific source. Rather, PM is a mixture of particles form different sources with different sizes, compositions, and toxicities. Therefore, personal exposure to PM should be characterized as personal exposure to PM from different sources rather than personal exposure to PM in different micro-environments. Occupational PM, indoor-generated PM, PM due to personal activities, and PM of ambient origin all may cause health effects. In order to develop accurate risk assessments and effective risk reduction strategies, it is necessary to know the concentrations and toxicities of PM from different sources. Reinterpreting personal exposure to PM in terms of personal exposure to particles from different sources helps eliminate the disagreement and confusion over whether ambient PM concentrations or personal exposure to total PM is the correct metric for epidemiologic study. In performing cohort studies with small groups, over short times, it might be possible to measure the personal exposure to PM generated indoors, PM due to personal activities, and PM of ambient origin. It is likely that the time patterns of these three exposures differ and it may be possible to determine which exposure pattern is most closely associated with the pattern of health effects. However, determination of exposure to PM of ambient origin is a challenging experimental problem. Exposure to ambient PM occurs both outdoors and indoors to ambient PM that has infiltrated indoors. In this paper we discuss an experimental approach that shows promise for the measurement and differentiation of indoor-generated PM and PM of outdoor origin. For longer time-series studies, measurements of the various components of personal exposure to total PM are not feasible, but they may be modeled based on measurement and surveys. Ambient PM concentrations are available, so their variations may be used as an index of community exposure to PM of ambient origin and that pattern may be compared with the patterns of community health parameters such as mortality or morbidity.

086 AUTOMATED MEASUREMENT OF $PM_{2.5}$ SULFATE, NITRATE AND CARBON.

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One of the difficulties in exposure assessment for $PM_{2.5}$ is the lack of daily or hourly data. Especially striking is the paucity of particle chemical composition data. Needed are automated methods that can measure the concentration of the chemical constituents of $PM_{2.5}$ on a routine basis, much as is now done for criteria gases such as ozone. Described here is an approach for automated, ten-minute time resolution measurement of the airborne concentration of $PM_{2.5}$ nitrate, sulfate and carbon. In most urban areas these three constituents comprise the majority of the dry $PM_{2.5}$ mass. The measurement method uses an integrated collection and vaporization cell whereby particles are collected by a humidified impaction process, and analyzed in place by flash vaporization. The evolved gases are quantitated using standard gas analyzers for NO_x, SO_2 or CO_2. The system provides six determinations per hour, each corresponding to an eight-minute sample collection. The nitrate system was fielded for six consecutive weeks in southern California in the summer of 1997. The approach proved to be robust, and provided nearly uninterrupted data. Operations were unattended apart from twice-weekly calibration checks. Preliminary results were available immediately. Nitrate concentrations agreed well with 24-hour averaged values obtained by parallel denuder filter systems. This approach has been extended to the measurement of sulfate and carbon, with initial field testing conducted in December 1998.

088 WHAT FRACTION OF $PM_{2.5}$ PERSONAL EXPOSURE IS ATTRIBUTABLE TO URBAN TRAFFIC?

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The characterization of $PM_{2.5}$ personal exposure in a non-smoking adult population has been carried out in Grenoble, France, in the framework of the European EXPOLIS study. The objective of this paper is to assess the fraction of the $PM_{2.5}$ personal exposure attributable to urban traffic emissions. Volunteers (n=40) carried out a personal exposure monitoring case and filled in questionnaires on their indoor environments, as well as time-activity diaries, during 48 hours (working days). Workplaces and places of residence have been classified in 2 categories using a Geographic Information System (GIS). Some volunteers have life environments that can be best represented by PM ambient air monitors located in urban background sites ; others by monitors situated close to high traffic density sites (proximity sites). A partial least square regression model estimated the $PM_{2.5}$ personal exposure (average=37.2 $\mu g/m^3$; standard deviation=23.2) as a function of the time spent in proximity (at work, home or commuting), the PM_{10} ambient air levels during the same days (background site average=35.5 $\mu g/m^3$; s.d.=16.6), and several confounders (passive smoking and indoor sources of particles) (R^2=0.6). The personal $PM_{2.5}$ exposure predicted by this model was compared to the background personal exposure, thus providing an estimate of the additional contribution of time spent near traffic sources. On average (% time spent in proximity=48.2), the $PM_{2.5}$ exposure attributable to traffic equals 35.4%. For the lower tercile (people spending less than 30% of their time in proximity) this contribution is 24.9% ; for the upper tercile (time greater than 60% in proximity) it is 44.1%. Given the spatial homogeneity of $PM_{2.5}$ concentrations across the city, these estimates seem robust although they are based only on winter data. There is a agreement with other results found in the corresponding literature.

094 MULTIPLE IMPUTATION FOR MULTIVARIATE TIME SERIES OF THE CHEMICAL CONCENTRATIONS OF AIR POLLUTION IN THE ARCTIC.

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Week-long samples of airborne particulate matter were obtained at Alert, N.W.T., Canada between 1980 and 1991. The data consist of the concentrations of 24 particulate constituents complicated by missing values that are either fully missing or below detection limits. To facilitate scientific analysis, it is appealing to create complete data by filling in missing values so that standard complete-data methods can be applied. However, applying the complete-data methods to complete data filled-in via *ad hoc* strategies, such as filling in fully missing values with the means and below detection limit values with zeroes or detection limits, generally leads to misleading results, especially when the data have certain intrinsic structures, such as correlations among variables and time or spatial patterns. Even if missing values are replaced by good estimates, the resulting inferences (i.e., confidence intervals and p-values) are generally invalid since they claim more precision than appropriate. We review commonly used strategies of handling missing values and focus on the multiple imputation approach, which generally leads to valid inferences when faced with missing data. Three statistical models are developed for multiply imputing the missing values in the samples of airborne particulate matter. We present our preliminary results, which demonstrate the advantages of multiple imputation.

095 EXPLORATION OF MEASUREMENT ERROR AND MICROSCALE VARIABILITY IN PM MONITORING IN PITTSBURGH

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Spatial models of ambient particulate matter (PM) have the potential to provide accurate and precise predictions of exposure to outdoor PM for regulatory purposes and to assess health effects. Spatial models require good estimates of measurement error and/or micro-scale variability. We explore the issue of measurement error and micro-scale variability in monitoring ambient particulate matter as PM10 in the Pittsburgh (USA) area. In the Pittsburgh area, seven of the available monitoring sites provide 'replicate measurements'; that is, measurements taken on the same day at the same location. However, differences exist between measurements that produce variability in replicate observations. For example, the height of the inlet through which air reaches the filter varies within a site. In addition, the types of filters used may differ. We will consider whether it is possible to differentiate pure measurement error from micro-scale variability. We model the variability within sites to determine whether the within-site variance is homogeneous over time and space and whether there is any systematic
relation between the level of PM and the variability (mean/variance relationships). We consider various hierarchical models to account for temporal and spatial dependence in the means and variances to obtain an estimate of measurement error and micro-scale variability.

101 AIRBORNE PARTICULATE MATTER IN THE CARDIFF AREA FOR 1958-1996 IN RELATION TO ENVIRONMENTAL FACTORS AND HEALTH .

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The measurement of airborne particulate matter in the UK atmosphere has traditionally been measured by the Black Smoke method, and

recently by automatic gravimetric TEOM monitors. Neither of these methods provides an effective indication of the mass of particles per unit volume of air, and so there is no routine data available on the size, composition and concentration of airborne particles. Recent evidence has implicated these characteristics, especially the ultrafine particles (Quality of Urban Air Review Group, 1996) in having the paramount effect on health. The current project is investigating a series of continuously recorded volumetric daily air samples, taken by Hirst-type traps from the center of Cardiff City and samples from selected sites around Cardiff. The set of slides is unique as it dates from 1954 to the present day, and contains data, which precedes any of the other routine measurements of PM10. The slides are analysed by an image analyser, which measures the size, composition and concentration of airborne particles. Image analysis has not been used previously to examine PM10 from slides taken by Hirst-type traps. The advantages of the technique are being able to perform simple but tedious calculations quickly, measuring a number of images simultaneously and quantifying parameters that would otherwise have been qualitative subjective comparisons. Environmental data including wind speeds, rain fall and temperature measurements are investigated to examine the influence on the temporal variation of the abundance and characteristics of airborne particulate matter. Confounding factors that have an impact on cardiovascular and respiratory illness are being examined including data on aeroallergens, nitrogen oxide, sulphur dioxide, and carbon monoxide. The project includes the analysis of the results in relation to the incidence of mortality and hospital admissions as a consequence of cardiovascular and respiratory illness.

102 HIERARCHICAL NON-PARAMETRIC POISSON MODELS IN ENVIRONMENTAL EPIDEMIOLOGY: INVESTIGATING THE ASSOCIATION BETWEEN PARTICULATE MATTER AND MORTALITY.

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There is a substantial controversy surrounding the recent findings that exposure to air pollution variables is associated with increased daily mortality. Key issues are the assumption of linear relationships between air pollution and mortality, and the adjustment for confounding factors such as weather, long-term trends, and age. In this article we introduce hierarchical non-parametric Poisson models to investigate the particulate matter/mortality association, with application to time-series data from several U.S. cities. At the first stage of the hierarchical model, daily mortality counts are modeled by a Poisson distribution. As an alternative to a fully parametric Poisson regression model, we allow for greater flexibility in the particulate/mortality association by assuming that the logarithm of expected value of the mortality counts is modeled by a sum of smooth functions of particulate matter, time, and weather variables. Specifically, we will model the association between particulate matter and air pollution via polynomials with several unknown knots to try to uncover possible thresholds. At the second stage of the model we describe the between-city variation in the particulate/mortality association by assuming that the coefficients and knots of the true city-specific curves follow Gaussian distributions with mean corresponding to population level coefficients and knots, respectively, and unknown covariance matrices. Our goal is to estimate the city-specific curves as well an overall curve borrowing strength across cities, and to attempt to identify city-specific and overall thresholds. We address practical issues related to the implementation of Markov chain Monte Carlo algorithms for sampling from the posterior distribution of the non-parametric components of the model and of the parameters of interest.

103 CALIFORNIAN'S PARTICLE EXPOSURE FROM ENVIRONMENTAL TOBACCO SMOKE.

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The inhalation exposure of nonsmoking Californians to particulate matter from environmental tobacco smoke (ETS) was evaluated for conditions in the late 1990's. Exposures for individuals were assessed by combining activity pattern data with microenvironmental concentration estimates. The latter were determined from measurements of ETS constituents and from indoor air quality models. The distribution of 24-h exposures among nonsmokers was determined by a stochastic (Monte-Carlo) simulation procedure. We estimate that 5.7-6.5 million nonsmoking Californians (19-21% of the entire nonsmoking population) are exposed to environmental tobacco smoke on any given day. For those exposed, the 24-h mean exposure concentration is estimated to be in the range 10-20 $\mu g\ m^{-3}$. Since particles in ETS are predominantly smaller than 2 μm, this result represents the estimated average increment in $PM_{2.5}$ exposure caused by ETS for exposed nonsmoking Californians. The range reflects differences in input data in the simulations and is expected to bracket the true value. Exposures vary widely among individuals exposed: the 10th percentile among exposed individuals receives approximately 1-3% of the exposure of the 90th percentile. Exposures vary much less among age groups. For children, 21-23% are exposed daily to a mean exposure concentration of 10-28 $\mu g\ m^{-3}$. For adolescents, the corresponding results are 33-35% exposed to a mean of 7-20 $\mu g\ m^{-3}$. And, for adults, the results are 16-19% exposed to a mean ETS particle concentration of 10-20 $\mu g\ m^{-3}$. Relative to conditions in the late 1980s, which were also simulated, the total population exposure of nonsmoking Californians to ETS is estimated to have declined by 60-75% for adults, 40-50% for adolescents, and 20-40% for children. These reductions are attributable to two causes: (1) an overall decline in smoking prevalence among California adults from about 22% in 1987-89 to about 18% in 1996-97; and (2) the implementation of a statewide ordinance (AB13) that effectively bans smoking in public buildings. Relative to the late 1980's, the proportion of the Californian population regularly exposed to ETS has been substantially reduced. Average exposures among the exposed have not changed much.

111 AMBIENT AEROSOL MEASUREMENT, SOURCE APPORTIONMENT AND APPLICATION IN HUMAN HEALTH RISK ASSESSMENT.

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Difficulties in developing defensible risk assessments of ambient air pollution requisite to informed risk management have been primarily two-fold. First, ambient data directly relevant to breathing space exposures that identify both the specific constituents of airborne particulates and their source(s) are lacking. Second, the toxicological basis for harm from fine particulates is not well developed. This paper explores an ambient air monitoring approach to help resolve these difficulties for a particular source. We describe the use of direct ambient exposure measurements including: site selection; data collection and source evaluation approaches including multivariate statistical techniques such as factor and principal component analysis (FA and PCA); chemical speciation of ambient aerosols; the development and use of toxicological endpoints; and the merging of these components into the risk assessment framework. A large number of ambient aerosol measurements and a broad suite of measured trace elements are critical to deconvoluting source-receptor relationships.

Analytes must include elements characteristic of particular source types. Higher frequency sampling is preferred to longer-integrated sampling because signal variation is less obscured. FA and PCA is used to reduce the suite of measured variables to a smaller set of indices that describe most of the data variability. Depending upon how different elements load on different factors, underlying factors such as marine sources, fuel combustion, and weathering can be elucidated. This analysis forms the basis for quantitative assessment of the relative contribution of source types to overall ambient aerosol mass concentrations. When constituents that may be causing health effects are uncertain, all trace substances associated with ambient particulates must be evaluated as part of the hazard assessment. EPA cancer slope factors and reference concentrations are used when available; values not available in current databases should be developed, if sufficient laboratory and/or epidemiological data exist. Dose-response modeling and CSFs/RfCs for various Ni species associated with ambient particulates are presented as an example.

117 CHARACTERIZATION OF AMBIENT PARTICLES.

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Acute exposure to inhaled ambient particles has been found to be associated worldwide with adverse health effects. Besides the PM-standard measurements of particle mass, the number concentration of particles has been proposed as a major factor contributing to these effects.

Therefore, particle size spectra and hygroscopicity of ambient aerosol particles were measured on-line and chemical components were analyzed from size-fractioned samples. Particles with diameters below 100 nm (ultrafine particles) determined the number con-centration at urban sites and particles with diameters between 0.1 and 1 mm (fine particles) determined the mass concentration. The concentration of ultrafine particles in urban air was much higher than at rural sites and could therefore best be used to characterize both sites. Fine and ultrafine particles can be hydrophobic or hydrophilic, however hydropho-bic particles in the ultrafine size range contribute nearly 80 % to urban particle concentra-tion. Mass spectrometric analyses of hydrophobic and hydrophilic particles and ion chromatographic analyses of anionic particulate compounds found that the major compo-nent in hydrophobic particles was carbon, the major component in hydrophilic particles was sulfate. Thus, thydrophobic carbon-related ultrafine particles and hydrophilic sulfur-related fine particles are suitable to simulate the major components of urban ambient particles in con-trolled exposure studies.

121 ANALYSIS OF HIGH-ALTITUDE, LIGHT- AND HEAVY-DUTY VEHICLE DIESEL PARTICULATE EMISSIONS AND THE IMPLICATIONS FOR PUBLIC HEALTH.

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During 1996 and 1997, a number of light- and heavy-duty vehicles were recruited in the Denver metropolitan area and subjected to emissions testing, principally for the purposes of assessing particulate emissions from vehicles operating at altitude. Little if any comparable data had been collected under high-altitude conditions, particularly from heavy-duty vehicles. Such data is an important resource, both for political entities involved in establishing compliance under the Environmental Protection Agency's new ambient air quality standards, and for public health officials involved in ongoing assessments of pollution impacts on local communities. The results of an extensive statistical analysis of all the particulate matter data obtained in the present studies is reported here. Most significantly, diesel particulate emissions are shown to be more variable than previously thought, suggesting that important sources of variation heretofore may not have been adequately taken into account. The public health implications are significant, because the results indicate that diesel particulate emissions levels cannot be reliably estimated through low-vehicle-count experimentation—an approach commonly taken. Diesel-powered vehicles—particularly, heavy-duty vehicles—contribute a substantial portion of the mobile source particulate emissions, and their contribution in the future is likely to increase as the result of government programs to promote the use of diesel as an energy security strategy.

122 THE EFFECT OF DIFFERENT FUELS, STOVES, AND VENTILATION ON PARTICLE COMPOSITION.

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[1]Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA, USA; [2]Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine, Beijing, China; [3]National Center for Environmental Assessment, US Environmental Protection Agency, Research Triangle Park, NC, USA.

The very high lung cancer rates in Xuan Wei, China, have been associated with indoor air pollution from burning smoky coal. In recent decades many people have switched from unvented firepits to stoves with various types of chimneys or ventilation. This study addressed the effect on particle composition of cooking with various fuels and combustion methods. Particulate material was collected for 5 days each in 104 homes in Xuan Wei, China. The three primary fuel types were smoky coal (5 communes), smokeless coal (2 communes), and wood. The stove types included firepit, portable stove, high stove, and low stove. In those homes with high or low stove, samples were collected for 3 days with the chimney in place, and for 2 days without the chimney. Samples collected over the 5 days in each home were composited for one analysis per ventilation condition; 183 composited samples were analyzed for benzo(a)pyrene and nickel. The BaP concentrations ranged from 7 to 3200 ng/m3, and the nickel concentrations from 11 to 140 ng/m^3. Both BaP and nickel concentrations were significantly different among the 3 primary fuel types and among the communes within the same fuel type. Stove improvement lead to decreases in BaP concentration, but not in nickel concentration.

128 INDOOR AND OUTDOOR PM10 AND ASSOCIATED METALS AND PESTICIDES IN ARIZONA: NHEXAS FINDINGS

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Appreciable PM10 was found indoors and out in a representative sample of 175 Arizona homes studied as part of Natl' Human Exposure Assessment Survey. Although 50th percentiles were only about 25 micg/m3 from 3-day low-volume impactor samples, indoors and out, the 90th & 95th percentiles indoors were above 50 and 100 micg/m3, respectively. These were significantly greater than micro-outdoor levels. Metals were sufficiently detected from these samples using XRF, ICP and ICP-MS, as will be shown. Chlorpyrifos was above detection levels only above the 75th percentile and contributed a small amount to the multi-media total exposure assessment. (Supported by EPA, though not reviewed by them).

130 ECOLOGIC EXPOSURE CHARACTERIZATION ERROR AND AIR POLLUTION-MORTALITY ASSOCIATIONS.
K Ito[1], GT Thurston[1], and AH Marcus[2]. [1]Nelson Inst. of Environ. Med., NYU School of Medicine, Tuxedo, NY; [2]National Center for Environ. Assess. US EPA, RTP, NC.

In time-series observational studies of air pollution health effects, there remains uncertainty as to how the choice of monitoring sites affect the estimated relative risks (RR). We investigated the relationship between exposure characterization error and air pollution-mortality association using multiple air monitoring stations for PM_{10} (11 sites), O_3 (10 sites), SO_2 (11 sites), NO_2 (4 sites), and CO (4 sites) during 1985-1994 in Chicago-Gary-Kenosha (CGK) Consolidated Metropolitan Statistical Area (CMSA). First, we characterized the ecologic level "exposure error", as determined from the site-to-site variation in daily fluctuations of each of the pollutants. Then, we empirically determined how the estimated error for each site relates to corresponding estimated RR. Poisson regressions were used to estimate pollution effects, adjusting for long-wave and seasonal fluctuations, day-of-week pattern, and non-linear temperature effects. The ranking of "reliability ratios", as estimated by the ratio of variance explained by the multiple site mean ("signal") to the total variance due to signal plus noise at each site was (median reliability ratio in parenthesis): O_3 (0.88) > NO_2 (0.73) > PM_{10} (0.68) > CO (0.53) > SO_2 (0.33). When individual site's data were used in Poisson regressions, the sites with smaller estimated error had larger and more significant RRs, except for NO_2, suggesting that having high correlation with other sites is not sufficient for mortality association. As expected, the average of multiple sites' data generally showed larger RRs than those for individual sites. For example, the estimated RR [95CI] per 50 $\mu g/m^3$ increase in multiple-site average PM_{10} was 1.031 [1.006 - 1.057], which was similar to the RR estimated (1.033 [1.006 - 1.061]) using Simulation Extrapolation method to back calculate error-free estimate, suggesting that averaging over multiple sites reduced error. These estimates are about 30% larger than the median estimate obtained from individual PM_{10} sites' data.

132 THE APPLICATION OF AN OPTICAL PARTICLE COUNTER AND AN AETHALOMETER TO DEFINE PM EXPOSURE SCENARIOS INSIDE COMMUTING VEHICLES.
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A multi-pollutant, in-vehicle study was conducted in California to estimate commuter exposures over 2 hour intervals, especially to PM. To more clearly define the sources of elevated $PM_{2.5}$, a PMS LAS-X optical particle counter and elemental carbon using a McGee Scientific Aethalometer were used in tandem with integrated measures. The LAS-X unit had been specially calibrated for optical response and the particle densities estimate by size, using real California ambient and vehicular aerosols. The tandem continuous monitor combination of particle count by size and black carbon, supplemented with CO data and video information from an on-board camera, greatly enhanced to the ability to characterize the elevated PM exposure scenarios, and their sources. Review of the measurement data and video tapes from the 5 highest $PM_{2.5}$ (of 29 total) commutes, showed that even during trips on heavily traveled freeways, the influence of emissions from single vehicles immediately in front of the test vehicle on in-vehicle concentrations, could be substantial. The signature concentration combinations of a) elevated particle counts in the 0.15 to 0.35 um size range, b) elevated elemental carbon, but c) minimal CO elevation, were consistently identified on the video as resulting from trailing poorly-tuned (usually visibly smoking, and/or odiferous) diesel-fueled, heavy-duty vehicles. Single, high-emitting vehicles (both heavy duty diesels and gasoline powered cars) in front of the test car, accounted for as much as 30 to 50% of the total $PM_{2.5}$ commute-average exposure. Well-tuned (and especially alternative clean fuel) individual vehicles appeared essentially transparent to the monitoring system. Another key finding was the significant reduction in pollutant concentration levels gained by using a carpool lane in Los Angeles.

133 PREDICTING POPULATION EXPOSURES TO PM_{10} AND $PM_{2.5}$
H.Özkaynak, M J Zufall, J M Burke, J Xue, J Zidek, USEPA, National Exposure Research Laboratory, RTP, NC, USA

An improved model for human exposure to particulate matter (PM), specifically PM_{10} and $PM_{2.5}$, is under development by the U.S. EPA/NERL. This model will incorporate data from new PM exposure measurement and exposure factors research. It is intended to be used to predict exposure of the general population and susceptible subpopulations to PM from both ambient and other sources. Two-stage Monte-Carlo simulation techniques will be used to characterize uncertainty and variability in the various model parameters and inputs. The initial version of this model was applied to Vancouver, Canada, following the statistical spatial interpolation of ambient PM_{10} data, to predict the distributions of PM_{10} exposures of both indoor and outdoor origin by cohort, age, activity type and microenvironment category. Exposures in homes were modeled using the information derived from the PTEAM study. The preliminary results showed wide ranges in the predicted personal exposures of various population cohorts due to influences from different human activities and contributions from indoor sources, such as smoking or cooking. Limitations of available data on PM measurements in schools, commuting environments, and different public places were identified as important sources of model uncertainties. Data from a recent review of PM concentrations in various non-residential microenvironments will be used to improve model estimates of indoor PM exposures in offices, schools, restaurants, public places and during commuting. Other planned model improvements include utilization of the results of recent research on: infiltration of ambient PM indoors, the effects of a personal cloud on PM exposure, and linkage with ambient air quality and dosimetric models. This is a proposed abstract and does not necessarily reflect USEPA policy.

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136 DETECTION AND QUANTIFICATION OF NITRIC OXIDE DESORBED FROM AMBIENT AIR PARTICLES AND PARTICLE EMISSIONS FROM VEHICLES.

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In light of the numerous reports of an association between particle air pollution and adverse human health effects, it is important to chemically characterize ambient and source-related particles. Among the gases which might be adsorbed onto such particles, nitric oxide is of interest because it naturally occurs in the human body and has physiological effects such as vasodilation. In this work, NO desorbed from four NIST reference materials, two ambient air particulate samples and two diesel particulate samples, is identified and quantified after heating the samples for one hour. Observed NO concentrations ranged from 0.005 ng NO / mg sample at 37°C to 1900 ng NO / mg sample at 140 °C. In addition, NO desorption was measured from particles collected from six late model spark-ignition vehicles after heating the particles at 120°C for one hour. NO concentrations ranged from 50 ng NO / mg to 560 ng NO / mg particulate sample. Measurements of NO desorption at 37 °C from particles collected from spark-ignition vehicles were not feasible because the particle mass emission rates for current technology vehicles are too low, around 1-2 mg/mile, to permit collection of adequate amounts of particulate material.

138 AEROSOL RESEARCH INHALATION EPIDEMIOLOGY STUDY (ARIES).

Tina Bahadori*, Michael Van Loy, Pradeep Saxena. EPRI, Palo Alto, CA

ARIES is a multi-faceted study in which the disciplines of monitoring, epidemiology, exposure assessment, health assessment, and modeling were considered collaboratively from the inception of study design. A state-of-the-art ambient monitoring will provide epidemiologists with a characterization of aerosol (gas and particle) physical, chemical and biological (aeroallergenic) properties that has not been available to them before. There are four components of ARIES:

- *Air Quality Characterization:* $PM_{2.5}$ mass concentration and composition, as well as related gas-phase and particle-phase pollutants, will be monitored for 18 months. The air quality field measurements also include SO_2, CO, NO, NO_2, NO_y, O_3, HNO_3, NH_3, and VOCs in the gas phase; major ions, including acidity, elements, water-soluble metals, and carbon in the particle phase; pollen and mold; and particle number and size distribution from nanometers to micrometers in diameter.
- *Air Pollution Mortality:* daily mortality data will be collected and analyzed in a multi-pollutant ecological time-series study.
- *Air Pollution Morbidity:* daily data on emergency room visits (for coronary and respiratory symptoms) will be collected from all the large hospitals in the Atlanta area. A parallel study will also be conducted to evaluate the physiologic responses of a group of patients with more severe cardiac conditions.
- *Exposure & Health Assessment:* a personal/indoor/outdoor exposure and health assessment study is planned to aid the epidemiologists in assessing how well ambient measurements can represent personal exposures. The field monitoring for this 18-month study (summer 1998-winter 1999) began in July in Atlanta. This is one of the few studies that will be in a position to provide valuable new monitoring as well as health data in time for EPA's next round of the reviews of the PM standard. EPA has selected Atlanta as its first designated 'Supersite.' The cooperative structure of ARIES allows for and fosters collaboration with EPA in integrating these private and public sector experiments. In this presentation, the design of the study and well as preliminary results will be discussed.

141 CHARACTERIZATION OF HUMAN EXPOSURE TO VERY FINE AIRBORNE PARTICLES

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Introduction and Aims: It is only very recently that attention in the field of airborne pollutants has been focused on very fine particles. At present, the measurement of PM2.5 (particles < 2.5 µm) is widely discussed and used. The different categories of diameter (TSP, PM10, PM4, PM2.5) are selected for a number of reasons, with pragmatic choice being based on the inhalation and exhalation properties of the human respiratory system, as well as technical problems in measuring and sampling particles. The number of particles is probably more important than mass in the pathogenesis of diseases related to airborne particles. Therefore, more attention must be paid to very fine particles with diameters below 1 µm to improve our understanding of the impact of exposure to particulate matter on human health. Once source of very these fine particles (which can penetrate indoor rooms) is combustion processes in car engines.

Methods: Although the load and distribution of aerodynamic diameters can be measured by particle counters, information on particle shape and type is neglected. Therefore, we used the method of isokinetic sampling on nucleopore filters with subsequent electron microscopy including image analysis and statistics.

Results:

Electron microscopy delivers a very detailed picture of air pollution by particles.

Different species of particles can be distinguished and soot particles can be identified.

The concentration and distribution of particle diameters can change over very short distances.

Soot particles change their shape and diameter as they age.

The traffic density in the immediate vicinity strongly influences the number, size and distribution of soot particles.

149 DESIGN OF A HIGH VOLUME IMPACTOR FOR TOXICOLOGICAL AND PARTICLE CHARACTERIZATION STUDIES.

IG Kavouras, ST Ferguson, JM Wolfson, and P Koutrakis. Environmental Science and Engineering Program, Harvard School of Public Health, Boston, MA, USA

The current sampling requirements for collection of ambient particles for toxicological and particle characterization include the following: a) high flow rate (1 m3/min); b) high capacity (one to seven day sample duration); c) sharp size-cutoff curve for the 50% impactor cutpoint; d) minimum particle bounce and particle re-entrainment (to assure valid size discrimination); e) minimum interference from components of the collection substrate; and f) minimum amount of collection substrate material. While some of these requirements are met by some of the existing sample collection methods, none of these methods completely satisfy all of them. We present here the design, development, and validation of a new high volume impaction method

that substantially meets all of the above requirements. The validation studies show high collection efficiencies for a wide range of particle sizes. The new impactor substrate has virtually no interference with typical toxicological testing methods and also has minimal interference with physico-chemical characterizations of the collected particles.

156 PERFORMANCE STABILITY OF THE HARVARD AMBIENT PARTICLE CONCENTRATOR.

Joy Lawrence, Petros Koutrakis, Mike Wolfson, Steve Ferguson and John Godleski. Harvard School of Public Health, Boston, MA. USA

The Harvard Ambient Particle Concentrator (HAPC) has been used routinely for exposure testing for a period of approximately two years. We investigated the stability of concentrator performance as a function of local meteorological conditions, ambient particle concentrations, composition, and size distribution. Concentrator performance is characterized by the concentration enrichment factor (CEF), a ratio of concentrated particle mass (or sulfate) concentration to the ambient concentration. Over two years of normal operation, the mass and sulfate CEFs averaged 27.9 and 28.6, respectively. The CEF was independent of the particle composition and size distribution. The majority of variability in the CEF was found to be related to the HAPC's total operating pressure. The concentrator operates optimally at a pressure drop of 2.5 inches of water per stage. Total negative pressure can range from 7.5 to 25 inches of water, depending on the alignment of the slits of the virtual impactors and other parameters. When the slits are improperly aligned, the operating negative pressure increases and the CEF decreases, mainly due to excessive particle loss by impaction on the edges of the collection slit. Other conditions beside misalignment can result in increased particle losses in the collection slit nozzle and thus in larger negative operating pressures and lower concentration efficiencies. In particular, the combination of high ambient fine mass concentration and high relative humidity can lead to excess particle losses, evidenced by deposition in the collection slit. Increased HAPC stage 3 minor flow negative operating pressure then results from obstruction of the collection slit. The HAPC minor flow pressures are thus monitored carefully, since they are the best indicator of concentration enrichment efficiency.

157 THE CHEMISTRY AND TOXICOLOGY OF FINE PARTICLES, PAHs, AND ORGANIC FREE RADICALS IN SOOT FROM COMBUSTION OF GASEOUS HYDROCARBONS AT PETROCHEMICAL PLANTS AND REFINERIES.

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There are >300 major (i.e., 30,000-60,000 product tons/yr) petroleum refining and petrochemical facilities in Louisiana. A number of these are located in and around Baton Rouge, which lies in the north-central section of what the national media have termed "cancer alley". Routine industrial activity at these sites results in large releases of VOCs , while sustained flaring and other incineration of light fractions and purgates produce a broad size range (0.01 - > 10 μm) of particulates. Work in our laboratories has confirmed that incomplete combustion of C2-C8 gaseous feedstocks can yield substantial amounts of carbonaceous particulates ("soot") and other partially combusted products (≥1% on a weight basis). At the larger plants, this can translate into 100s to 1000s of pounds of soot/yr. Adsorbed to soot from these sources is an array of PAHs including known or suspected human carcinogens (mass = 252 amu), and higher molecular weight PAHs (mass > 276 amu, e.g., anthanthrene, dibenzopyrenes) that are frequently present in substantial amounts (e.g., 1000□ 5000 cigarette equivalents of perylene, benzofluroanthenes+benzopyrenes). If nitrogen oxides are present during or after combustion, nitration of the soot-adsorbed PAHs generates nitro- and polynitro- phenols, toluenes, and PAHs. In many cases, the soot particles also contain a solid state free radical, as detected by ESR analysis. The free radical in 1,3-butadiene soot is stable for more than year in the solid state, extractable into organic solvents (e.g., toluene or DMSO) and stable there for days. The butadiene soot also can oxidize biomolecules (e.g., ascorbate). The toxicological and long-term health consequences resulting from exposures to combustion mixtures of the type described here are unknown. Preliminary experiments in our laboratory have demonstrated that DMSO extracts of this soot are both cytotoxic and genotoxic to cultured human bronchial epithelial cells.

160 A FACILITY FOR CONTROLLED HUMAN EXPOSURES TO ULTRAFINE PARTICLES.

D. Chalupa, F. R. Gibb, P. E. Morrow, G. Oberdörster, E. Riesenfeld, R. Gelein, M. J. Utell, M. W. Frampton; University of Rochester School of Medicine

Ultrafine particles (UFP, less than 100 nm) may contribute to the morbidity associated with exposure to ambient particulate matter. Controlled human exposure studies with UFP are needed to assess their impact on human health, and to determine whether particle number is a more appropriate exposure metric than particle mass for determining health risk. We have developed a facility for experimental exposure of humans to UFP of varying composition, which permits the quantitative determination of the exposure levels, respiratory intakes, and depositions of the aerosol. Particles are generated in an argon atmosphere using an electric spark discharge between two electrodes made of the material to be generated. The aerosol is conducted through a ^{85}Kr deionizer, and mixed with the incoming air in a stainless steel intake manifold. The system consists of an "overflow" line to the exhaust, designed to cope with the excess aerosol produced, and of a parallel conducting line to the non-rebreathing valve, which provides aerosol according to the demands of the subject. The inspired and expired aerosol concentrations can be analyzed in real time. The aerosol analyses are provided by two Condensation Nuclei Counters, sampling at 300 cm^3/min, which provide quantitative data on the particle number concentration, a Particle Size Classifier, and an Aerosol Mass Balance. Aerosol size classification measurements alternate between the inspired and expired side of the subject. Electronic integration of a pneumotachographic airflow transducer continuously provides tidal volume, respiratory frequency, and minute ventilation measurements. These real-time respiratory data, together with the aerosol measurements, uniquely permit quantitative airway deposition measurements of particle number, particle size and particle mass. Particle surface area deposition is a derivative of these measurements. Initial exposures in our laboratory are being conducted with pure carbon, with a count median diameter of 24 nm, GSD of 1.8. Ultrafine metal and metal oxides can be generated using the appropriate electrode and mixing oxygen with the argon.

161 LTRAFINE PARTICLE CONCENTRATIONS IN A HOSPITAL.

E. Riesenfeld, D. Chalupa, F. R. Gibb, G. Oberdörster, R. Gelein, P. E. Morrow, M. J. Utell, M. W. Frampton; University of Rochester School of Medicine

Ultrafine particles (UFP, less than 100 nm) may be important contributors to the morbidity associated with exposure to ambient particles, but few data are available on ultrafine particle numbers in indoor air, where the most susceptible subjects spend the majority of their time. We measured fine particle number (3 μm and smaller), UFP size distribution (0.8 μm and smaller), and total suspended particulate (TSP) mass within Strong Memorial Hospital, a large tertiary care hospital in Rochester, NY, in two locations: the General Clinical Research Center (GCRC), a hospital floor for inpatient research subjects, and an environmental exposure chamber on the same hospital

unit, with filtered intake air. Outdoor air above a construction site just outside the hospital was also sampled. Mass and number concentrations were run continuously in each location over 70-110 hours. Mean±SD particle number concentrations were 3.63±1.15 x 10^3 particles/cm^3 in the GCRC, 5.86±2.11 x 10^3 particles/cm^3 in the environmental chamber, and 3.05±6.65 x 10^4 particles/cm^3 outside the hospital. Fine particle number in the GCRC was almost completely attributable to UFP (3.56±1.93 x 10^3 particles/cm^3). In the GCRC, particle number and mass declined steadily during the evening hours when the unit was less active, with particle number reaching a low of 1.15 x 10^3 particles/cm^3. The highest peaks in particle number (2.78 x 10^4 particles/cm^3) were reached in the morning hours and coincided with intensity of activity on the unit. Although changes in fine particle number generally tracked changes in TSP mass, peaks in one metric were often not reflected in the other. Outdoor fine particle numbers above a construction site were highly variable, and reached peaks of greater than 1.7 x 10^6 particles/cm^3. These data suggest that particle numbers within an acute care hospital vary with intensity of local activity, are dominated by UFP, and are small relative to outdoor particle numbers. Higher particle counts may be present in hospital units dominated by technology, such as intensive care and dialysis units.

167 A PERSONAL ENVIRONMENTAL MONITORING SYSTEM FOR SIMULTANEOUS MEAUSUREMENTS OF $PM_{2.5}$, $PM_{1.0}$, PM_{10}, OZONE, NITROGEN DIOXIDE AND SULFUR DIOXIDE.

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Fixed site outdoor measurements of air pollutants are insufficient for complete characterization of human exposures. There is a need for sampling devices that provide adequate sensitivity and accuracy for personal exposure measurements with little interference with normal personal activities. In order to determine accurately the particulate matter and criteria gases, the measuring devices should be located reasonably close to the breathing zone of the subject. Such a monitoring system has to be sufficiently compact and light-weight in order to not interfere with the normal activities of the subject. A personal environmental monitoring system has been developed, which allows simultaneous measurement of particulate matter and criteria gases. The system, which is based on sampling collection methods previously developed and validated in our laboratory, consists of impactors to remove particles smaller than $PM_{1.0}$ $PM_{2.5}$ and PM_{10} and a single elutriator to minimize entrainment of fibers and other substances related to personal activities. Additionally, passive samplers are integrated into the system to collect ozone, sulfur dioxide, nitrogen dioxide and other pollutant gases. Particulate matter is collected on filters for measurement of mass and trace elements. The system has a modular design with the basic unit for particle mass/elemental analysis and with optional features for simultaneous measurements of particle speciation and passive measurement of pollutant gases. The system uses a single personal sampling pump. Sensitivity is adequate for 24 hr durations. Preliminary results from laboratory tests with reference methods demonstrate the suitability of the developed system for exposure assessment studies.

168 SIZE SELECTION AND ANALYSIS OF INDIVIDUAL PARTICLES FROM 10 NM TO 2 MICRONS.

RV Mallina, AS Wexler, KP Rhoads, and MV Johnston, University of Delaware, Newark, DE USA

RSMS-II is a unique characterization technique that is aimed at analyzing the chemical content of individual airborne ultrafine particles in real time. Although based on earlier versions, the newest implementation offers crucial enhancements including a smart data acquisition system and a completely redesigned particle inlet. The particle inlet is based on a dynamic focusing mechanism that selectively transmits a narrow particle size range in the form of a high speed particle beam. The optimally transmitted particle size is dynamically altered by changing the nozzle source pressure; thus particles over a wide size range may be selected. Inherent in the design of dynamic focusing mechanisms is the ability to size-select particles based on their aerodynamic characteristics; thus obviating the need for additional sizing components. The principle, design and calibration of a variable pressure inlet is presented in the current work. Characteristics are estimated employing a theoretical approach based on the Stokes number definition, and supported with numerical simulations using CFD tools. Results from a preliminary effort in calibrating the inlet using two different sources of monodisperse aerosol are presented. Results indicate that the size resolving capability of the inlet is comparable with that of an Electrostatic Classifier. Finally, the capability of RSMS-II as a characterization technique is demonstrated by analyzing ultrafine atmospheric particles from a moderately polluted episode.

Session 2. Relevant PM Properties Related

006 RESIDUAL OIL FLY ASH (ROFA)-INDUCED PULMONARY INFLAMMATION AND MUCOUS CELL METAPLASIA IN RATS CORRELATES WITH LEACHABLE VANADIUM CONTENT.

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Increases in particulate air pollution are linked with increased hospital admissions and emergency room visits for cardiopulmonary diseases. Intratracheal instillation of residual oil fly ash (ROFA), an emission source particulate, induces pulmonary inflammation, cytokine/chemokine expression, and airway epithelial injury. F344 rats were intratracheally instilled with 0, 50 or 200μg of ROFA containing high (ROFA6) or low (ROFA2) amounts of leachable Vanadium to examine the effect of this transition metal in ROFA-induced inflammation and mucous cell metaplasia. Rats were sacrificed 3 d and 7 d after instillation. Right lung lobes were lavaged and recovered fluids analyzed for inflammatory cells, LDH activity, and total protein content. RNA from right lung lobes was analyzed for MIP-2 and MCP-1 mRNA levels. The left lung was processed for light microscopy and morphometric quantitation of intraepithelial mucosubstances in pulmonary airways. Both ROFA2 and ROFA6 induced dose-dependent increases in recovered neutrophils and lymphocytes that were greatest 7 d after instillation. All ROFA6-instilled rats had significantly more neutrophils than ROFA2-instilled rats. Only ROFA6 induced an increase in LDH activity and total protein levels in lavaged fluids. Both ROFA6 and ROFA2 induced dose- and time-dependent increases in MCP-1 and MIP-2 mRNA levels. ROFA6, but not ROFA2, induced mucous cell metaplasia with significant increases in stored mucosubstances in the surface epithelium lining main axial pulmonary airways. These data suggest that ROFA-associated inflammation and mucous cell metaplasia is dependent on the amount of Vanadium associated with the particles and emphasizes the need to consider the chemical/physical characteristics of ambient air particulates when assessing potential pulmonary toxicity.

014 AUPHEP ON THE WAY.

Austrian Project on Health Effects of Particulates
H Hauck and O Preining, Austrian Academy of Sciences, Clean Air Commission, Vienna, Austria
Interdisciplinary team: Aerosol Physics, Atmospheric Chemistry, Environmental Hygiene, Epidemiology, Paediatric Pulmonology

Goal:
Contribution to answer the question: What is the impact of anthropogenically produced ultrafine particles on the health of the exposed population?
Method:

a) Monitor continuously TPM, PM10, PM2.5, PM1, CN for 12 months.
b) Specify the chemical composition of the size fractions at 2x2 locations.
c) Measure periodically the lung function of toddlers and schoolchildren, each group about 100 in the monitored areas.
d) Investigate the statistics of hospital admissions, doctor visits and other parameters describing the health situation in the monitored areas.
e) Use questionnaires on the respiratory symptoms of elementary schoolchildren (parents).

The project started January 1, 1999, measurements started March 1, 1999, first results may be expected early 2000.

023 EFFECTS EXERTED BY PM IN MINUTES TO HOURS, INVOLVING IMMUNOLOGICAL AND ELECTROPHYSIOLOGICAL MECHANISMS, CAN ACCOUNT FOR EPIDEMIOLOGICAL ASSOCIATIONS OF DAILY MORBIDITY AND MORTALITY WITH 24-HOUR-AVERAGE PM IN AIR.

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Daily airborne PM-10 concentrations within the 150-μg/M NAAQS are associated with morbidity and mortality. The U. S. EPA has changed the size and concentration, but not the 24-hour averaging time, of PM addressed by the standard. We report on emerging evidence of shorter-term mechanisms of PM toxicity, justifying consideration of a shorter regulatory averaging time, such as one hour, in addition to 24-hour and annual averaging times in the NAAQS. PM instilled intratracheally or inhaled caused morbidity in animals within minutes, including apnea and electrophysiological effects in dogs. PM killed rats within one hour to a few hours via electrophysiological mechanisms. In clinical settings, PM effects have occurred in asthmatics during brief exercise or, in one study, rest. Allergenic bioaerosols (fragments of mesquite pollen and dried alfalfa) incapacitated 300 people, many within minutes following exposure outdoors. Cockroach allergen in airborne PM indoors was identified as a major cause of asthma among urban children in the U. S. Daily asthma symptoms were most strongly associated with the maximum one-hour average airborne PM concentration during the day, whereas the association lost strength with dilution into eight-hour and then 24-hour averaging times. In Australia, daily mortality was associated with daily maximum one-hour, but not 24-hour, PM concentrations. Documented PM excursions lasting minutes to hours frequently reached concentrations eliciting effects noted above. We conclude that PM epidemiological effects are consistent with causation by mechanisms of PM action acting over shorter times than the NAAQS now regulates, including electrophysiological and immunological processes. Further research is required into PM toxicity, and into the relative importance of identified mechanisms in contributing to the epidemiological observations. However, real-time PM data and toxicological evidence indicate that, despite 24-hour PM control, potentially health-significant PM excursions occur over shorter time frames, such as one hour, whose regulation therefore should be considered.

054 LABORATORY-GENERATED SURROGATE PARTICLES FOR TOXICOLOGY STUDIES.

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Ann E. Aust, Kevin R. Smith, Utah State University

Studies of toxicological mechanisms are facilitated by being able to perform multiple, replicated biochemical and particle physical characterization assays on a homogeneous sample from a known source. Surrogate particles can be generated in the laboratory under controlled conditions that are selected to test specific toxicological hypotheses. For example, samples of size-fractionated particles from coal combustion, from soils, and from mine tailings have been used in a collaborative project to study the role of iron in the production of reactive oxygen species. Bulk samples of size-fractionated particles have been collected from both a pilot-scale furnace simulating power plant combustion and from a tumbler simulating mechanical attrition of minerals. The samples were enriched in particle size ranges corresponding to the epidemiology literature, that is, over 10 μm, 2.5 to 10 μm, less than 2.5 μm, and less than 1 μm. A preseparator, long-slot virtual impactor, and filter were used to produce 500 mg samples of coal fly ash enriched in submicron particles. Electron microscopy verified

that half of the sample volume was in particles smaller than 1 μm aerodynamic diameter. Work is ongoing to characterize these samples, and to correlate chemical speciation data with iron mobilization under physiologically-relevant conditions.

056 LACK OF CONCORDANCE BETWEEN REPORTED LUNG-CANCER RISK LEVELS AND THE OCCUPATION-SPECIFIC POTENTIAL FOR DIESEL EXHAUST (DE) EXPOSURE.

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Some epidemiologic studies of occupational groups show an association between surrogates of DE exposure and increased lung cancer, but causality has not been established. Causality would be supported by finding a linear relationship between DE exposure levels and reported lung-cancer risk. Although none of the epidemiologic studies had concurrent measurements of DE concentrations, data are available from more contemporary studies. We compared information on the reported lung-cancer risk (Bhatia *et al.*, 1998) with estimated DE concentrations for various occupations (Mine Safety and Health Administration, 1998). Particle concentration measurements cluster the occupations in three, overlapping "order-of-magnitude" groups:

Truck drivers, dockworkers, railroad workers (non-shop)	1's – 10's μg/m³
Bus garage workers, railroad shopworkers	10's – 100's μg/m³
Underground miners	100's – 1,000's μg/m³

There is an approximately two-orders-of-magnitude difference in potential DE particle exposure, yet, the epidemiologic relative risks (RR) cluster in a narrow range. The Bhatia *et al.* summary meta-analysis RR value is 1.33 for all DE occupations, with a range of 1.11 to 1.49 in the subanalysis by occupation. If DE were causally increasing lung-cancer risk by 50% for low exposure occupations (*e.g.* truck drivers, RR = 1.49), then the lung-cancer risk in a more heavily exposed population (*e.g.* railroad shopworkers) should be much higher; however, the shopworkers experienced a RR = 1.08 (Crump, 1998). Similarly, the added lung-cancer risk for bus garage workers (RR = 1.24) is half that of truck drivers, but DE concentrations are considerably higher. Such a lack of concordance between reported risk and estimated exposure argues against a causal role of DE in the epidemiologic associations.

057 A PARALLEL TIME-SERIES STUDY OF AIR POLLUTION IN THE LOS ANGELES AIR BASIN.

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We report on a time-series study looking at the association between daily ambient air pollution and acute admissions to the hospital for cardiovascular (CV), chronic respiratory (CR) and acute respiratory (AR) disease in the South Coast Air Basin (SoCAB) for 1995. A novel approach was used to assign exposure whereby each member of the Kaiser Permanente Southern California (KPSC) health plan in the study area and exposure were mapped into one of 89 - 10 km x 10 km exposure grids. KPSC members were assigned to a grid based on residential zip code and pollutant exposure for each grid was assigned to the grid using either data from a monitor within 5 km, if available, or a interpolation of the three closest monitors. Final exposure assignments were made by joining contiguous grid squares that were highly correlated and had equivalent absolute levels of the primary pollutants into 57 exposure groups. In addition to criteria pollutants, $PM_{2.5}$ data were obtained for a portion of the year and coarse fraction (CF) was calculated by subtracting the $PM_{2.5}$ from PM_{10}. After controlling for meteorologic conditions, and temporal and spatial effects, the RR per 10 unit change of pollutant was significantly associated between CV and PM_{10} (RR=1.2), $PM_{2.5}$ (RR=1.2), CF (RR=3.3) and ozone (RR=2.0). No associations were observed overall for CR or AR diseases. However, strong seasonal effects were observed. For all but NO_2, effects for all criteria pollutants and CF were significantly associated with all three outcomes in the May to September period. In the October to December period only PM_{10} and CF were associated with CV outcomes. While others have found similar associations, our finding of associations with coarse fraction suggest that it may play an important role in adverse health effects.

067 A PRELIMINARY ANALYSIS OF AIR POLLUTION AND MORTALITY OUTCOMES IN PHOENIX, ARIZONA.

TF Mar, JQ Koenig, GA Norris [1] and TV Larson
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The association between elderly mortality outcomes and $PM_{2.5}$, PM_{10}–$PM_{2.5}$, particulate matter elemental concentrations, organic particulate carbon, elemental particulate carbon, particulate matter sources, and gaseous pollutants will be evaluated in Phoenix, Arizona using 3 years of daily data (1995–1997). Phoenix is in the arid southwest and is the 6th largest city in the US with a population of 1.2 million residents. Particulate matter data were obtained from EPA National Exposure Research Laboratory Platform in central Phoenix. Gaseous pollutant data were obtained from the EPA AIRS Database: carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), and sulfur dioxide (SO_2). The three year average ± standard deviation and maximum daily average concentrations of $PM_{2.5}$ was 12.02 ± 6.59 ?g/m³ and 32.62 ?g/m³, CO was 1.46 ± 0.83 ppm and 3.68 ppm; NO_2 was 0.03± 0.01 ppm and 0.05 ppm; O_3 was 27.11 ± 10.43 ppb and 57.01 ppb, and SO_2 was 3.11± 2.15 ppb and 6.32 ppb. Correlation analyses show that $PM_{2.5}$ was correlated with the daily CO ($r = 0.85$), NO_2 ($r = 0.79$), O_3 ($r = -0.50$) and SO_2 ($r = 0.41$). The average number of non-accidental deaths in Phoenix in the zip code region selected for this analysis for all residents and residents older than 65 years were 12 and 8.6 per day, respectively. Poisson regression analysis will be used to evaluate the association between air pollution and elderly non-accidental, ischemic heart disease, and respiratory mortality. The results from this study will address associations between mortality and particulate mass, and non-traditional exposure variables such as elemental concentrations, and particulate matter sources. This is a proposed abstract and does not necessarily reflect U.S. EPA policy.

077 INFLAMMATION CAUSED BY ULTRAFINE CARBON BLACK PARTICLES IS INDEPENDENT OF TRANSITION METALS OR OTHER SOLUBLE COMPONENTS.

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Various workers have suggested that the following characteristics are amongst those that influence the toxicity of PM:- ultrafine particles, transition metals, endotoxin, primary particles, secondary particles. We have examined the potential role of ultrafine particles by using ultrafine carbon black (ufCB 14nm primary particle diameter) and fine carbon black (CB 260nm primary particle diameter) and have demonstrated that ufCB has enhanced ability to cause a number of effects compared to CB. These include:- inflammation after instillation at low mass in rats (at high mass the fine caused more inflammation than the ultrafine), inflammation after short-term inhalation exposure in rats, GSH depletion in alveolar epithelial cells and altered Ca^{++} flux in epithelial cells. It could be that these effects are driven by transition metals associated with the surface of the ultrafine particles. We therefore set

out to determine whether or not ufCB causes inflammation via a mechanism that is dependent on transition metals. We did the following:- tested leachates for transition metal using desferioxamine (DFB); assessed the inflammogenicity of ufCB pre-treated with DFB in the rat lung; leachates of ufCB were tested for ability to cause inflammation; tested the effect of pre-treating ufCB with DFB on its ability to cause Ca^{++} flux in epithelial cells. There was no evidence for a the role of transition metals or any soluble component in the inflammogenicity or pro-inflammogenic effects of ufCB.

081 COMPOSITION OF AMBIENT PARTICULATE MATTER AS DETERMINANT OF CELLULAR RESPONSE.

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We have previously reported that exposure of pulmonary epithelial cells to size-fractionated ambient air particulate matter (PM) activates cell signaling pathways leading to expression of the inflammatory cytokine, interleukin-8 (IL-8). In this study, we further investigated the influence of composition on the cellular response. In Sep., 1997 and Feb., 1998, 114 24-hr samples of coarse (2.5-10 μm) and fine (<2.5 μm, corresponding to PM2.5) particles were collected on Teflon filters from 4 locations in southwestern Taiwan using dichotomous samplers. The concentration of anions (SO_4^{2-}, NO_3^-) and 20 relevant elements were determined using ion chromatography and x-ray fluorescence, respectively. Confluent monolayers of A549 cells were exposed to the coarse and fine particles for 2 hrs at 100 μg/ml. Immediately after exposure, intracellular pH (using the fluorescent dye BCECF), reactive oxygen intermediate (using the fluorescent dye DCF) production, and expression of IL-8 mRNA (using RT-PCR) were measured. On an equal mass basis, fine particles produced greater cellular response than coarse particles in samples collected from a sampling site which was downwind from a major highway. Particle size had no effect for samples collected from other locations. For samples collected from another location next to a highway, cellular responses were correlated with the concentration of sulfur but not with metals. The results suggest a complex relationship between cellular response, physico-chemical characteristics, and measurement sites of ambient PM.

(Supported by USEPA R82-5268-010 and a grant from the National Research Council, Taiwan)

083 HEALTH EFFECTS OF PRIMARY AND SECONDARY PM COMPONENTS IN TWO NEW YORK STATE METROPOLITAN AREAS.

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Epidemiological analyses of hospital admissions and mortality data indicate that adverse effects of particulate matter (PM) air pollution on human health can occur at ambient levels. PM is chemically non-specific, and a determination of its more toxic components may help elucidate possible mechanisms for the observed PM effects. However, to date, few observational epidemiological studies have investigated the impacts of PM components on human health. To address this knowledge gap, an environmental and health effect database was constructed for Buffalo-Rochester and for New York City, NY during 1988-90. Daily counts of both hospital admissions and mortality were grouped into the categories: asthma, respiratory, circulatory and total. Daily measurements of H^+, $SO_4^=$, PM_{10}, O_3, SO_2, NO_2, CO, CoH, temperature and relative humidity were also considered. Poisson and Negative Binomial time-series regression methods, controlling for potential confounders (such as season, day-of-week and weather) were used to determine air pollution-health effect associations. Results from these mortality and hospital admissions analyses indicate consistent air pollution-health effect associations across outcomes and locales. Due to the inter-correlations of the pollutants, a definitive identification of one causal pollutant was not possible. However, $SO_4^=$, an indicator of acidic secondary PM, demonstrated associations coherent across health effect outcomes and consistent across cities. In contrast, CoH, an indicator of the carbonaceous primary particles, yielded the weakest PM associations. For example, for respiratory mortality in NYC, a 33 $\mu g/m^3$ max. minus mean $SO_4^=$ concentration increase was significant (RR=1.20, 95%C.I.:1.04-1.39), while a 1.4 CoH max. minus mean concentration increase was not (RR=1.00, 95%C.I.:0.91-1.09). These results suggest that, in these Northeast U.S. cities, the secondary sulfate aerosol component of PM plays a greater role than primary carbon soot in the adverse health effects of PM.

087 OZONATION OF DIESEL EXHAUST PARTICLES AFFECTS LUNG RESPONSES.

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Ambient particulate matter (PM) has been reported to be associated with morbidity and mortality due to cardiopulmonary toxicity. The components of PM that induce the health effects have not been clearly characterized, but may include organic compounds. Diesel exhaust particles (DEP) constitute part of ambient PM, and have a relatively high organic content. We used DEP to examine the role of organics in inducing lung toxicity. Additionally, the role of co-pollutants in modulating PM -induced toxicity is not well defined. Ozone (O_3) co-exists with PM, and has been documented to react with some organic compounds found on DEP. We examined the effects of ozonation on DEP-induced lung toxicity. Sprague-Dawley rats (male, 60 day old) were intratracheally instilled with saline vehicle, DEP, or DEP previously exposed in vitro to O_3 (48 hr x 0.1 ppm; OzDEP). At 24 hr post instillation, lungs were lavaged, and total protein (TP) levels, LDH activity, and cell differential counts determined in the lavage fluid. With up to 500 μg instilled, both particle types induced significant dose-dependent increases in TP, LDH, and neutrophils (compared to vehicle). However, OzDEP was more potent at 10 μg than DEP in the induction of neutrophilia and an LDH increase. The increased toxicity of the OzDEP was not due to oxidation by air, as air-exposed DEP (48 hr) had the same activity as unexposed DEP. Exposure of DEP to high O_3 concentrations (1.0 ppm x 48 hr) decreased the toxicity compared to OzDEP. Preliminary studies with ^{18}O-labeled O_3 show that the O_3 does bind to DEP, and that the DEP-associated ^{18}O remains bound for at least 24 hr at room temperature. These data suggest that low, ambient concentrations of O_3 can react with DEP to produce an altered, stable particle that is more toxic in a rat model than the unexposed DEP. O_3 and at least some PM may therefore chemically react, and play a role in PM-induced morbidity and mortality. [This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.]

090 RELATING DAILY MORTALITY DIRECTLY TO SPECIFIC SOURCES OF AIRBORNE PARTICULATE MATTER: AN EXPLORATORY ANALYSIS

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We are exploring relationships between daily mortality and the major sources of airborne particulate matter (PM) using a variant of a recently developed approach which employs Factor Analysis (FA) with Poisson Regression (PR). We hypothesize that by adding information on PM chemical speciation and source apportionment to the typical PM health epidemiological analysis, we will be able to identify and evaluate the impact of those PM sources that cause adverse health effects. Previous PM health studies have generally used PM mass or a

single PM component, such as sulfate, as exposure metrics. In our approach, FA is used first to convert multiple, highly correlated chemical speciation variables, such as trace metals, to a smaller number of linearized sums of the individual variables, *i.e.*, a smaller number of factors. The factors, which are related to the sources of the PM via chemical markers, are then used as exposure metrics in Poisson regression with weather variables included for confounder control. The method has been applied to a unique PM data set with extensive chemical speciation, including measurements of nine trace metals, sulfate, particulate organic matter and meteorological data made in three New Jersey cities (Camden, Newark and Elizabeth) from 1981 to 1983 (Lioy and Daisey, 1986). Mortality data for total, cardiovascular and respiratory causes of 1981-1983 were retrieved from the mortality data tapes from the U.S. Department of Health and Human Services. Accidental deaths and homicides were excluded from the total mortality. We have found statistically significant associations(p-value £ 0.10) between daily mortality and several of the seven FA-derived PM factors/sources. These PM sources were residual oil burning, industry sources, sulfate aerosol, and motor vehicles emissions. In Newark and Camden, there were statistically significant associations between total mortality and the factor related to residual oil burning. Cardiovascular death was also significantly related to oil burning in Camden. In Newark, both industrial sources and sulfate had statistically significant associations with total mortality and cardiovascular mortality. The motor vehicle emissions source was a significant predictor of cardiovascular death only in Camden. For Elizabeth, no statistically significant relationships were found between total mortality and any of the source emissions metrics. The relative risks for the FA/PR model are higher than those obtained for simpler PM exposure metrics including inhalable and fine PM mass without consideration of sources.

096 AIR POLLUTION, POLLENS AND HOSPITAL ADMISSIONS FOR COPD IN THE SEATTLE METROPOLITAN AREA.

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Three approaches were used to investigate the association between air pollution, pollens and respiratory hospital admissions in King County. In the first, time series analyses employing generalized additive models were used to investigate the association between pollution, pollens and daily counts of admissions for COPD in King County hospitals between 1987 and 1995. In the second, a cohort of individuals was chosen and a history of admissions for COPD was constructed for each member. The case-crossover design was used to investigate the association between pollution, pollens and admissions in this cohort. Finally, a point process approach, which is a generalization of the case-crossover design, was used for analysis of the same cohort. In single and multi pollutant models, carbon monoxide showed the strongest and most consistent association with admissions, whereas PM showed only weak and inconsistent association with admissions. Tree pollens were strongly associated with admissions.

106 AIRWAY CYTOKINE EXPRESSION AS A FUNCTION OF CHEMICAL COMPOSITION OF INHALED METAL PARTICLES.

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Inhalation of specific metal oxide particles produces cytokine associated changes in airway fluid cells. The expression of cytokines could be due to mechanical particle effects on, and/or chemical interactions with, the airway epithelium. This experiment was designed to test the hypothesis that zinc-oxide particles (ZnO) would result in changes in airway fluid cytokine expression as compared to both magnesium-oxide (MgO) and filtered-air [(FA): Control]. In a counter-balanced, repeated-measures, single-blind design, 10 healthy subjects (3 Females, $\bar{x} \pm$ SD; Age = 32.0 ± 7.3 yr) were each exposed, at rest, for 60 min to each of; 1) ZnO ($\bar{x} \pm$ SD; 20.0 ± 10.0 mg m^3), 2) MgO ($\bar{x} \pm$ SD; 51.9 ± 42.5 mg m^3), and 3) FA. Sputum-induction (3% saline; t = 20 min) was performed 65 h pre- and 6 h post-exposure. Between-condition comparisons were made using the post- minus pre-exposure delta value. For ZnO, there was a significant (P<0.05) increase, compared to both MgO and FA, in TNF ($\bar{x} \pm$ SE; 46.4 ± 18.5 pg ml vs 0.5 ± 1.1 pg ml and 2.1 ± 1.7 pg ml), IL-1β ($\bar{x} \pm$ SE; 444.4 ± 152.7 pg ml vs 0.3 ± 0.8 pg ml and 1.5 ± 1.2 pg ml), IL-6 ($\bar{x} \pm$ SE; 170.2 ± 66.3 pg ml vs -15.1 ± 25.3 pg ml and 0.3 ± 4.5 pg ml), and IL-8 ($\bar{x} \pm$ SE; 3386.9 ± 838.4 pg ml vs 773.7 ± 474.9 pg ml and 392.6 ± 633.6 pg ml). Within ZnO, there was no pre-to post-exposure change in IL-10 or ENA-78. These results indicate that the chemical composition of inhaled metal particles is a primary factor controlling changes in specific airway fluid cytokines.

109 STRONGER EFFECTS OF COARSE PARTICLES IN MEXICO CITY.

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Several recent epidemiologic studies suggest a stronger effect of fine particles ($PM_{2.5}$) than of coarse particulate matter. To examine the support for such a differential effect, we conducted a daily time-series analysis of mortality in relation to measurements of $PM_{2.5}$, PM_{10}, and $PM_{10\text{-}2.5}$ in southwestern Mexico City in the years 1992-1995. A generalized linear model based on Poisson regression was used to control for weather and periodic cycles. The mean concentrations of $PM_{2.5}$ and PM_{10} were 27.4 μg m^{-3} 44.6 μg m^{-3}, respectively, and the mean concentration of $PM_{10\text{-}2.5}$ was 17.2 μg m^{-3}. PM_{10} was highly correlated with both the fine and coarse fractions (correlation coefficients 0.89 and 0.84, respectively), but $PM_{2.5}$ and $PM_{10\text{-}2.5}$ were rather weakly correlated with each other (coefficient 0.52). We used the average concentration of the previous five days as the index of particle exposure because it was most strongly associated with mortality in analyses of lag structure. All three particle size fractions were associated individually with mortality: a 10 μg m^{-3} increase in PM_{10} was associated with a 1.83% increase in total mortality (95% CI -0.01-2.96), and an equal increment in $PM_{2.5}$ was associated with a 1.48% increase in deaths (95% CI 0.98-2.68%). The largest effect was observed for a 10 μg m^{-3} increment in $PM_{10\text{-}2.5}$; mean daily mortality increased 4.07% for each 10 μg m^{-3} (95% CI 2.49-5.66%). These patterns persisted after adjustment for O_3 and NO_2. To assess the independent contribution of $PM_{2.5}$ and $PM_{10\text{-}2.5}$, we added both variables simultaneously to the regression model. The effect of $PM_{10\text{-}2.5}$ was maintained at 4% per 10 μg m^{-3} (95% CI 1.96-6.02%) while the effect of $PM_{2.5}$ virtually disappeared (0.2% change per 10 μg m^{-3}). Our findings suggest that the relative effects of coarse and fine particles on mortality should be examined in more cities with a wider variety of climates, population characteristics, and air pollutants.

119 A LONGITUDINAL STUDY WITH DOGS EXPOSED TO AN ACID AEROSOL.

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To study the effects of atmospheric acidity on lung structure and functions, 8 adult male beagle dogs were daily exposed in 2 whole body chambers for 6 hours and more than a year to an acid aerosol (sodium hydrogen sulfate): 0.95 μm mass median aerodynamic diameter, 2.09 geometric standard deviation, 5.2 mg m^{-3} total mass concentration, 23 μMol m^{-3} particle-associated H^+. Thus, during the entire exposure period the lungs of each dog received a H^+-mass similar to that of a person

breathing lifelong ambient urban aerosols. Another 8 animals served as controls. They were housed in 2 chambers under clean air conditions. To establish baseline data prior to exposure all animals were housed for several months in the chambers while they were ventilated with clean air. The exposure did not cause an influx of inflammatory cells into the lungs. Neither the permeability of the alveolar-capillary membrane nor the integrity of the alveolar epithelium (early indicators of lung injury) were altered. The production of reactive oxygen metabolites by alveolar macrophages such as superoxide was increased after stimulation with opsonized zymosan. However, there was no increase of oxidized proteins in the epithelium surface layer so that no redox-imbalance due to an exposure-related inflammatory response occured. On the other hand, there were changes regarding airway resistance, clearance and lung morphology. However, all these "effects" were subtle effects and therefore of no pathophysiological consequences. Parts of these changes were even compensated during exposure by adaption. In conclusion, it is unlikely that the inhalation of acid ambient particles by healthy adults is associated with a health risk.

126 DAILY MORTALITY AND AIR POLLUTION IN SANTA CLARA COUNTY, CA: 1989-1996.

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Since the last revision of national particulate standards, there has been a profusion of epidemiological research showing associations between particulates and health effects, mortality in particular. Supported by this research, EPA promulgated a national standard for $PM_{2.5}$. Nevertheless, the San Francisco Bay Area may meet this new standard. This study investigates the relationship between daily mortality and air pollution in Santa Clara County (a Bay Area county) using techniques similar to those utilized in earlier epidemiological studies. Statistically significant associations persist in the early 1990s when the Bay Area met national air pollution standards for every criteria pollutant. Of the various pollutants, the strongest associations occur with particulates, especially ammonium nitrate and $PM_{2.5}$. The continuing presence of associations between mortality and air pollutants calls into question the adequacy of national standards for protecting public health.

127 A CHEMICAL AND TOXICOLOGICAL COMPARISON OF URBAN AIR PM_{10} COLLECTED DURING WINTER AND SPRING IN FINLAND.

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Our aim is to compare the chemical characteristics and biological effects of urban air inhalable particles (PM_{10}) collected during this winter and spring in Helsinki, Finland. A high-volume particle collection system has been developed, in which a weekly collection of ambient air PM_{10} is made at 1000 liters/min on a high-capacity substrate. The particles are extracted from this substrate into aqueous solution by sonication. Ambient air quality during the weekly PM_{10} collection periods is characterized by continuous measurements of CO, NO_x, PM_{10}, $PM_{2.5}$ and black carbon. There is also a reference PM_{10} collection of 10 liters/min with the Honeycomb denuder sampler. A chemical characterization of the collected PM_{10} (about 200 mg / week) is made by ion chromatographic analysis of the most abundant ions and by inductively coupled plasma mass spectrometric (ICP-MS) analysis of the most abundant elements. The screening of biological effects is made *in vivo* in male KTL:NIH/S mice, which are intratracheally instilled with 50 µl of the suspension of collected PM_{10}. After 24 hrs the bronchoalveolar airways of exposed mice are lavaged (BAL) and the lungs of some animals are taken for histological examination. The cytotoxic and inflammatory effects of PM_{10} samples are investigated by determination of leucocyte distribution from BAL fluid and of lactate dehydrogenase and protein from BAL fluid supernatant. In addition, the BAL cells are incubated at 37 ¡C for 24 hrs and the production of nitric oxide, TNF-α and IL-6 are determined. The mechanisms of cytotoxic and inflammatory effects of PM_{10} are investigated further *in vitro* by exposing cultured murine RAW 267.4 macrophages to the same PM_{10} samples as used in *in-vivo* screening tests and in chemical characterization.

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144 ACUTE EFFECTS OF AMBIENT AIR PARTICLES AND GASES ON MORTALITY: PRELIMINARY RESULTS OF A META-ANALYSIS

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While there have been several reviews of acute effects of air pollution on mortality, some of which have involved quantitatively combining effect estimates from primary studies, many of these have been restricted in scope with respect to pollutants or geographic areas considered. In this paper, we critically review studies from around the world which link airborne particulate matter and gaseous pollutants to daily mortality. Studies were identified using electronic searches of bibliographic databases, review of reference lists of all relevant articles, contact with selected investigators, and searches of the authors' own files. Inclusion criteria were as follows: publication date≥ 1985, language = English or French, and design = time-series analysis of daily mortality and daily air pollution level expressed as a continuous variable. In order to avoid publication bias, we did not restrict our review to published studies. Data extraction from 77 studies which met the inclusion criteria was carried out independently by two authors. Disagreements were resolved by discussion and reference to a third author if necessary. Studies were evaluated with respect to methods used to adjust for confounding factors, including: weather, other air pollutants, and coincident temporal trends such as season and influenza epidemics. The primary measure of effect was percent excess mortality (PEM) per unit change in pollutant concentration (24h average unless specified otherwise). We quantitatively assessed heterogeneity of effect estimates among studies and calculated pooled effects estimates where appropriate using a random effects model. Our analysis yielded the following pooled estimates of PEM (standard error): PM_{10}, 0.049 (0.011) per µg/m^3; $PM_{2.5}$, 0.082 (0.032) per µg/m^3; hydrogen ion, 0.039 (0.012) per nmol/m^3; sulfate ion, 0.194 (0.037) per µg/m^3; carbon monoxide, 0.001 (0.0004) per ppb; nitrogen dioxide, 0.06 (0.022) per ppb; ozone, 0.041 (0.017) per ppb 1h max.; and sulphur dioxide, 0.058 (0.02) per ppb. Significant statistical heterogeneity among individual estimates was observed for a number of pollutants. The influence of methodological differences between studies, season, cause of death, and age at death are examined.

150 EFFECT OF SIZE-FRACTIONATED AMBIENT PM ON RELEASE OF GM-CSF BY HUMAN BRONCHIAL EPITHELIAL CELLS.

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Pollutants have been proposed as a cause of the increase in asthma in the urban population. Human bronchial epithelial cells (HBECs) are one target for PM and have the capacity to release granulocyte-macrophage colony-stimulating factor (GM-CSF). Importantly, GM-CSF affects the recruitment and maturation of dendritic cells, the most potent antigen presenting cells. This property may result in localized altered immune responses in the airway. We hypothesized that size-fractionated ambient PM would enhance the airway immune response via the induction of GM-CSF in HBECs. HBECs were

derived from endobronchial brushing of normal human volunteers and cultured in serum-free, hormonally-supplemented medium. Size-fractionated ambient PM (<0.18μm,ultrafine, UF; 0.18-1.0μm; and 1.0-3.6μm) was collected from New York City air with a micro-orifice uniform deposition impactor. Particles were resuspended in media and used within 3 days. UF PM elicited a dose-dependent increase in GM-CSF (commercial ELISA) with UF (11 μg/cm^2) eliciting a 7 fold increase in GM-CSF release (n=3, P<0.05). Exposure to larger particles (0.18- 3.6μm) resulted in a significant, albeit only two-fold increase in GM-CSF. We have previously demonstrated that optimal generation of GM-CSF mRNA and protein by HBECs is mediated by a protein kinase C, extracellular-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) pathway. Since GM-CSF release induced by UF was greater than that induced by TNF- or IL-1 (36 vs.15% of maximal PMA response), size-fractionated ambient PM, particularly UF, may stimulate a PKC/ERK MAPK pathway. These data begin to describe mechanisms by which ambient PM may generate an airway milieu conducive to the development of allergic asthma.

151 IS SO_2 A CAUSATIVE FACTOR FOR THE PM ASSOCIATED HEALTH RISKS IN THE NETHERLANDS?

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Associations between serious health risks and PM have been found in numerous studies, including studies in the Netherlands (Verhoeff et al., 1996). More recent European studies have also found associations with gaseous components (Katsouyanni et al., 1997, Hoek et al., 1997), of which SO_2 is one of the gasses. A recent report in the UK (COMEAP, 1998) quantifies that in ambient air SO_2 leads to an increase in total mortality of 0.6% per 10 μg/m^3. Although these statistical associations have been found, it remains questionable whether the associations are causal. A careful analysis of a nine years Dutch time series (Hoek et al.,1997) by successive exclusion of the highest concentrations indicates that SO_2 is probably not causally associated with the health effects, but that it is correlated ('smoking gun'). During these nine years 90 percent of the daily average SO_2 concentrations in the entire Netherlands were under 22 μg/m^3. Including all SO_2 data (N=3068) in the analysis shows an increase in total mortality of 0.7% per 10 μg/m^3. Excluding the 29 days with moving weekly average SO_2 concentrations higher than 50 μg/m^3, shows a mortality increase to 1.4%. Further exclusion of even lower SO_2 concentrations shows a still higher increase in total mortality (2.1%) per unit of mass. These results seem to indicate a higher toxicity for lower concentrations of SO_2. This contradicts with a geographical analysis of the mortality data: Dutch urban situations with a mean SO_2 concentration of 18 μg/m^3 show an increase of 1.1% and rural concentrations of 10 μg/m^3 show an increase in mortality of 0.7%, both calculated for the same unit of mass. Another analysis of the mortality over different years, indicates that 9 years of SO_2 lead to a much lower relative risk than a separate analysis of the last 3 years of SO_2 with the lowest SO_2 concentrations. The conclusion that in the Netherlands SO_2 does not seem to be a causative factor for PM associated health effects will be substantiated by further circumstantial evidence, in combination with biological and physical arguments, indicating that a factor correlating with SO_2 (probably PM) might explain the observed associations with total mortality.

152 CHRONIC INHALATION STUDY IN RATS EXPOSED TO CAR EXHAUST ORIGINATING FROM FERROCENE CONTAINING FUELS.

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Gasoline engine exhaust resulting from the combustion of fuel with 30 ppm ferrocene additive was compared to exhaust from commercial fuel without ferrocene. Rats inhaled the exhaust after dilution at ratios of 1:20 and 1:40 for 18 hrs/day, 5 days/week for up to 24 months plus additional 6 months of clean air. The highest exposure group (1:20 dilution) was the highest exhaust concentration technically feasible in an inhalation study as the limiting factor was the relative humidity of the exposure atmosphere. The exhaust was characterized by particle mass, size distribution and measurement of aliphatic hydrocarbons, polycyclic aromatic hydrocarbons, aldehydes and other components(phenols, ammonia, iron, platinum, NO_x, CO). In defined intervals parameters of clinical chemistry, hematology, bronchoalveolar lavage were measured as well as lung clearance. Histopathological investigations were done in 200 animals per group. In none of the investigations conducted, differences in the toxic effects of the exhausts, deriving from fuel without or with 30 ppm ferrocene, could be detected.
The study was funded by VEBA-Oel AG, Germany

155 MODELING HUMAN EXPOSURE TO PM10 AND CHRONIC BRONCHITIS IN DOWNTOWN MEXICO CITY.

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The objective of this project was to develop parameters to extrapolate from area measurements and time activity patterns the predicted human exposure and related disease. We developed a time activity daily log, that was applied to 600 diverse socieconomically and demographic inhabitants of downton Mexico City. Personal exposure and household indoor monitoring was performed on a subsample of them. Data was collected on housing conditions, job history, duration of residence, and on acute and chronic symptoms. Several indices were developed accounting for ventilation, indoor and outdoor emission factors. Multivariate least square models were developed to predict exposure in those without personal monitoring. Chronic Bronchitis risk models were estimated in the monitored population, and later on with the population without personal monitoring. Accumulated exposure was estimated to those having long term residency and plausible continuous activity patterns. Acute symptoms did relate to exposure, but less so the chronic bronchitis. Models had to be adjusted for several variates. Outdoor concentrations were poorly related to personal concentrations, questioning their use on exact polulation based risk estimates.

169 EFFECTS OF AIRBORNE PARTICLES ON THE RESPIRATORY TRACT OF HEALTHY ADULT RATS.

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Ambient exposure to particulate matter (PM) has been associated with adverse health effects involving the cardiopulmonary system. We wished to determine if exposure to ammonium nitrate (AN) and carbon (C), two common components found in California PM, would affect the respiratory tract of healthy adult rats. Sprague Dawley rats were exposed to filtered air (FA), PM (0.15 mg/m^3 AN and 0.2 mg/m^3 C), ozone (0.2 ppm) or PM plus ozone for 6h/day for 3 days. Randomly selected animals were implanted with bromodeoxyuridine (BrdU) pumps one day prior to the start of exposures. Animals were

examined immediately following exposure for changes in reduced gluthathione levels (GSH) and cell permeability along the airways and lung parenchyma. Animals implanted with BrdU pumps were examined 18 hr later for cell proliferation within site-specific regions of the lung airways and parenchyma. Immediately following 3 days' of exposure, GSH levels and cell permeability failed to demonstrate significant changes from the FA controls. The main axial airway path of the right infracardiac lobe was examined in paraffin sections prepared for immunohistochemistry to determine anti-BrdU labeling. The frequency of BrdU uptake into cells was determined as a labeling index for epithelial cells along the airways as well as at airway bifurcations. Interstitial cell labeling for each of these same regions was also determined. The percentage of labeled epithelial cells along the airways was 2.5 ± 0.5% (mean ± SEM) in FA and was not significantly changed following exposure. In contrast, the labeling index of epithelial cells on airway bifurcations was significantly increased 2-fold following exposure to PM and PM plus ozone. The percent of labeled interstitial cells was also significantly increased following exposure to PM plus O_3. At the level of the terminal bronchiole (TB) a significant increase in epithelial labeling index was noted only in the lungs of animals exposed to ozone. However, alveolar regions within 400 μm of the TB, significant increases in the total parenchymal cell labeling index were observed for all treatments groups compared to FA: FA 5.2±0.5, O_3 10.1±1.6, PM 8.7±1.1, p=0.01; PM+$O_3$9.0±1.4, p=0.03. We conclude that (1) AN and C cause injury to the lungs and (2) airway bifurcations and central acinar regions are important sites of injury to inhaled particulate matter.

Session 3. Mechanism Related

012 A PILOT STUDY OF CONTROLLED HUMAN EXPOSURES TO CONCENTRATED AMBIENT FINE PARTICLES IN METROPOLITAN LOS ANGELES.

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We installed a particle concentrator (Sioutas et al., J Aerosol Sci 1993; 28:1057-71), with $PM_{2.5}$ size-selective inlet and 2 virtual impactor stages, in a movable human exposure lab (Avol et al., JAPCA 1979; 29:743-5) in Los Angeles. Concentrated ambient fine particles (size range 0.1-2.5 μm) are supplied to a modified body plethysmograph of 2000-L volume at flow 220 L/min and pressure 10 cm H_2O below atmospheric. Multiple pilot exposures, lasting 20 to 120 min with 15-min exercise, were performed with an investigator (male, age 53, with ectopic heartbeats but no known cardiopulmonary disease) as subject. Chamber $PM_{2.5}$ concentrations, measured by nephelometer with allowance for humidity artifacts, ranged from 100 to 200 μg/m³, typically 7-8 times outdoor concentrations. Ammonium nitrate and organic carbon accounted for >80% of total mass. In-chamber concentrations were 25% below inlet concentrations due to inhalation and wall losses; gradients within the chamber were small. In-chamber temperature remained comfortable with no special controls under existing mild weather conditions. No changes in lung function, blood pressure, or arterial Q saturation occurred during exposures. More frequent ectopic beats occurred during some studies, but were difficult to interpret because of unblinded exposures and lack of appropriate control studies. The only reported symptoms were transient mild substernal irritation after exercise on one occasion, and mild ear discomfort from pressure changes upon opening or closing the chamber. Conclusions: Concentrated fine particle exposures of human volunteers are feasible in Los Angeles; more pilot tests will be conducted to refine the methodology.

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020 INHALATION OF FINE PARTICULATE MATTER CONTAINING HYDROGEN PEROXIDE INDUCES TISSUE INJURY AND MODIFIES THE ACTIVITY OF ALVEOLAR MACROPHAGES.

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Inhalation of fine particulate matter (PM) has been positively correlated with mortality and morbidity in humans. The overall objective of our studies is to analyze the role of particle-associated peroxides in fine PM-induced toxicity. An aerosol consisting of ammonium sulfate (AS), a major component of fine PM in the eastern US, with and without hydrogen peroxide (H2O2) was used as a model. The aerosol was generated by atomizing an aqueous solution of AS with and without H2O2. The aerosol was then conditioned to 85% relative humidity and introduced into a 6-port nose-only exposure chamber. The resulting aerosol contained 420μg/m³ AS with a particle number distribution that peaked at 0.46 μm in diameter, as measured by Lasair optical particle sizer, with and without 18 ppb of gas-phase H2O2 in equilibrium with the particle phase. Gas-phase and particle-phase H2O2 concentrations were calculated using total peroxide concentration, Henry's law constant and aerosol water content. Sprague-Dawley rats were exposed to particle-free air, gas phase H2O2, or aerosols of AS or AS+H2O2 for 2 h. Rats were sacrificed 22 h post-exposure and bronchoalveolar lavage (BAL) fluid and cells collected. We found that BAL fluid from rats exposed to particle-free air contained 104μg/ml protein and 96% alveolar macrophages (AM). Treatment of rats with AS or H2O2 alone had no effect the number, viability or composition of cells or protein levels in BAL fluid. Lactate dehydrogenase levels in BAL fluid and serum were also unchanged relative to air exposed rats. In contrast, exposure of rats to AS+H2O2 significantly increased the total number of AM (2-fold) as well as protein in BAL fluid (30-35%). These data indicate that AS+H2O2 induces tissue injury. To determine if AM functional activity was altered by the exposures, we quantified cellular oxidative metabolism. Inhalation of AS aerosol, gas phase H2O2 or AS+H2O2 resulted in decreased production of superoxide anion as well as nitric oxide by AM relative to cells from air exposed rats. Decreased production of cytotoxic oxidants by AM following PM exposure may underlie increased host susceptibility to infections (Health Effects Institute and NIH NRSA ES05810).

021 AMBIENT PARTICULATE MATTER ($PM_{2.5}$) INDUCES DOSE-RELATED CHANGES IN PROTOONCOGENE AND PROAPOPTOTIC GENE EXPRESSION IN MURINE ALVEOLAR TYPE II EPITHELIAL CELLS

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Our recent work shows that $PM_{2.5}$ causes activation of the cJun kinase signaling cascade and transcriptional activation of AP-1-dependent gene expression in lung epithelial cells *in vitro* (Timblin et al., Cancer Res 58: 4543-4547, 1998). In studies here, we employed a differentiated murine alveolar type II epithelial cell line (C10) to examine patterns of AP-1 binding to DNA and expression of AP-1 family genes and apoptosis-associated genes using a ribonuclease protection assay in cells exposed to noncytotoxic (10 μg/cm²) and apoptotic (50 μg/cm²) concentrations of $PM_{2.5}$. $PM_{2.5}$ samples were collected on filters from the Burlington, VT monitoring station, characterized for their size and inorganic elemental surface chemistry, and added to confluent cultures of C10 cells for time periods of 8 and 24 hrs. At low concentrations, $PM_{2.5}$ increased levels of *c-jun*, *junB*, *junD*, *c-fos*, *fra1*, and *fra2* mRNAs after 24 hrs of exposure. At higher doses of $PM_{2.5}$, increased mRNA levels of several proapoptotic genes, including caspase 8, FAS, and FADD, were observed. We also demonstrate using the oxidant probe, 2'7'-dichlorofluorescein, that oxidative stress precedes increased expression of AP-1 family genes, i.e. *fos*, *jun*, and proapoptotic genes. We are presently confirming these observations in transgenic AP-1-luciferase reporter mice exposed to $PM_{2.5}$. Our studies suggest that $PM_{2.5}$ initiates signaling pathways in epithelial cells of the lung that are dose-dependent and may be related to oxidant-dependent proliferation, cell injury and survival. Research is supported by grants (R01 ES/HL09213; R01 39469; R01 522563) from NIEHS and NHLBI.

024 PARTICLE-ANTIOXIDANT INTERACTIONS IN EPITHELIAL LINING FLUID

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Many of the absorbed materials on the surface of particles are recognised oxidants. These oxidants probably interact with antioxidants present in lung epithelial lining fluid (ELF). These reactions have the potential to both modify the character of the particles as well as decreasing the antioxidant screen in the airways. To examine this possibility we investigated the ability of 5 well characterised particles (3 carbon black particles [CBPs] plus amorphous and crystalline silicon dioxide [SiO_2]) to modify the ELF antioxidant screen using a model exposure system. A composite antioxidant solution (urate [UA], ascorbate [AA], and reduced glutathione [GSH] at concentrations of 200, 200, and 400 ?mol/L, respectively) was incubated with particles (150μg/ml) at 37°C for up to 6h under mixed conditions, and antioxidant consumption followed with time. The following observations were made: (1)UA was not consumed by any particle.(2) AA was consumed in a near-linear fashion, with time by all CBPs, but not by either form of SiO_2. Significant differences in the rate of AA consumption by the 3 CBPs were noted, and could be ascribed to differences in their surface area and chemical composition.(3) Complex GSH consumption kinetics were noted with all CBPs but no GSH depletion was

observed by SiO_2 particles. Similarly to AA, GSH consumption rates by the 3 CBPs differed significantly.(4) Addition of chelators reduced AA consumption rates associated with all three CBPs, but had no effect on GSH consumption rates. Together, these data demonstrate that particle size and surface area are important factors when considering particle/antioxidant interactions in the airways. Moreover, these data demonstrate that both GSH and AA represent significant substrates for CBPs. Deposition of CBPs within the respiratory tract lining fluid may therefore compromise the airways antioxidant defensive screen and accelerate epithelial injury.

029 EXPOSURE TO COAL FLY ASH, BUT NOT URBAN PARTICULATE MATTER, CAUSES PERIPHERAL AIRWAYS HYPERREACTIVITY IN DOGS. A N Freed, S McCulloch and T Myers. The Johns Hopkins Medical Institutions, Baltimore, MD

The purpose of this study was to examine the immediate and delayed changes in canine peripheral airway function that result from local insufflation of coal fly ash (CFA) and urban particulate matter (UPM). A bronchoscope was used to isolate sublobar airways in anesthetized dogs and record peripheral airway resistance (R_p). After testing airway reactivity to aerosolized histamine, either a 5 or 10 mg dose of dry CFA or UPM was delivered directly into a sublobar airway. The immediate effect on R_p was recorded, and R_p and airway reactivity were recorded at 24 h and one week after the local particle exposure. The experiment was repeated using bronchoalveolar lavage instead of airway reactivity as the endpoint. Five mg of either CFA or UPM produced little if any immediate effect on R_p or on airway reactivity 24 h later, but resulted in significant neutrophilic inflammation at that time. The 10 mg dose of CFA significantly increased R_p immediately after exposure, and resulted in airways hyperreactivity and neutrophilia 24 h later. Unlike CFA, UPM did not alter R_p immediately after exposure, nor did it alter airway reactivity at any time point thereafter despite the development of significant neutrophilic inflammation at 24 h post exposure. Unlike CFA, the inflammatory response to UPM appeared to be dose-dependent, and UPM increased the total inflammatory cell number recovered in BALF. These data suggest that local exposure to CFA causes airway hyperreactivity in a dose-dependent manner, and this increase in airway reactivity appears to be unrelated to neutrophilic inflammation. We speculate that the presence of endotoxin in the UPM but not the CFA sample accounts for the different functional effects produced by these two PM samples;*i.e.*, endotoxin may inhibit the development of PM - induced hyperreactivity.

036 A CARDIOPULMONARY RAT GENE ARRAY FOR SCREENING ALTERED EXPRESSION PROFILE IN PARTICLE-INDUCED LUNG INJURY.
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Lung injury and repair processes involve cellular events, including inflammation, cell growth, differentiation and remodeling of extracellular matrix. These processes are governed by coordinated expression of multiple mediators. Literature survey shows that more than 200 genes are implicated, either directly or indirectly in varied cardiac and pulmonary diseases. In order to analyze the tissue expression profile of a large number of genes, we developed a mini-gene array filter using the gene array technology. This array contained 30 genes representing inflammatory and antiinflammatory cytokines, growth factors, adhesion molecules, stress proteins, transcription factors and antioxidant enzymes. Using rat gene specific PCR primer pairs, cDNAs for these 30 genes were amplified and cloned into TA vector. Plasmids with recombinant cDNA inserts were purified and blotted onto a nylon membrane. Total RNA was isolated from lung tissues recovered from rats exposed by intratracheal instillation to saline, residual oil fly ash (ROFA; 8.8 mg/kg) or its equivalent metallic constituents found in one instillate of ROFA: nickel (nickel sulfate; 3.3 µmol/kg) and vanadium (vanadium sulfate; 5.7 µmol/kg). ^{32}P-labeled cDNA was generated using total RNA in a reverse transcription reaction and subsequently hybridized to the array blots. Densitometric scans of the blots revealed a 2 -3 fold induction of adhesion molecules and growth factors, such as VCAM, fibronectin-EIIIA and PDGF-AA ligand. The altered expression observed in this array screening is being confirmed by northern blot analysis. Developing a customized gene array to study tissue specific markers provides a quick tool to screen for gene expression profile of the target tissue. (This abstract does not reflect EPA policy).

037 METALS IN THE INDUCTION OF APOPTOSIS OF HUMAN AM
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We reported that residual oil fly ash (ROFA) particles and the well characterized particles 1648 and 1649 (NIST) can induce apoptosis of human alveolar macrophages (hAM) in a dose and time dependent manner. ROFA particles were more bioactive and contain greater amounts of soluble metals than ambient particulate matter (PM), supporting the hypothesis that metals on PM may be an important bioactive component. In order to further test this hypothesis we evaluated the ability of four different ROFA particles to induce apoptosis and necrosis of hAM. In addition, dose response curves for $NiCl_2$, Na_3VO_4 and $VOSO_4$ were also examined. Since scavenger receptors (SR) appear to play an important role in the recognition of particles and induction of apoptosis in AM, the role of SR in ROFA induced apoptosis was examined. The results indicated that there was a difference in the relative potency of the four ROFA particles to induce apoptosis and necrosis of hAM. However, this toxicity did not correlate with the soluble or total fraction of metals or the individual amounts of Ni, Fe, or V. The dose responses of the metal solutions resulted in a relative potency of $VOSO_4 > Na_3VO_4 > NiCl_2$, indicating that the form of the metal is also important. However, the concentrations of individual metals causing apoptosis were 10-20X higher than the soluble metal fractions present in the ROFA particles. Finally, the toxic effects of ROFA were able to be partially blocked using polyinosinic acid (SR antagonist) without affecting the phagocytosis of the ROFA particles. Taken together, these results suggest that while metals such as Ni and V can cause apoptosis of hAM, they do not appear to account for all of the effects of ROFA and probably not for ambient PM as well. This work was supported by EPA grant G8K10427.

043 OXIDATIVE EFFECTS OF INHALED PARTICULATE MATTER: INFLUENCE OF EXTRACELLULAR LINING FLUID ANTIOXIDANTS.
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Residual oil fly ash (ROFA), an airborne particle emitted from oil burning power plants, contains soluble Ni, V, and Fe, and appears to cause an injury that is at least partly oxidative in nature. Since most Inhaled particles first make contact with mucus or surfactant, we have synthesized an artificial lung lining fluid (ALL) and exposed it to concentrations of ROFA that might be encountered in the human lung. ROFA catalyzes oxidation of lipids and proteins in ALL which is detectable as incorporation of ^{18}O following exposure to 20% $^{18}O_2$ or $H_2{}^{18}O_2$ (<200 µM). In this study, we altered concentrations of GSH (5-500 µg/ml), ascorbate (C) (5-500 µg/ml) and ROFA (<200 µg/ml) in a matrix design to determine whether certain antioxidant ratios might pose greater risks to oxidative stress. We found that high [C] at any [GSH] and high [GSH] combined with low [C] led to the greatest oxidation from 20% $^{18}O_2$ and ROFA (4 hr. exposure, 25°C). A 10 fold range in oxidation was observed. $H_2{}^{18}O_2$ induced oxidation (25°C, 1 hr) followed a different pattern: C decreased oxidation in a dose dependent fashion, while GSH had little or no effect at any concentration. The dose response behavior of ROFA (10-200 µg/ml) confirmed that antioxidants either diminish or enhance oxidation, depending on concentration and oxidant, with [C] being the most critical factor. Based on these results and previous measurements we and others have made of actual levels of antioxidants in lung lining fluids under different conditions, we suspect that allergic responses, or excess consumption of vitamin C, might predispose individuals to ROFA -induced oxidation and injury.
(Abstract funded by U.S. E.P.A., and does not reflect EPA policy).

044 AIRWAY EPITHELIAL CELL RESPONSES TO METAL CONSTITUENTS OF PARTICULATE MATTER (PM) AND THE POTENTIAL FOR METAL INTERACTIONS

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Uncertainties exist as to whether or not ambient air PM metal content contributes significantly towards the health effects epidemiologically associated with PM exposure. In addition to better characterization of PM chemical constituents — one must also elucidate the toxicodynamic consequences of metal exposure on a pulmonary cell specific basis. A wide variety of transition metals have been detected in particulate material, be it of emission source or ambient air origin. Furthermore, the quantities and combinations of metals vary considerably. The purpose of these experiments, therefore, was to determine the relative effects of a variety of individual transition metals on the airway cells, and to begin to elucidate biologic effects of specific metal "mixtures". Rat tracheal epithelial (RTE) cells in primary culture were used as a model for differentiated airway epithelial cells. Results demonstrated that of the three principal transition metals contained in a residual oil fly ash (ROFA) sample, namely iron (Fe), nickel (Ni), and vanadium (V), RTE cells were most sensitive to the effects of V in terms epithelial cell gene induction and production of inflammatory cytokines, altered solute permeability, and overt cytotoxicity. The addition of Ni to V-exposed cells, neither abrogated nor enhanced V-induced effects. However, exposure to fly ash samples containing similar quantities of V with negligible Fe or Ni content, resulted in significantly greater cytotoxicity than exposure of cells to a V+Fe+Ni-containing fly ash sample. Results suggest that other metals present (e.g., iron) were inhibiting the overall airway toxicity of V. Additional studies have focused on two of the principal metals recovered in water extracts of ambient PM collected on high volume filters, namely zinc (Zn) and copper (Cu). Results demonstrated that significant increases in epithelial permeability and cytotoxicity occurred after exposure to a combination of Zn + Cu, at concentrations that were not cytotoxic on an individual metal basis. Thus, the overall biologic effects of the various metals contained in PM are likely to be complex. The RTE cell culture system, however, provides a useful model with which to determine potential mechanisms by which metal exposure may result in airway injury and inflammation. (This abstract does not reflect US EPA policy).

046 PARTICULATE MATTER AND CHARGED SYNTHETIC POLYMER MICROSPHERE ANALOGUES ACTIVATE BRONCHIAL EPITHELIAL CELLS AND SENSORY NOCICEPTIVE NEURONS.

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Residual oil fly ash (ROFA), an industrial particulate pollutant, causes airway inflammation and *hyperresponsiveness* in rodents. Recent data showed that exposure of human bronchial epithelial cells (BEAS-2B) to ROFA elicited inflammatory effects (e.g. increases in intracellular calcium, cytokine transcript and release), which were inhibited by capsazepine (CPZ), an antagonist of capsaicin receptors, and by amiloride, an antagonist of acid receptors (Veronesi et al., Toxicol. Appl. Pharmacol., 154(1),1999). In this study, we examined how physicochemical characteristics of particles differentially contributed to inflammatory changes recorded in culture. Electrophoresis showed that field ROFA particles carried a negative surface potential, as indicated by their zeta potential of -28 mV. Synthetic polymers microspheres (SPM) were synthesized, that resemble ROFA particles with 3-7 ?m diameter and a zeta potential of -29 mV. The effects of ROFA and SPM were examined on BEAS-2B cells and sensory dorsal root ganglion (DRG) neurons. ROFA and SPM caused an immediate increase in intracellular calcium. In addition, exposure of BEAS-2B cells and DRG neurons to ROFA or SPM caused the release of IL-6, which was inhibited by CPZ and amiloride. In both cell types capsaicin and pH 6.5 increased intracellular calcium and caused the release of IL-6 in a receptor-mediated fashion. The SPM-induced $[Ca^{2+}]i$ responses were correlated with the presence of acid-sensitive and/or capsaicin receptors in DRG. We propose that the acidic environment associated with the negatively charged colloidal particles can activate capsaicin and acid receptors. Activation of these irritant receptors triggers the subsequent release of inflammatory cytokines and neuropeptides which initiate and sustain neurogenic inflammation in the airways. (This abstract does not reflect EPA policy.)

047 PHYSICOCHEMICAL COMPONENTS OF PARTICULATE MATTER CONTRIBUTE DIFFERENTIALLY TO IMFLAMMATORY RESPONSES IN SENSORY NEURONS.

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Particulate matter (PM) consists of complex aggregates of elemental and organic carbons, metals, sulfates and microbial contaminants. Itsheterogeneous composition has complicated identifying the mechanism(s) which underlie symptoms of airway inflammation. We examined the physicochemical characteristics of various urban and industrial PM in relationship to their inflammatory effects on cultured sensory dorsal root ganglion neurons (DRG) and human bronchial epithelial cells (BEAS-2B). The components of various suspended PM were separated by centrifugation and filtration, and characterized in terms of particle size, surface charge and pH in buffered solutions. The results show that most PM particles remain acidic in solution, even after multiple washings. The different PM contained particles of variable sizes, including ultrafine particles. The suspended PM particles, their washed particle cores and the ultrafine particles, obtained after 22 μm filtration, contained variable surface charges as indicated by their zeta potentials. Exposure of DRG to these components of PM caused a differential release of the proinflammatory cytokine IL-6 depending on the source and the fraction of PM tested. Finally, pretreatment of DRG and BEAS-2B cells with antagonists for the capsaicin receptors (i.e., capsazepine) or acid-sensitive receptors (i.e., amiloride or benzamil) inhibited the PM-induced IL-6 release in a differential manner. Collectively, these data support our hypothesis, that PM initiate inflammation by activation of capsaicin and acid-sensitive irritant receptors located on various airway target cells. In addition, the data indicate that both soluble, and charged particle components of PM contribute to the inflammatory effects. *(This abstract does not reflect EPA policy.)*

051 PULMONARY TOXICITY OF PARTICLES COLLECTED FROM THE UTAH VALLEY.

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Recent epidemiology studies have shown an association between short-term ambient PM_{10} pollution and cardiopulmonary-related mortality/morbidity. Hospital respiratory admissions in the Utah Valley have been associated with PM_{10} levels from April 1985 - February 1988. During that time, a steel mill which was a major contributor of particles in the valley closed and reopened. We tested the hypothesis that particles sequestered from Utah Valley filters during this time period would demonstrate differences in metal content, oxidative stress, and lung injury after instillation in humans. Aqueous extracts from the filters were lyophilized. Metal concentrations were increased before the mill closed and after its reopening relative to when it was not functioning. Corresponding to metal concentrations,*in vitro* measures of oxidant generation (thiobarbituric acid reactive products of deoxyribose) were increased in particles collected from filters before the mill closed and after its reopening Finally, after bronchoscopic instillation of 500 g into a lingular subsegment, the influx of inflammatory cells were similarly increased in particles collected from filters before the mill closed and after its reopeningrelative to when the mill was not operational. We conclude that the inflammatory lung injury in human volunteers after exposure to an aqueous extract collected from a filter can correspond to metal concentrations and oxidant generation. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

053 MECHANISMS OF PARTICLE EFFECTS ON HUMAN LUNG EPITHELIAL CELLS.

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The long term objective of this research is to identify the characteristics of ambient air particulates responsible for and mechanisms which contribute to specific biological effects in human airway epithelial cells in culture. Particulate air pollution contains iron, and some of the pathological effects after inhalation may be due to reactive oxygen species produced by iron-catalyzed reactions. In the studies presented here coal fly ash was generated and size fractionated to investigate whether the bioavailability of iron from the particles was related to the amount of iron present, the size of the particles, or the source of the coal. The size fractions examined were fine (<2.5 μm), coarse (2.5-10 μm), and >10 μm. Human lung epithelial cell (A549) were treated with coal fly ash, and the amount of the iron storage protein ferritin was determined after 24 h. Ferritin levels increased by as much as 11.9 fold. The amount of bioavailable iron was related to the coal being combusted and the size of the particles (with fine having the most bioavailable iron), but not the total amount of iron in the particles. Work is currently underway to characterize the iron speciation in these samples. We are also investigating the particle characteristics which might be responsible for the induction of inflammatory mediators in A549 cells. We have found that the coal fly ash will induce the production of interleukin 8 (IL-8) and prostaglandin E_2 (PGE_2) after exposure for 24 h and are currently investigating the particle characteristics responsible for this induction. This work was supported by a grant from the Health Effects Institute.

070 PULMONARY INFLAMMATION INDUCED BY IRON-CONTAINING PARTICLES.

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Reactive oxygen species (ROS) catalyzed by transition metals associated with inhaled particles may in part be responsible for acute inflammatory effects of particulate air pollution. We instilled catalytically active Fe_2O_3 particles made in our laboratory into the lungs of healthy human volunteers (n=10) and found a transient influx of neutrophils (PMN) (27.1 ± 6.7% vs. 2.5 ± 0.5% in control), and transient elevations in total protein, lactate dehydrogenase (LDH) and Interleukin-8 in bronchoalveolar lavage fluid (BALF) at one day post-instillation (PI). The method employed to make the particles (hydrolysis of $FeCl_3$), led us to suspect that ROS catalyzed by residual soluble iron (Fe^{3}), or an intermediate oxide of iron capable of participation in Fenton-catalyst reactions, was the cause of the inflammation. These particles were compared to a commercial iron III oxide (Fe_2O_3) for 1) soluble iron content, 2) oxidant generation assessed by formation of thiobarbituric acid (TBA) reactive products of deoxyribose, and 3) induction of pulmonary inflammation in rats. We found soluble iron (240 ± 3.0 ng sol. Fe/mg of particles) associated with our particles but not with the commercial Fe_2O_3. Significantly greater levels of TBA adducts were associated with the particles than with the commercial product (OD = .194 ± .006 vs. .008 ± .001 respectively, $p<0.05$) indicating greater oxidant generating capacity. Intratracheal instillation of the particles in rats induced an influx of PMN in BALF at one day PI, but not the commercial Fe_2O_3 product. Thus, residual soluble iron and associated oxidant generation was likely responsible for the acute inflammation seen in the human volunteers. These findings support the hypothesis that iron associated with particulate air pollutants may induce ROS and inflammation in the lungs of humans. This abstract does not necessarily reflect EPA policy.

082 EFFECT OF ACUTE EXPOSURE TO ACID AEROSOL ON HEART RATE.

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A number of studies have shown that exposure to elevated levels of particulate air pollution (PM) is associated with a small but significant increase in heart rate. The physio-chemical properties of PM that cause this increase are not known. The purpose of this project was to determine whether inhalation of sulfuric acid aerosol, a component of PM, affects heart rate. Male F344 rats (8 mo old) with implanted EKG transmitters were exposed to air (n=5) or acid aerosol (n= 6) for 6 hours. The target concentration of the acid was 200 μg/m3 and the particle size was < 0.3 μm . Heart rate was monitored every 5 min for 24 hr before, during, and for 18 hrs after exposure. Following exposure, the acid-exposed rats had a small but significant (P<0.004) increase in heart rate over pre-exposure baseline vaues as compared to air-exposed rats. This increase persisted for the entire 18 h post-exposure monitoring period. There was no change during exposure. These results are preliminary and need to be replicated. However, this data suggests that acid aerosols can cause a persistient small increase in heart rate.

Post Exposure Change in Heart Rate from Baseline (mean ± SEM)

Exposure	1-6 hrs	7-12 hrs	13-18 hrs
Air	20.1 ± 6.5	-0.9 ± 4.3	-18.4 ± 3.5
Acid	37.6 ± 5.0	16.0 ± 3.8	-1.1 ± 4.7

100 OXIDATIVE DNA DAMAGE AS MEASURED BY 8-OXO-7,8-DIHYDRO-2'-DEOXYGUANOSINE FORMATION IN THE DNA OF HUMAN AIRWAY EPITHELIAL CELLS AFTER EXPOSURE TO OIL FLY ASH PARTICLES.

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Epidemiologic studies have shown causal relationships between ambient air pollution particles and adverse health effects in susceptible subpopulations. Residual oil fly ash (ROFA) particles are a component of ambient air particulate pollution, and ROFA may contribute to the effects of air particulates. Using x-ray fluorescence (XRF) analysis, we analyzed ROFA along with several other particles for elemental content and their solubility. The major proportion of elements in most particles were insoluble Si, Al, Ca, and Fe. In contrast, ROFA had high concentrations of soluble V and Ni. Kinetics and formation of oxidative DNA base lesion, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) in response to metal species V (vanadyl and vanadate forms), Ni(II), Fe_2O_3, and ROFA in aqueous buffered suspensions containing 2'-deoxyguanosine (dG) in presence of molecular oxygen was measured by HPLC/UV-EC detection. Metal species catalyzed maximum 8-oxo-dG formation at 15 min with yields ranging from 0.05 to 0.17% respectively. Insoluble Fe_2O_3 had no effect. Consistent with these results ROFA particles rich in soluble V and Ni concentrations showed highest levels of 8-oxo-dG with 1.25% yield. DMSO, a hydroxyl radical scavenger inhibited, and metal ion chelators, DTPA, ferrozine and deferoxamine, blocked the 8-oxo-dG formation. ROFA particles both in cell-free system and response to human airway epithelial cells induced 8-oxo-dG in a dose dependent manner. The results suggest that soluble metals associated with particles can catayze 8-oxo-dG formation and this DNA damage may contribute to particulate air pollutant-induced toxicity and carcinogenicity. [This abstract does not necessarily reflect EPA policy].

105 PARTICULATE SURFACE - PHOSPHOLIPID SURFACTANT INTERACTIONS AFFECTING THE EXPRESSION OF TOXICITY.

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Respirable mineral dust and diesel exhaust soot interactions with dipalmitoyl phosphatidylcholine (DPPC) surfactant and subsequent expression *in vitro* of cellular membranolysis and genotoxic activities are reviewed. Incubation of respirable particulate material with DPPC, a major component of pulmonary surfactant, dispersed in saline is used to model the environmental interface conditioning of particles depositing in the bronchioalveolar depths of the lung. Diesel exhaust particulate material generated by some engines and operating conditions can express genotoxic activity after incubation in DPPC dispersion, as measured by procaryotic cell mutation assay and eucaryotic cell assays for

chromosomal or DNA damage. This activity is expressed by the surfactant-solubilized (coated) soot particles rather than by a surfactant extract of the particles. Adsorption of DPPC on insoluble mineral particles inhibits prompt membranolytic activity for pulmonary macrophage *in vitro*. The time-courses of enzymatic digestive removal of adsorbed surfactant and of restoration of dust toxic activities are compared.

107 LPS-PRIMING AMPLIFIES LUNG MACROPHAGE TNF PRODUCTION IN RESPONSE TO AIR PARTICLES.

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We postulate that in the inflammatory milieu of diseased lungs, AMs may be 'primed' for enhanced responses to stimuli such as inhaled air particles. To test this hypothesis in vitro, we first cultured normal AMs with or without lipopolysaccharide (LPS). We then incubated the cells with particle suspensions (urban air particles (UAP, Washington, D.C.), residual oil fly ash (ROFA), concentrated respirable-size ($PM_{2.5}$) air particles (CAPs), and inert TiO_2) and compared rat and human AM production of the critical pro-inflammatory mediator, tumor necrosis factor (TNF). LPS-priming caused a concentration-dependent increase in TNF production by rat AMs in response to UAP (e.g., control vs. LPS-primed: 0.6 ± 0.4, 8.6 ± 5.6 ng/ml), but not inert TiO_2. Priming also amplified responses of AMs to CAPs, although the potency of CAPs samples collected on different days varied (e.g.,TNF, ng/ml, control vs. LPS-primed human AMs: 0.9, 47.2 (CAPs # 1); 1.5, 72.2 (CAPs # 7)). The soluble fraction of UAP and CAPs suspensions showed minimal potency for induction of AM TNF production, suggesting AM-particle interactions were essential for cytokine stimulation. The antioxidants NAC and DMTU blocked particle induction of TNF production (e.g., DMTU 20, 10, 2, mM in primed AMs + UAP: 97, 51, 9 % inhibition, respectively). The data suggest that AMs activated by extant pulmonary inflammation can promote further inflammation by an enhanced, oxidant-dependent, cytokine response to inhaled ambient air particles.

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112 ARDIOVASCULAR MORTALITY RESPONSE TO AIR POLLUTION IS STRONGEST FOR HEART FAILURE AND THROMBOTIC CAUSES OF DEATH.

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Numerous studies have documented associations between daily variations of outdoor air pollution and cardiopulmonary mortality. Little is known about the mechanism of particularly the cardiovascular response. Most studies have analyzed the association of all cardiovascular causes combined with air pollution. The purpose of this study was to investigate whether the association found for 'total cardiovascular mortality (CVD)' was due to predefined specific causes. We collected data on daily mortality, air pollution, weather and influenza from 1986 1994 for the entire Netherlands (mean daily CVD count 142). We analyzed CVD combined mortality (ICD-9 codes 390 448), myocardial infarction (410), other ischemic (411-414), a combination of ischemic causes (410-414, 428, 435, 437.1), dysrythmia (427), conduction disorders (426 and 427), heart failure (428) and 'thrombotic' causes (415.1, 433, 434, 444, 452, 453). We used the generalized additive model to adjust for confounding by season, weather and influenza, using Poisson regression. Total CVD mortality was associated with ozone (O_3), sulfur dioxide (SO_2), black smoke (BS) and nitrogen dioxide (NO_2). Relative Risks associated with the range in exposure were between 1.02 and 1.03 with the exception of ozone lag1 for which a RR of 1.05 was found. Significant associations of these components of a similar magnitude were found for ischemic heart disease and cerebrovascular disease. No statistically significant associations were found for dysrythmia and conduction disorders. Substantially higher RR's were found for heart failure (RR for SO_2 lag1 1.07, RR for ozone lag0 1.12) and 'thrombotic' causes (RR for SO_2 lag1 1.08, RR for ozone lag1 1.14). These findings suggest that especially heart failure and changes in blood viscosity play a role in the cardiovascular mortality response to air pollution.

115 MECHANISMS OF PARTICLE-INDUCED LUNG INJURY: INDUCTION AND ACTIVATION OF MATRIX METALLOPROTEINASE (MMP)

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Early pathological changes caused by exposure to particular matter (PM) are characterized by pulmonary edema, alveolar hemorrhage, and infiltration of inflammatory cells. Such changes suggest alterations in the structural integrity of the lung. Metalloproteinases (MMP) are capable of degrading major components of basement membrane. Elevated protease activity has been implicated in a wide range of lung diseases. We hypothesize that induction and activation of MMP may be a common mechanism involved in early pathogenesis of PM-induced lung injury. To test this hypothesis, Sprague-Dawley rats were exposed to a combustion particle (residual oil fly ash, ROFA) or an ambient air PM (8.3 mg/kg b.w.) or saline by intratracheal instillation and sacrificed at 3 to 72 hr post-exposure. Pulmonary histopathology, gene expression, proenzyme activation, and protein localization were examined following exposure to ROFA or ambient air PM. Similar patterns of pathology were observed in rats following exposure to these PM samples. The injury was characterized by focal hemorrhage, pulmonary edema, and inflammation although the extent of injury varied. Gene expression for matrilysin and gelatinase B was increased following exposure to both PM samples. Pulmonary gelatinase A and interstitial collagenase gene expression was also increased in ROFA-exposed animals while TIMP1 and TIMP2 were not significantly affected by either PM. Moreover, both PM samples were capable of activating matrilysin from its proenzyme to its active form. The activation of matrilysin was observed as early as 3 to 6 hr following PM exposure, which preceded the peak of inflammatory response and coincided with histopathological lesions such as alveolar hemorrhage and pulmonary edema. In addition, similar patterns of pulmonary MMP and TIMP localization were demonstrated by immunocytochemistry following exposure to both PM samples. Thus, the early induction/activation of pulmonary MMP and possibly an imbalance between MMP and their intrinsic inhibitors, TIMP, may be a common mechanism involved in combustion as well as ambient air PM-induced early lung injury processes. (This abstract does not reflect EPA policy)

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116 LONGITUDINAL STUDIES WITH DOGS EXPOSED TO PARTICLE-ASSOCIATED SULFURIC COMBUSTION PRODUCTS AT LOW CONCENTRATIONS.

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Particle-associated sulfur IV (S-IV) and particle-associated hydrogen ions are supposed to be biologically effective products of fossil fuel combustion. To assess the response pattern of the healthy lung to these products three consecutive long-term exposure studies were carried out in dogs. Eight male adult beagle dogs were exposed over a period of about one year either to a neutral S-IV aerosol (sodium sulfite, 0.22 mg m^3 particle-associated S-IV), or to an acidic sulfur VI aerosol (sodium-hydrogen-sulfate, 23 µmol m^3 particle-associated H^+), or to a combination of both. The pulmonary response to the S-IV aerosol is characterized by a non-reactive loss of alveolar surface area. There were functional and morphometrical indications of early stages of lung emphysema with no obvious inflammatory response or repair processes. Restrained alveolar macrophage functions and an altered integrity of the alveolar-capillary membrane were observed. No tissue damage but adaptive changes within physiological boundaries are characterizing the response of the

healthy lung to chronic H^+ aerosol exposure. The response induced by the combined exposure to S-IV and H^+ aerosol is characterized by repair processes and morphological indications of early fibrotic lesions in the alveolar region of the lung without affecting respiratory lung function. Therefore, the pulmonary response pattern observed after exposure to the S-IV aerosol only was substantially modified by the additional inhalation of hydrogen ions which by themselves caused no pathophysiological responses. In vitro studies show, that effects of S-IV and H^+ on the synthesis of lipid mediators, especially PAF, LTB_4, and PGE_2, appear to be involved in the different reaction patterns observed in response to particle-associated sulfur.

118 A MORPHOLOGIC STUDY ON THE FATE OF ULTRAFINE PARTICLES IN J774A.1 CELLS.

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The distribution pattern of inhaled particles in tissues is an important factor for the evaluation of health effects. In this study, we morphologically investigated the fate of intracellular agglomerated UFP using in vitro systems. Metallic silver (Ag) was chosen as a test particle, since it can be easily produced and detected by elemental and morphologic analyses. Method: Ag-UFP generated by the electric spark generator in an argon atmosphere were collected by PTFE filters. The particles were suspended in PBS and adjusted to 5 different concentrations (1µg/100µl - 100µg/100µl). J774A.1 macrophage-like cell suspensions (200000 cells in 400 µl RPMI 1640 medium) were plated in small chambers (Lab-Tek "chamber slide" 177445). Eight hours later, 100 µl of the silver-PBS suspension was added to each chamber. Until day 9, the chamber slides were examined daily by an inverted microscope in order to estimate cell growth and to detect agglomerated particles in the cell. The medium was changed every day. At day 1, 3, 5, 7 and 9, cells in the chambers were fixed with 2.5 % buffered glutaraldehyde and examined ultrastructurally. Results: 1). Dose-dependent presence of agglomerated particles was observed in J774A.1 cells. The size and form of particles remained unchanged throughout the observation period. Electron microscopy with x-ray microanalysis confirmed that Ag was the only element of singlet and agglomerated particles observed in the dilated phagolysosome of J774A.1 cells. 2). At day 1, 2 and 3, degenerative and necrotic cells were frequently observed in the 10 µg-, 32 µg- and 100 µg-groups. At day 4, the cells began to proliferate and after day 5, the cell growth was comparable to that in the control group. In the 1- and 3.2 µg-groups, even at day 1 cells proliferated well like those of the control group. These results suggest that no rapid solubilization of Ag-UFP occurred in this system. Ag is a suitable test material for examining the in vivo distribution pattern of ultrafine particles in tissues.

123 INFLAMMATORY RESPONSES FOLLOWING EXPOSURE TO DIESEL EXHAUST- A STUDY OF TIME-KINETICS USING INDUCED SPUTUM

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Particulate matter (PM) pollution is of considerable concern for airway health as several epidemiological studies have revealed strong associations between ambient levels of PM pollution and increased morbidity and mortality in respiratory diseases. In a recent bronchoscopy study, we detected a pronounced airway inflammation following exposure to diesel exhaust (DE). The present study was conducted to evaluate the time kinetics of the inflammatory response following exposure to DE using induced sputum, a non-invasive and easily repeatable method to investigate airway inflammation. Fifteen healthy non-smoking volunteers were included. Each subject was exposed to DE (PM_{10} 300µg/m³) and air during one hour at two separate occasions. Sputum induction with hypertonic saline was performed 6 and 24 hours after each exposure. Analyses of sputum differential cell counts and soluble proteins were performed. Six hours after exposure to DE, a significant increase was found in the percentage of sputum neutrophils together with increases in the concentrations of IL-6 and methyl-histamine. Twenty-four hours after exposure, there was a significant increase in the percentage of lymphocytes, whereas the early increase in neutrophils had subsided. The present study demonstrates that exposure to DE induces a time-dependent inflammatory response in healthy human airways, represented by an early increase in IL-6, methyl-histamine and neutrophils followed by a later increase in lymphocytes. The data shown here correspond well with result from previous bronchoscopy studies. Induced sputum thus has the capacity to be an important tool to investigate the inflammatory effects of diesel exhaust.

125 EXPOSURE TO DIESEL EXHAUST ENHANCES THE EXPRESSION OF IL-8 AND GRO-a IN THE BRONCHIAL MUCOSA OF HEALTHY SUBJECTS.

T Sandström, C Nordenhäll, S Salvi*, J Pourazar, A Blomberg, S Wilson*, FJ Kelly**, AJ Frew*, ST Holgate*. Univ Hospital, Umeå, Sweden; *Southampton General Hospital, UK; **St Thomas' Hospital, London, UK

Diesel exhaust (DE) is a common air pollutant and a major source of particulate matter pollution. Short-term exposure to DE has been shown to induce an acute inflammatory response in human airways, involving increases in mucosal neutrophils, mast cells and lymphocytes (Salvi *et al. Am J Respir Crit Care Med*, 1999). This study was conducted to investigate the role of various neutrophil chemoattractive cytokines in the inflammatory response to DE. Fifteen healthy non-smoking volunteers were exposed to DE (PM_{10} 300mg/m³) and air during one hour at two different occasions, separated by at least three weeks. Bronchoscopy with endobronchial biopsy sampling was performed at 6 hours post exposure. Inflammatory cytokines were analysed with immunohistochemistry on biopsies processed in GMA resin and immunostained with monoclonal antibodies for inflammatory cytokines. Furthermore, biopsies were frozen in liquid nitrogen for analysis of cytokine mRNA expression with ELISA-PCR. Immunohistochemistry revealed a significant increase in the expression of IL-8 ($p<0.05$) and GRO-a ($p<0.01$) in the bronchial epithelium following exposure to DE compared with after air. ELISA-PCR analysis showed an increase in IL-8 mRNA expression ($p<0.05$) and a trend towards a significant increase in IL-5 mRNA ($p=0.09$). In conclusion, this study shows that short-term exposure to DE enhances the expression of the neutrophil chemoattractive cytokines IL-8 and GRO-a in the bronchial mucosa. These cytokines may thus be of importance in the recruitment of neutrophils following exposure to DE. Further, these data suggest that the epithelial cells, as the first cellular target to inhaled air pollutants, may be involved in the early inflammatory response to DE.

134 METHODS TO STUDY THE EFFECTS OF ATMOSPHERIC PARTICULATE MATTER ON PERIPHERAL BLOOD LEUKOCYTES IN VITRO.

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In vitro methods were developed that use human peripheral blood to study the effects of atmospheric particulate matter on leukocyte function. The effects of pre-treatment with National Institute of Standards and Technology (NIST) urban PM #1648, diesel PM #1650, silica PM and a locally collected sample (New Jersey PM10) on phagocytosis, cell structure, release of granule contents and the release of histamine were examined. Pre-treatment of cells with NIST urban and diesel PM decreased the rate of uptake of bacteria by granulocytes. Pre-treatment with NIST urban PM resulted in a decrease in the light scattered by the granulocytes. Examination by transmission electron microscopy suggested that the decreased light scatter associated with NIST urban PM treatment is the result of degranulation. In additional assays, the release of contents of primary and secondary granules and the release of histamine from the cells was measured. In these assays, *Staphylococcus aureus* bacteria, a known inducer of degranulation and histamine secretion, was used as a positive control. Bacteria were found to induce the release of primary granules, as determined by the release of p-nitrophenyl-N-acetyl-beta-D-glucosaminidase and myeloperoxidase, secondary granules as determined by the release of lactoferrin, and histamine. Pre-treatment with NIST urban PM did not induce the release of primary granule contents, but did induce the release of lactoferrin from secondary granules. Interestingly, NIST urban PM and the locally-

collected New Jersey PM10 were potent inducers of histamine release. Experiments were repeated with PM fractionated into material soluble and insoluble in phosphate-buffered saline solution. Both soluble and insoluble New Jersey PM10 and NIST urban PM induced histamine release in cells. Histamine is an endogenous bioactive amine associated with effects on cardiac tissue and vascular tone. We hypothesize that systemic release of histamine mediated by atmospheric PM constituents contributes to effects on cardiac function that have been reported elsewhere (Godleski, 1998). This hypothesis should be tested *in vivo*.

Authors are members of the Environmental and Occup. Health Sci. Inst.

135 COMBUSTION-DERIVED METALS ACTIVATE THE EGF RECEPTOR SIGNALING PATHWAY IN HUMAN AIRWAY EPITHELIAL CELLS.

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We have previously shown that exposure to combustion-derived metals rapidly activates mitogen-activated protein kinases (MAPKs), including ERK, in the human bronchial epithelial cell line BEAS. To study the mechanisms responsible for metal-induced activation of ERK, we examined the effect of noncytotoxic exposures (500 M) to As, Cu, V or Zn on upstream kinases of ERK in EGF receptor signaling pathway. Western blotting using a phosphospecific antibody showed that these metals induce a rapid (within 20 min) phosphorylation of MEK1/2. Kinase activity assays conformed the activation of MEK1 by metal treatment. The selective MEK1 inhibitor PD98059 blocked metal-induced phosphorylation of ERK1/2. Immunoprecipitation studies using specific antibodies to the EGF receptor demonstrated that As, Cu, V or Zn induce phosphorylation of the EGF receptors. Furthermore, the EGF receptor-specific tyrosine kinase inhibitor (PD15035) significantly blocked the phosphorylation of MEK1/2 initiated by metals. Interestingly, we did not observe evidence of Raf-1 activation in BEAS cells treated with. Finally, transfection assays showed that PD98059 could inhibit trans-activation of Elk, a transcription factor in the ERK pathway.These data show that As, Cu, V and Zn can activate the ERK pathway via the EGF receptor and induce MEK1/2 phosphorylation in BEAS cells, which was associated with activation of Elk. These findings suggest that combustion-derived metals can activate the EGF receptor signaling pathway in human airway epithelial cells and that this mechanism may participate in pulmonary responses to combustion-derived metal inhalation. (This work does not necessarily reflect the EPA policy, EPA CR817643)

137 ACTIVATION OF MAPK SIGNALING PATHWAYS IN RATS TREATED WITH ROFA.

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Residual oil fly ash (ROFA) is a highly metallic particulate pollutant produced in the combustion of fuel oil. Exposure to ROFA induces a strong lung inflammatory response in rodents, is associated with adverse respiratory effects in humans and induces inflammatory mediator expression in cultured human airway epithelial cells (HAEC).Vanadium, a major component of ROFA, activates components of the mitogen-activated protein kinase (MAPK) signaling cascades in HAEC. ROFA and vanadium increase protein tyrosine phosphate levels in HAEC. In order to study MAPK activation in response to *in vivo* metal exposure, we examined levels of phosphorylated protein tyrosines (p-Tyr) as well as phosphorylation of the MAPKs JNK, P38 and ERK1/2, in lung sections from rats intratracheally exposed to ROFA. Results were compared to the effects of ROFA exposure on p-Tyr, p-JNK, p-ERK and p-P38 levels in primary HAEC cultures analyzed by Western blotting. After a 4 hr exposure to 500 ug ROFA, there was evidence of mild perivascular edema but no incursion of inflammatory cells. There was an accompanying increase in specific immunostaining for p-Tyr and p-ERK in alveolar epithelial cells and resident macrophages relative to saline-instilled control animals. In addition, there were small increases in p-JNK and p-P38 in airway and alveolar epithelial cells and macrophages. After 24 hrs, there was a strong inflammatory response to ROFA instillation that included severe edema and a large increase of inflammatory cells. Alveolar epithelial cells and inflammatory cells showed a marked increase in immunostaining with anti- p-Tyr, p-JNK, p-P38 and p-ERK antibodies. The airway epithelium and pleura had comparatively smaller elevations in immunostaining. Vascular structures had a modest increase in expression of p-ERK and p-JNK. By comparison, primary cultures of HAEC exposed to 100 ug/ml ROFA for 20 minutes resulted in marked increases in p-Tyr, p-JNK, p-P38 and p-ERK, which remained elevated at 24 hrs of continuous exposure. P-Tyr levels continued to increase up to 24 hr. These results demonstrate *in vivo* activation in cell signaling pathways in response to pulmonary exposure to particulate matter, and support the relevance of *in vitro* studies on mechanisms of lung injury induced by pollutant inhalation.This **abstract of a proposed presentation does not necessarily reflect EPA policy.**

142 BRADYCARDIA SIGNALS IMMINENT DEATH IN SENESCENT-PRONE AKR/J MICE.

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Susceptibility to cardiac mortality is increased in elderly populations. We hypothesize that bradycardia is a cardiac biomarker that predicts individual susceptibility to imminent death. To test this hypothesis, we assessed heart rate (HR) in AKR/J (AK) inbred mice prone to early senescence, and maintained on a 12/12 light/dark cycle. Radiotelemeters were surgically implanted in male AK mice at 200 d of age to provide an ECG signal from which HR was measured at 30 min intervals during weekly 48 h periods until the animal's natural death. In each animal ($n = 9$), average HR was assessed at least 14 d following surgery and 3 d prior to death. The average (mean ± SEM) life-span of surgically-treated animals (339 ± 15 d; $n = 17$) did not differ from untreated controls (317 ± 17 d; $n = 25$). The daily average HR following surgery was 612 ± 7 bpm, which was reduced significantly ($p < 0.01$) to 460 ± 38 bpm 3 d prior to death. Thus, a 25% lower daily HR occurred 3 days prior to death suggesting that bradycardia signals imminent death in AK mice. This decline in cardiac homeostasis may be due to sympathetic-parasympathetic imbalance in the nervous control of HR or altered pacemaker cell function at the sinus node. In conclusion, the findings suggest that bradycardic mechanisms may contribute to increased susceptibility to cardiac mortality in the elderly. Future studies will explore the role environmental stressors pose to exacerbate cardiogenic risk. Support: EPRI-WO8203-01

145 HEALTH EFFECTS OF CONCENTRATED AMBIENT PM10 IN HEALTHY GUINEA PIGS.

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Previous work from this and other labs has demonstrated that guinea pigs may be particularly sensitive to the adverse pulmonary effects of inhaled particles and gases. The present study examined the response of healthy guinea pigs to urban PM2.5. Concentrated PM atmospheres were generated with a Gerber centrifugal concentrator. Animals were exposed nose only to filtered air or 318 ug/m^3 concentrated PM for 6 hours and then examined for changes in pulmonary function and cellular and biochemical parameters in bronchoalveolar lavage fluid. At 3 and 24 hours after exposure to air or PM, lung volumes (inspiratory, vital, and total lung capacity) and DLco were measured in anesthetized and tracheotomized animals. No significant differences between air- and PM-exposed animals were observed for any pulmonary function parameter. Similarly, no evidence of pulmonary injury (protein or LDH activity in lavage fluid) or inflammation (PMNs in lavage fluid) was noted in animals examined 24 hours after exposure to PM. Thus, a single 6 hour exposure to a relatively high concentration of PM2.5 produced no adverse pulmonary changes in adult guinea pigs. This research was funded in part by HEI and the USEPA.

147 VEREXPRESSION OF CATALASE INHIBITS VANADIUM-INDUCED MOBILIZATION AND p38-DEPENDENT TRANSACTIVATION OF NF-κB IN AIRWAY EPITHELIAL CELLS.

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Metals associated with particulate matter (PM) have been suggested to mediate PM-induced adverse health effects, including inflammation of the lower respiratory tract. Expression of many inflammatory genes, such as IL-8, is regulated at the transcriptional level by NFκB. Many studies suggest that reactive oxygen intermediates (ROI) are involved in NF-κB activation and subsequent gene expression. In this study, we compared the role of oxidant stress in NF-κB activation and IL-8 gene expression in normal human bronchial epithelial (NHBE) cells treated with either TNF-α or vanadium, a transition metal often associated with PM. Stimulation with vanadium, but not TNF- α, imposed a detectable oxidant stress. While both stimulants induced nuclear translocation of NF-κB and increased steady-state IL-8 mRNA abundance, overexpression of catalase selectively inhibited the response to vanadium. In contrast, overexpression of Cu,ZnSOD had no effect on NFκB activation or IL-8 expression evoked by either stimulus. Both vanadium and TNF- α enhanced κB-dependent transcription and this response was inhibited by overexpression of a dominant-negative mutant of the stress responsive MAP kinase p38 or of its upstream activator MAPK kinase 6 (MKK6). The p38 inhibitor, SB203580, had no effect on vanadium or TNF-α -induced NF-κB nuclear translocation or DNA-binding activity. These data indicate that activation of the p38 MAPK pathway by either vanadium or TNF-α enhanced the transactivation potential of NF-κB in NHBE cells, but did not affect NF-κB mobilization or DNA-binding activity. In addition, these results suggest that vanadium and TNF- α activate an overlapping subset of signaling cascades, but by oxidant stress-dependent and independent mechanisms, respectively. (This abstract does not reflect EPA policy)

148 TYROSINE PHOSPHATASES AS POTENTIAL TARGETS IN PM-INDUCED SIGNALING IN HUMAN AIRWAY EPITHELIAL CELLS (HAEC).

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We have previously shown that exposure to metal-laden combustion particles disregulates protein tryosine phosphate homeostasis and results in the activation of phosphorylation-dependent signaling pathways in cultured human airway epithelial cells. In order to study the role of protein tyrosine phosphatases in these effects, we examined the effect of As, V and Zn on tyrosine phosphate catabolism in BEAS S6 cells or human bronchial epithelial cells. Western blots and immunocytochemical analyses showed that non-cytotoxic levels of As, V or Zn increase levels of protein phosphotyrosines in HAEC. Protein tyrosine phosphatase activity, measured using [^{2}P]-labeled polyGlu:Tyr as a substrate, was markedly inhibited in cells treated with V or Zn, but was unaffected by exposure to As (See table, MEAN ± SEM, *$p<0.01$, $n=5$). FPLC fractionation and subsequent in-gel phosphatase activity assay of HAEC protein extracts revealed numerous tyrosine phosphatases of varying molecular weight which were effectively inhibited by exposure to V and Zn ions. In marked contrast, As had no discernible effect on these enzymes. Similarly, the protein tyrosine phosphatase PTP1B, immunoprecipitated from human airway epithelial cells, was inhibited by V and Zn but not by As ions. These data show that V and Zn may induce protein tyrosine phosphate accumulation through inhibition of tyrosine dephosphorylation, while implicating kinase activation as a mechanism in HAEC exposed to As. These findings also demonstrate that metal exposure can activate signaling pathways through multiple and distinct mechanisms, and suggest a biologically plausible toxicological mechanism that may contribute to the pulmonary effects of PM inhalation. **This abstract of a proposed presentation does not necessarily reflect EPA policy. EPA CR817643**

159 PAHs LEVELS AND MUTAGENIC ACTIVITY IN BACTERIA AND HUMAN CELLS OF ORGANIC EXTRACTS FROM SANTIAGO'S OF CHfLE AIRBORNE PARTICLES.

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The air in Santiago of Chile is among the most highly polluted in the world. Due to the high levels of airborne particles, CO and 03 and the high incidence of respiratory diseases specially in the most susceptible groups, Santiago has been declared as saturated zone for PM10, 03, and CO. The levels of the 16 polycyclic aromatic hydrocarbons (PAHs) were determined by HPLC in organic extracts from PTS and respirable particles. Particulate matter contain high levels of PAHs including six classified by IARC as carcinogenic, which represented around *50%* of total PAHs concentrations. In addition a seasonal effect was oberved with higher values in months with lower temperatures. Strong declining of their levels both in PTS and PM10 were observed in the last years, but the levels of carcinogenic PAHs are still higher than those reported in Japan, USA and Europe cities. Airborne particles were highly mutagenic and contained direct and indirect mutagens which produce both frameshift and base sustitution mutations in *Salmonella thyphimurium*. In spite of the important decrease on PAHs in the period 1991-1996, direct mutagenic response do not changed significantly, suggesting that the levels of direct mutagenic pollutants have not decreased considerably during the last years. Airborne particulate were also highly mutagenic at the tk locus in hlAlv2 human lymphoblasts in culture. These results suggest a high risk for Santiago's inhabitants since compounds adsorbed in the particles are highly mutagenic and can damage DNA.

162 OARSE (PM-10) AND FINE (PM-2.5) FRACTIONS OF AIRBORNE PARTICULATES FROM „POLLUTED" AND „CLEAN AIR" AREAS INDUCED STRONG GENOTOXIC EFFECTS ON HUMAN BRONCHOEPITHELIAL CELLS (BEAS-2B) IN VITRO

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Epidemiological studies disclose serious problems concerning adverse effects on human health
by permanent inhalation of coarse (PM-10) and fine (PM-2.5) fractions of airborne particulates and especially a higher incidence of lung cancer in industrialized areas. The epithelium of the respiratory tract is the major target of airborne particulates and the location of the most common cancer in man, the bronchogenic cancer.
Airborne particulates of the coarse (PM-10) and fine(PM-2.5) fraction were collected with a Low Volume M-10 Dichotomous Sampler (Graseby-Andersen) equipped with glass fiber filters in urban areas (Düsseldorf/ Leipzig, Germany) an industrialized area (Duisburg), and a rural „clean air" area (Borken). Chemical substances were extracted from glass fiber filters with dichloromethane in a Soxhlet apparatus and after evaporation of the solvent in a rotation evaporator the residual substances were dissolved in dimethyl-sulfoxide (DMSO) for cell culture experiments. As target cells in vitro we utilized the human bronchoepithelial cell line BEAS-2B, and as sensitive bioassay of genotoxicity the induction of „Sister Chromatid Exchanges" (SCE). The fractions PM-10 and particularliy PM-2.5 caused a strong, dose-related, highly significant induction of „sister chromatid exchanges". In comparison to the samples from the urban and industrialized areas revealed the fractions PM-10 and PM-2.5 from the „clean air" area a lower, but still remarkable genotoxic activity.

164 HEART RATE VARIABILITY ASSOCIATED WITH PARTICULATE AIR POLLUTION

Pope CA III, Verrier RL, Lovett EG, Larson AC, Raizenne ME, Kanner RE, Schwartz

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Epidemiologic studies have linked fine particulate air pollution with cardiopulmonary mortality, yet underlying biological mechanisms remain unknown. Changes in heart rate variability (HRV) may reflect changes in cardiac autonomic function and risk of sudden cardiac death. This study evaluated changes in mean heart rate (HR) and HRV in humans associated with changes in exposure to particulate air pollution. Repeated ambulatory ECG monitoring was conducted on seven subjects for total 29 person-days prior to, during, and following episodes of elevated pollution. Mean HR, the standard deviation of NN intervals (SDNN), the standard deviation of the averages of NN intervals in all 5-minute segments of the recording (SDANN), and the square root of the mean of squared differences between adjacent NN intervals (r-MSSD) were calculated for 24-hour and 6-hour time segments. Associations of HRV with particulate pollution levels were evaluated using fixed-effects regression models. After controlling for differences across subjects, elevated particulate levels were associated with: 1) increased mean HR; 2) decreased SDNN, a measure of overall HRV; 3) decreased SDANN, a measure that corresponds to ultralow frequency variability; and 4) increased r-MSSD, a measure that corresponds to high frequency variability. The associations between HRV and particulates were small but persisted even after controlling for mean HR. This study suggests that changes in cardiac autonomic function reflected by changes in mean HR and HRV may be part of the pathophysiological mechanisms or pathways linking cardiovascular mortality and particulate air pollution.

165 HUMAN VOLUNTEERS DEMONSTRATE NO DECREMENTS IN PULMONARY FUNCTION AND NO ABNORMAL EKG CHANGES AFTER EXPOSURE TO FINE PARTICLES CONCENTRATED FROM CHAPEL HILL AMBIENT AIR

AJ Ghio, C. Kim, RB Devlin. NHEERL, US EPA, Research Triangle Park, NC.

Epidemiologic investigation has associated human morbidity and mortality with exposure to ambient air pollution particles. Exposure of canines to concentrated ambient particles for 3 days (6 hours/day) were associated with both inflammation in lavage samples and EKG changes. We tested the hypothesis that exposure of healthy humans to concentrated ambient air pollution particles can be associated with symptoms, changes on physical exam, pulmonary function decrements, and abnormalities on EKG. Eight volunteers (6 males and 2 females, 28.0$\pm$ 5.8 years old) were exposed to particles concentrated from the air around the EPA Human Studies Facility in Chapel Hill, NC. Particle concentrations in the chamber during these 8 exposures ranged from 40 -330 mg/m^3. While in the exposure chamber, the volunteers alternated between exercise (15 minutes) and rest (15 minutes) for a total exposure time of 2 hours. Exercise was on a bicycle at a workload calculated to obtain a ventilation rate of 50 l/min. No symptoms were noted by volunteers during or immediately after the exposure. Similarly, there were no abnormal cardiopulmonary findings upon examation of subjects following exposure. There were no decrements in FVC or FEV$_1$ (0.7$\pm$3.5% and 7.5$\pm$7.4% increases above pre-exposure values, respectively; not significant). Finally, physician examination of EKGs taken during exercise periods and post-exposure revealed no obvious abnormalities. We conclude that healthy human volunteers exposed to moderate levels of ambient air particles while exercising do not experience clinical signs of cardiopulmonary distress. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

170 INVOLVEMENT OF CALCIUM AND CALCIUM CHANNELS IN RESIDUAL OIL FLY ASH AND SILICA-INDUCED STIMULATION OF HUMAN ALVEOLAR MACROPHAGES

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Residual oil fly ash (ROFA) and silica induce a calcium (Ca^{++}) dependent oxidative burst in human alveolar macrophages (AM). To investigate whether irritant receptors are involved in the influx of Ca^{++}, AM were exposed to 100 ug particles and luminol-dependent chemiluminescence assessed in the presence or absence of capsazepine(cpz), an irritant receptor channel blocker. Inhibition of chemiluminescence was seen with cpz only at 10^{-4}-10^{-5} M, concentrations which also may interfere with other Ca^{++}channels. To elucidate the possible involvement of specific Ca^{++} channels, various channel blockers were used. While Nifidepine (specific L-type channel blocker) decreased the particle-induced chemiluminescence more than 80% at concentrations ranging from 10-7 - 10-14 M, blockers of the N-type Ca^{++} channel had no effect on chemiluminescence. In addition, various inhibitors of tyrosine kinases and protein kinase C significantly decreased the ROFA or silica-induced oxidate burst. These results suggest that ROFA and silica signal oxidant production through pathways involving L-type Ca++ channels and phosphorylation events. (This is an abstract of a proposed presentation and does not reflect EPA policy.)

171 SOLUBLE COMPONENTS OF UTAH VALLEY PARTICULATE POLLUTION ALTER ALVEOLAR MACROPHAGE FUNCTION IN VIVO AND IN VITRO.

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Soluble extracts of Utah valley dust (UVD), have been found to cause lung inflammation in both humans and rodents, which correlates with themetal content in the extracts. The PM collected over a three year span varies in total metal content (Fe, Cu, Zn, Pb, Ni, Va) with year 1= year 3>year 2. Previous studies of human alveolar macrophages (AM) exposed *in vitro* to particles high in metals have shown an inhibition of phagocytosis and oxygen radical production. In the present study the phagocytic activity and oxidative response of AM was investigated following in vivo or in vitro exposure to the various UVD. AM obtained by bronchoalveolar lavage, were studied 24 hrs following instillation of either saline (right lobe) or UVD (left lobe). Using flow cytometry, phagocytosis of FITC-labeled S.cerevisiae by AM exposed to the extracts was inhibited (inh.) as compared to unexposed AM by year 1 UVD (61% inh.) but not by years 2 and 3. Oxidant generation by the cells was also only affected (decreased) following exposure to year 1 UVD (48% inh.). Following exposure of AM to the UVD for 20 hours in vitro, the % AM phagocytizing particles was significantly decreased by year 1 UVD (30%), while no significant effect was found with the other two extracts. On the other hand, all three extracts inhibited AM chemiluminescence to a similar extent in vitro. The detrimental effects on AM host defenses were possibly due to apoptosis evident in cells exposed to the year 1 UVD and to a much lesser extent with AM exposed to year 2 and 3 UVD. Since year 1 and year 3 UVD are similar in their metal content, but differ in their effects on AM, we suggest that metals may not be the culprit in effects of particulate matter on AM host defenses. This is an abstract of a proposed presentation and does not reflect EPA policy.

Session 4. Dosimetry Related

013 DETAILED FLOW IN THE HUMAN NASAL PASSAGE.

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A quantitative understanding of particle deposition in the human nasal passage is important for its inherent health implications and for determining the particle concentration that enters the lungs. To quantify the total and regional particle deposition in the nasal passage, the fluid flow in the airway must first be understood in detail. The complex geometry of the nasal passage has previously limited such a detailed experimental study, and consequently, the available computational models have not been sufficiently validated. In this work, an anatomically accurate, optically transparent replicate model of an adult male human nasal passage has been fabricated by using rapid prototyping techniques. Particle Image Velocimetry (PIV), a non-invasive technique for measuring fluid velocities over global domains, was used to obtain two-dimensional instantaneous velocity vector fields in planes throughout the model. In brief, the working fluid's index of refraction was matched to that of the models' and was seeded with tracer particles. By illuminating these particles with laser light pulsed twice in rapid succession, two subsequent positions of each particle were recorded. Post-processing of the recorded data yielded two-dimensional instantaneous velocity vectors. The flow field in the human nasal passage has proven to be complex due to the complex geometry of the airway. The flow is characterized by flow separations, reverse flows and relatively stagnant regions. It was found that the olfactory slit, which is a sensitive region responsible for olfaction, sees relatively small flow velocities. The physiological implication is that this sensitive region is protected from particle deposition and is primarily exposed to vapors and odors whose transport is dominated by diffusion. The region of the highest flow rate was found to be along the floor of the passage, and an eddy was found to form where the nasal valve expands into the main part of the passage.

018 DEPOSITION PATTERNS OF AMBIENT POLLUTANTS IN CHILDREN'S LUNGS.

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Children have been identified as a sensitive subpopulation to be addressed in the determination of regulatory standards for air pollutants. For ethical and legal reasons, however, experimental data describing the deposition patterns of inhaled particulate matter in children are rare. Therefore, we have developed an age-dependent theoretical model to predict particle dosimetry in children's lungs. Algorithms that define the morphologies of growing human lungs have been derived; that is, computer codes describe the dimensions of individual airways and the geometries of branching airway networks within lungs. Likewise, breathing parameters have been formulated as functions of human subject ages. The results of computations indicate that the deposition patterns of inhaled air pollutants are affected by particle sizes and subject ages. For example, for 1 μm particles inhaled under resting conditions, total (i.e., lung) deposition is 24% in an adult but is 33% in a 22 month old subject, a relative increase of 38%. The model also partitions total deposition into its tracheobronchial (TB) and pulmonary (P) components. For toxicological reasons it is important to determine compartmental dose because the TB and P airways have different clearance processes (i.e., mucociliary transport and macrophage action, respectively). The results of such simulations provide a basis for integrating children into regulatory standards for air pollutants. DISCLAIMER: This abstract has been internally cleared and does not necessarily reflect EPA policy. ACKNOWLEDGMENTS: C. J. Musante was funded by the EPA/UNC Toxicology Research Program, Training Agreement CT902908, with the Curriculum in Toxicology, University of North Carolina at Chapel Hill.

019 COMPARISON OF PARTICLE DEPOSITION IN HEALTHY SUBJECTS AND COPD PATIENTS USING COMPUTER SIMULATIONS AND HUMAN DATA.

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The US EPA has identified COPD (chronic obstructive pulmonary disease) patients as a susceptible group to be addressed in regulatory standards. To investigate the observed increase in deposition in COPD patients relative to healthy subjects, a computer model was used to calculate the deposition fractions of particulate matter (PM) within human lungs. The model, which uses the Weibel lung morphology, has been previously validated with other human subject data. Human subject variation was accounted for by scaling Weibel lung dimensions based on measured FRC (functional residual capacity) values. COPD was modeled via airway diameter reduction using airway resistance measurements. Simulations were performed for V_T (tidal volumes) of 360, 500, and 1000 ml, respiratory times (T) from 2 to 8 s and particle sizes of 1, 2, 3, and 5 μm. Deposition patterns depended on the particular ventilatory parameters, particle sizes and level of disease. For example, when V_T = 500 ml and T = 2 s, the deposition of 1 μm particles was 100% greater for COPD patients. This may be attributed to increased efficiencies of specific deposition processes in different regions of the lung. The model is used in risk assessment efforts to estimate the inhalation hazards of airborne pollutants. Disclaimer: This is an abstract of a proposed presentation and does not necessarily represent EPA policy. Acknowledgements: RAS is funded by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT826512010, NCSU.

030 METHODOLGY FOR SITE-SPECIFIC DELIVERY AND KINETICS OF CLEARANCE OF INSOLUBLE PARTICLES TO SUBLOBAR LUNG SEGMENTS.

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In 5 mixed breed, anesthetized dogs a bronchoscope was inserted through an endotracheal tube and placed (nonwedged) within a sublobar bronchus located in the right or left lower lobes. Dogs secured in a supine position were mechanically ventilated and imaged from the ventral aspect by γ-camera. A ventilation scan (^{133}Xe gas) demarcated ventilated lung regions. Aqueous, *insoluble* sulfur colloid particles (labeled with ^{99m}technetium) were generated by ultrasonic nebulization and delivered directly through a channel of the scope into the sublobar segment at a flow rate of 200 ml/min. During aerosol delivery (15 sec) the respirator was stopped at end-expiration, followed by additional 15 sec of breath-hold time, before the respirator was turned back on. Clearance of particles was measured continuously for 60 min and dogs were then permitted to recover. At 6 and 24 hr post deposition the dogs were re-imaged for subsequent measures of residual retention. During the initial hour, clearance from sublobar segments was disparate with clearance more effective in lower left segments, i.e., 51% (±5% SE) of particles as compared to 23% (±6% SE) from right segments. At 6 h post deposition clearance was more comparable between the segments, i.e., left segments had cleared 60% of particles vs. 51% for lower right segments. Clearance appeared complete with no additional changes in retention at 24 h post deposition. The methodology assesses delivery and clearance of particles, is reproducible, and permits restudy of the airway. NIH RO1 HL50579 and NIEHS ES-03819.

064 AGGREGATED FINE AND ULTRAFINE PARTICLES IN LUNGS OF MEXICO CITY RESIDENTS.

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Epidemiological evidence has associated inhalable particulate (PM_{10}) air pollution with cardiopulmonary morbidity and mortality. The biological mechanisms underlying these associations are not clear nor is the relationship between ambient levels PM_{10} and retained particles in the lung. We compared the mineral particle content in the parenchyma of 5 autopsy lungs from never smoking, female, long-term residents of Mexico City, a region with high ambient PM_{10} levels (3 year mean = 65 $\mu g/m^3$), with 5 control residents of Vancouver, BC, a region with relatively low PM_{10} levels (3 year mean = 14 $\mu g/m^3$). Autopsy lungs were dissolved in bleach and particles were identified and counted by analytical electron microscopy. Total particle concentrations in the Mexico City lungs (geometric mean [GM] = 1085 x 10^6 particles/g dry tissue, geometric standard deviation [GSD]= 2.0) were significantly (p=0.01) greater than in the Vancouver lungs (GM = 243 x 10^6 particles/g dry tissue, GSD = 2.2). While lungs from both sites contained a variety of crustal particles (silicates, mica, talc, iron and titanium oxide), the Mexico City lungs contained numerous particle aggregates. Such aggregates made up an average of 23% of the total particles in the Mexico City lungs and were composed of carbonaceous spheres with or without sulfur, particles analyzing as an aluminum silicate resembling kaolinite, or particles analyzing as iron plus silica. If the individual particles comprising the aggregates are considered separately, then these make up the vast majority of the retained particles in the Mexico City lungs. The geometric mean size of the carbonaceous particles in the aggregates was 0.067μm. The kaolinite particles had a geometric mean diameter of 0.11μm. Particles of these types were never observed in lungs from Vancouver. These observations indicate for the first time that residence in a region of high ambient PM_{10} results in pulmonary retention of large quantities of particles, including fine and ultrafine aggregates. At least some of these aggregates appear to be combustion products.

065 EFFECT OF PARTICLE SIZE ON THE PULMONARY TOXICITY OF CADMIUM CHLORIDE

The application of a multiple path particle deposition model (MPPDep V1.1)

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Epidemiological studies on the health effects of PM mainly investigated exposure-response associations based on PM mass concentrations, since PM is regulated by mass concentrations of particles with an aerodynamic diameter of less than or equal to a nominal 10 μm. Although this is a size-specific pollutant indicator, it does not discriminate for the three size-fractions within PM_{10}: coarse (2.5 - 10 μm), fine (0.1 - 2.5 μm) and ultrafine ($\leq$0.1 μm). Moreover, there is no straightforward relationship between ambient or personal exposure (what gets measured or estimated) and the effect-relevant dose delivered to the target site (respiratory tract). Several dosimetry models have approached to describe this complex relationship. A recently introduced human and rat airway PM deposition model (MPPDep V1.1) has been developed by the Chemical Industry Institute of Toxicology (CIIT) and the RIVM, based on the work of Raabe et al. (1975) and Anjilvel and Asgharian (1995). This paper describes studies using the toxic model aerosol cadmium chloride ($CdCl_2$) (a) to investigate the role of particle size in the development of toxic effects in the lung and, (b) to evaluate the dosimetry model by comparing calculated $CdCl_2$ deposition data with measured deposition of $CdCl_2$ in the lung. Rats were exposed for 4 hr/day for two consecutive days at various $CdCl_2$ sizes. Initial pulmonary $CdCl_2$ deposition was determined right after the first 4 hrs of exposure. Toxicity, as measured by lactate dehydrogenase, N-acetyl glucosaminidase and protein levels in bronchoalveolar lavage fluid was determined 20 hr after the last exposure. The first preliminary results suggest that the pulmonary toxicity of $CdCl_2$ is independent of particle size, and relates more to the intrinsic properties of the aerosol. In addition the measured $CdCl_2$ deposition could be predicted accurately by using the MPPDep V1.1. Addition results will be presented.

068 MODELING PARTICLE DEPOSITION IN THE NONHOMOGENEOUSLY OBSTRUCTED LUNG.

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Relative to healthy individuals, persons with obstructive lung diseases have marked ventilatory inhomogeneity and increased intrathoracic particle deposition. Moreover, deposition is frequently found to be nonuniform, with high localized deposition. Mathematical models for particle deposition in the normal human lung have been developed and validated by comparison with experimental data. Only limited attempts have been made to model particle deposition in the obstructed lung and generally only for the case of uniform airway constriction, which rarely occurs in real obstructive lung disease. We have considered the partial obstruction of the airways in some regions of the lung with other regions remaining relatively unaffected and used a simple model of respiratory mechanics to determine the distribution of ventilation between regions. Using Weibel's Model A morphology, airway constrictions were applied to the small airways ($8 \leq Z \leq 16$) and a combination of large and small airways ($3 \leq Z \leq 16$). The fraction of obstructed to normal lung was varied from 0 to 100% in 25% increments. Obstructed and normal regions were separated at the 2nd generation of the lung for all calculations. For our initial model, pulmonary compliance was assumed to be constant and equally partitioned. Total deposition and surface doses were calculated for particles sizes ranging from 0.02-5 μm with a 500 ml tidal volume and breathing frequency of 14 min^{-1}. As expected, we found total deposition increased with total airway resistance and that much of this increase was attributable to high localized deposition in obstructed airways; in many cases >10 times normal airways surface dose. These findings were less pronounced for particles sizes with minimal intrinsic mobility (0.5 to 1μm). Within normal regions (with no existing obstructed regions) there was a moderate increase (<50%) in proximal airway deposition of coarse particles ($\geq$ 1μm) relative to a lung free of any obstruction. The additional ventilation to normal regions (due to increased time constants of obstructed regions) also caused more aerosol to penetrate and deposit in the peripheral lung, though surface doses were small. Findings suggest that compared to normal, nonuniform airway obstruction results in an increased surface dose of unobstructed as well as obstructed lung regions. (Funded by USEPA coop. CR824915)

069 EFFECT OF VENTILATION DISTRIBUTION ON COARSE PARTICLE DEPOSITION IN PATIENTS WITH OBSTRUCTIVE AIRWAYS DISEASE.

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The importance of ventilation distribution on the partitioning of particle deposition between obstructed and relatively normal regions of the lung is unclear. At the extreme, when a lung region becomes so obstructed such that it receives little or no flow, the deposition in healthier regions may be enhanced due to their increased ventilation. On the other hand, there may also be enhanced local deposition in obstructed airways associated with poorly ventilated regions. We compared the regional deposition (RD) of ^{99m}Tc labeled 5.0 μm MMAD (σ_g=1.25) particles to regional ventilation (RVent) assessed by ^{133}Xe washout in 18 COPD patients and 19 healthy controls. Lung regions corresponding to 1/3 of the lung height were evaluated. The lower left region could not be analyzed due to activity from swallowed material in the stomach. Not surprisingly, due to the location of large airways, deposition in middle lung regions was increased relative to other regions in both patients and controls,

though significantly more in the patients. In attempting to discern any association between ventilation and deposition, it was necessary to discard the middle regions from further analysis in favor of more anatomically similar regions. RD was computed for the upper regions and the lower right lung region as the fraction of aerosol deposited within a compartment normalized to compartmental volume (fraction of ^{133}Xe equilibrium counts within compartment). RVent for each compartment was also determined by normalizing the ^{133}Xe washout rate for that compartment by the total washout rate for the 3 compartments combined. Correlation coefficients were computed between RD and RVent across subjects for each compartment, e.g. right top. There were no significant (positive or negative) correlations for any of the regions in either group of subjects implying that RD of coarse particles does not follow RVent. In the COPD patients, the lung region having on average the lowest RVent also had the highest RD. Furthermore, this same region was also found to have the lowest 24 hour retention of particles. These data suggest that the coarse particles are preferentially deposited in obstructed airways associated with poorly ventilated lung regions. Further studies are underway investigating deposition patterns of fine and ultrafine aerosols. Funded by USEPA Cooperative Agreement (CR824915).

071 TOTAL AND REGIONAL DEPOSITION OF INHALED PARTICLES IN CHILDREN.

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Epidemiological studies suggest that children may be more susceptible than adults to effects of inhaled particulates. While children may be predisposed to acute effects, they may also receive an increased particle dose to lung tissue compared to adults. In a recent study we measured fractional deposition (DF) of fine particles in children, age 7-14, adolescents, age 14-18, and adults, age 19-35, who inhaled 2um monodisperse particles while following a breathing pattern previously determined by respiratory inductance plethysmography for that subject (i.e. that subject's spontaneous pattern at rest). Among the children there was no variation in DF with subject age or height, but DF was dependent on inter-child variation in tidal volume. On the other hand, the rate of deposition normalized to lung surface area, nDrate, was greater (35%) in the children vs. the combined group of adolescents and adults for resting breathing of these particles. The increase in nDrate in the children was due to their higher minute ventilation in relation to their lung size. Though total deposition may not be much different between children and adults, regional deposition of particles within the respiratory tract may be more affected by age. For example we also found recently that extra- vs. intra-thoracic deposition of radiolabeled coarse particles (4.5 um) was enhanced in a group of children vs. adults with cystic fibrosis but normal pulmonary function. Extrathoracic (laryngeal and mouth) deposition increased with decreasing age and height of the subjects suggesting that the upper airways act to filter inhaled particles more efficiently in children compared to adults. Finally, to assess exercise-related effects on total and regional deposition in children we are currently determining oro-nasal switching points associated with exercise in children and DF of fine particles for exercise breathing by both mouth and nose. These results may prove useful in determining relative risks associated with the inhalation of airborne particulates. Supported by USEPA Cooperative Agreement CR824915.

078 REGIONAL DEPOSITION DISTRIBUTION OF INHALED ULTRAFINE, FINE, AND COARSE MODE PARTICLES IN HEALTHY HUMAN LUNGS.

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Accurate estimation of dose is an integral part of health risk assessment for inhaled particulate matters. However, specific quantitative data are lacking for local dose estimation in human lungs. We measured total (TDF) as well as detailed regional deposition values in healthy adult men (N=22) and women (N=22) for ultrafine (UF; 0.04, 0.06, 0.08 and 0.10μm dia.), fine (F; 1 μm dia.), and coarse (C; 5μm dia.) particles using serial bolus delivery method (J. Appl. Physiol. 81:2203, 1996). A laser aerosol photometer was used to detect F and C, and a modified ultrafine condensation nuclei counter (TSI 3025A) was used to detect UF. The subject inhaled a series of bolus aerosols with a tidal volume (Vt) of 500 and 1000 ml at a respiratory flow rate (Q) of 150 or 250 ml/s. Regional deposition values were obtained for 10 sequential volumetric lung regions (Vp=50-500 ml at 50 ml interval). Regional deposition pattern varied markedly with particle size and breathing patterns. Peak deposition was found in the conducting airway region (Vp=100-150 ml) for C and in the proximal alveolar regions (Vp=250-300 ml) for F with Vt=500 ml at Q=250 ml/s. Sites of peak deposition of UF were in the distal airway region, Vp=150-200 ml. With Vt=1000 ml, sites of peak deposition shifted toward the deeper lung regions. Deposition dose per unit surface area (RDF/S) was largest in the proximal airways and decreased rapidly with an increase in Vp regardless of particle size used. Peak surface dose was 3-9 times greater than average lung dose depending on particle size tested. In men, TDF was 0.55±.07, 0.18±.03 and 0.76±.04 at Q=150 ml/s and decreased to 0.49±.07, 0.10±.02 and 0.7±.04 at Q= 250 ml/s for UF (D_p=0.04 μm), F and C, respectively with Vt=500 ml. With Vt=1000 ml, TDF was 0.70±0.06, 0.27±0.05, and 0.78±0.04 for UF (D_p=0.04 μm), F and C, respectively at Q=250 ml/s. In women, deposition pattern was similar to those in men, but the magnitude of peak deposition was amplified particularly for very small UF and C. TDF also was greater for these particles compared to men. These distinctions in total and local deposition dose may have significant implications in assessing health risk of inhaled particles. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

079 DEPOSITION OF FINE PARTICLES IN HUMAN LUNGS: EFFECT OF BREATHING PATTERN AND GENDER.

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The respiratory deposition of inhaled particles is a key factor in assessing health risk of particulate air pollution. In this study we investigated the variability in total lung deposition fraction (TDF) in healthy men (N=10) and women (N=7) with three different particle sizes and widely varying breathing patterns. The subject inhaled monodisperse fine (F; 1 micron dia.) and coarse particle aerosols (C; 3 and 5 micron dia.) with varying tidal volumes (Vt=350, 500, 750, 1000 and 1500 ml) and flow rates (Q= 175, 250, 500, 750 and 1000 ml/s) from functional residual capacity; the minute ventilation was thus varied from 4.5 to 30 l/min. Particle concentration and flow rate were measured continuously by means of a laser aerosol photometer and a pneumotachograph, respectively. It was found that for a fixed flow rate TDF increased with increasing tidal volume for both F and C. For a fixed tidal volume TDF decreased with increasing flow rate for Dp=1 and 3 micron, but no significant changes were observed for Dp=5 micron. For a fixed breathing frequency of 15 breaths/min TDF increased with increasing tidal volume for C but no significant changes for F. The pooled TDF data was correlated reasonably well with a composite breathing parameter, $Dp^{1.5}Q^{-0.4}Vt^{1.3}$, by using a logistic function. There was no significant difference of TDF for F between men and women, but TDF of C in women was consistently higher (8-36% for 3 micron, 4-22% for 5 micron; $p<0.05$) than that in men for all Vts and Qs used. For a given tidal volume the difference of TDF between man and women was greater with increasing flow rate. Our results suggest that mode of breathing significantly affects respiratory deposition dose, and that women may have a greater health risk to particulate pollutants depending on particle sizes. Supported by USEPA Cooperative Agreement CR824915. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

080 TOTAL LUNG DEPOSITION OF INHALED ULTRAFINE PARTICLES IN HEALTHY MEN AND WOMEN.

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Ultrafine particles (Dp < 0.10 _m in dia.) are present in a great number in polluted urban air and this may pose a potential health risk. Previous studies measuring lung deposition of ultrafine particles have used theoretical models or have only tested a few subjects, without evaluating gender differences. In this study, the total deposition fraction (TDF) of non-hygroscopic sebacate oil aerosol (Dp = 0.04, 0.06, 0.08, and 0.10 _m and _g = 1.3) have been measured in a group of young healthy adults (11 males and 11 females). TDF was obtained with 3 different breathing patterns: tidal volume (Vt) of 1 liter at respiratory flow rate (Q) of 250 ml/s and Vt = 500 ml at Q of 150 and 250 ml/s. An on-line computer automated system was used to control the aerosol valve, to integrate the flow signals, and to display the flow/volume and inhaled aerosol signals for the subject to view their breathing pattern during the maneuver. The results show that TDF was 0.34, 0.40, 0.44, and 0.53 at Vt = 500 ml and Q = 150 ml/s, and decreased to 0.26, 0.30, 0.35, and 0.44 at Vt = 500 ml and Q = 250 ml/s for 0.10, 0.08, 0.06, and 0.04 _m particles, respectively. At Vt = 1000 ml and Q = 250 ml/s, TDF was 0.40, 0.47, 0.56, and 0.66 for 0.10, 0.08, 0.06, and 0.04 _m particles, respectively. TDF was greater for females than males at 0.04 _m for all breathing patterns ($p < 0.05$), and the difference was marginal at 0.06 _m, whereas no significant differences were observed for the 0.08, nor 0.10 _m particles. The results suggest that TDF of ultrafine particles increases with breathing patterns of longer respiratory time, and that there may be a differential health risk between males and females. This is an abstract of a proposal presentation and does not necessarily reflect EPA policy.

097 A SOFTWARE PACKAGE FOR MULTIPLE-PATH MODELING OF PARTICULATE MATTER DEPOSITION IN HUMAN AND RAT LUNGS.

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Characterizing the dosimetry of particulate matter in the respiratory tract is critical to evaluating health risks due to airborne particulate matter toxicants. Lack of resolution in current dosimetry models introduces uncertainty in estimates of risk to human health based on animal data. We developed a user-friendly software package based upon a multiple-path model developed previously by S. Anjilvel and B. Asgharian (Fundam. Appl. Toxicol. 28, 41, 1995). The software calculates lobar-specific and airway-specific deposition of monodisperse and polydisperse aerosols in human and rat respiratory passages. The multiple-path model is capable of incorporating all the asymmetries in the airway branching structure if the morphometric details are available. Our analysis indicates that deposition is very different among the lung lobes for both species. In the human lung, the lower lobes accounted for the highest deposition fractions. Within a lobe, the middle airways received similar doses. Deposition in the upper bronchial airways and pulmonary airways differed significantly across lobes, however, with differences being larger for coarse particles. As particle size increased beyond 3 mm, deposition in the upper bronchial airways increased significantly. The deposition of ultrafine particles (0.1 mm) was high and confined to a relatively small number of pulmonary acini. Assuming uniform deposition across all acini as in the single-path models, neglect of local heterogeneity in the airway branching structure leads to an underestimation of the health risk. Increased regional resolution in the deposition model reduces uncertainty in assessment of risk to human health from particulate matter.

099 AIR POLLUTION PARTICLES: PYSIOCHEMICAL PROPERTIES, DNA DAMAGE AND DETECTION IN HUMAN AND CANINE TISSUE.

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Air pollution particles (APP) represent a heterogeneous complex mixture. We characterized particles for elemental content and their solubility from ten natural and APP. We also evaluated and compared physicochemical properties of particles as detected in human autopsy (n=50) and dog lungs (apical and basal) (n=17) collected in different regions in Mexico City. Particle analysis was carried out by 1) x-ray energy fluorescence (XRF); tissue analysis by 1) XRF, 2) inductively coupled plasma (ICP) analysis 3) and energy dispersive spectrometry scanning electron microscopy (EDS-SEM). The major proportion of elements in fugitive, urban air and coal fly ash particles were Si, Al, Fe, Ca, and S. In contrast, residual oil fly ash had high concentrations of S, Fe, Ni, and V. In situ EDS-SEM tissue analysis revealed Al, Si, and Fe-rich particles in canine and human samples. By ICP analysis, lung metal burdens were as follows Fe>Al>Zn>Cr>Cu>Ni ranging from 2-1161 ppm in human tissue. The relative advantages of applying multi-instrumental analysis for determining particulate chemistry, dose and size in tissue in relation to age, smoking status, and occupation will be discussed. Oxidative and polycyclic aromatic hydrocarbon-derived damage for a sub set of samples will be presented. [This abstract does not necessarily reflect EPA policy].

110 BIOKINETICS OF AN ULTRAFINE SILVER AEROSOL INHALED BY RATS.

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The biokinetics of inhaled ultrafine particles deposited in the respiratory tract is poorly understood. To quantify this biokinetics, male F344/Crl rats, 12 wk of age, were exposed by inhalation to a silver aerosol radiolabeled with ^{110m}Ag (count median diameter = 25 nm). Rats received an initial body burden (IBB) of approximately 130 kBq at a particle mass of < 1 ng from the 35-min exposure. Immediately thereafter, then at 2, 6, 24, and 72 hr after exposure, groups of four rats were sacrificed to quantify the ^{110m}Ag in selected tissues. The ^{110m}Ag was cleared rapidly, with 53% and 25% of the IBB present at 24 and 72 hr, respectively. Over 98% of the ^{110m}Ag was excreted through the feces. As a percentage of the sacrifice body burden, the activity in lungs was 27%, 9%, and 2% at 6, 24, and 72 hr after exposure, respectively; the activity in the GI tract was 43% at both 24 and 72 hr; and the activity in liver was 10% and 6% at 24 and 72 hr, respectively. A tissue digestion and ultracentrifugation technique was used to determine whether the ^{110m}Ag activity was present in selected tissues in a particulate or soluble form. Preliminary data suggest that the activity consisted predominantly of particles in the lung and liver, and was dissolved in the blood. Increased numbers of exposed grains were present in autoradiograms of liver. These grains were located over scattered hepatocytes and foci of hepatocytes 24 and 72 hours after ^{110m}Ag inhalation, and were not present in hepatic autoradiograms of companion rats administered ^{110m}Ag by gavage. These data describe the biokinetics of an inhaled ultrafine aerosol in the rat and suggest that ultrafine particles can be translocated from lung to liver. Research supported by the US DOE Office of Energy Efficiency and Renewable Energy and Office of Biological and Environmental Research.

163 OBSERVATIONS ON PARTICULATE MATTER RETENTION ALONG WELL-DEFINED AIRWAY PATHS IN A SAMPLE OF 117 AUTOPSY SUBJECTS.

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To elucidate mechanisms that can explain the role of particulate matter (PM) episodes on human morbidity and mortality, experimental studies in animals and cell lines are being performed. Once mechanisms have been successfully identified, dosimetry models will be needed to make critical risk assessment extrapolations between the exposure regimen in a controlled experiment and the exposure scenarios that might be expected to result in similar health consequences in humans. At present, only limited information is available on particle deposition and retention in humans. Lung tissue samples for pathologic, morphometric and mineral dust analyses have been collected (July 1994-April 1996) from 117 autopsies of Hispanic males (16-73 years of age; median age=29 years) at the Fresno County (CA) Coroner's office. Morphometric analyses have been completed in 43 cases and generally show that retention is greatest in the respiratory bronchioles (rb) and that the observed retention in 3 generations of rbs decreases as the generations proceed distally. Severity scores for histological features such as fibrosis, muscle hypertrophy and wall inflammation are positively correlated with the indicators of retention: they are more pronounced in the rbs than at other sites and decrease as rb generation number increases. For example, a 73 year old subject lived in Fresno County for 29 years and was a general laborer on a farm for 25 years. At a relatively apical site in the left lung, black pigment was observed to be dense in the 1st rb, moderate in the 2nd, and slight in the 3rd. This subject had corresponding scores for fibrosis in the 3 rbs. No polarized pigment was observed. For comparison, histological results for the same site in three students (18-19 years old) who had lived in Fresno County all their lives and had never worked showed no black pigment in any of the 3 rbs (except a slight accumulation in one of the 3 subjects in the 1st rb). In these subjects also the fibrosis score was the same as the black pigment score except in one case: slight fibrosis was observed with no black pigment in a 2nd rb in one subject. Interestingly, no fibrosis was observed in that subject in the 1st rb. In addition to providing a relatively large database for a morphometric study, the age range of the subjects in this database permits examination of the progression of structural remodeling and changes in particle retention patterns over the lifetime of adult humans.

Session 5. Susceptibility Related

004 ARE MALES MORE SUSCEPTIBLE TO AMBIENT PM THAN FEMALES?

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Recent epidemiologic studies of modern air pollution show a statistically significant relationship of daily mortality to daily ambient concentrations of particulate matter (PM) at low concentrations. A review of historic smoke-fog (smog) episodes (Meuse Valley, Donora, London) was conducted to identify common characteristics of victims of very high concentrations of air pollution to help identify a susceptible cohort for an effect of the lower PM concentrations. The Meuse Valley episode had been investigated in depth, but the published report did not detail the age and sex of the victims (Firket, 1931). We obtained the complete set of death certificates from all the Belgian villages in the area over the period of the smog (December 1-7, 1930). These data on age and sex from the Meuse Valley are provided here for the first time. The results show a male mortality excess of 50% (36♂, 25♀) consistent with the finding of excess male deaths in both the Donora Fog (15♂, 5♀) and the 1952 London Fog (autopsied cases; 419♂, 287♀). In all three episodes mortality was predominantly amongst the aged, and most of those autopsied presented with pre-existing cardio-pulmonary lesions. Modern air pollution time-series epidemiology studies at the much lower air pollution concentration levels than occurred during those historic smog incidents also show a male excess mortality (20%) amongst the aged in cardio-pulmonary cases (Schwartz, 1994). The data sets on age and sex are presented for the three historic episodes and the possible reasons for the apparent excess male vulnerability to the smog are discussed. A possible confounder or effect modifier in these historic studies may be a higher tobacco smoking rate amongst males than females in the aged at-risk population. Although no data are available on the prevalence of smoking amongst these populations in general, or the deceased in particular, recent epidemiologic studies have found no statistically different risk of mortality from modern PM exposures for smokers and nonsmokers (Pope et al., 1995). We therefore hypothesize that males may be more susceptible to the effects of ambient PM exposures than females, and that an excess of male smoking, if present in these populations, would not be a major factor in creating the excess in male mortality that we have observed.

005 EFFECTS OF PM10 ON EXERCISE-INDUCED CHANGES IN SPIROMETRIC LUNG FUNCTION OF SYMPTOMATIC CHILDREN.

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In order to find out how ambient air pollution affects bronchial responsiveness, we examined 30 primaryschool children who participated in exercise challenge tests at most five times during the winter of 1994. All the children had had chronic respiratory symptoms during the preceding year or had asthma. The exercise test lasted 8 minutes and it was done outdoors at the schoolyard with a bicycle ergometer. The exercise test was done once indoors as a baseline test for each child before the series of outdoor tests. The spirometric lung function of the children was measured before, and 3 and 10 minutes after exercise. For analyses, the largest change between pre- and post-exercise recordings was calculated, and the baseline response was subtracted from this. Ambient 24-h PM10 concentration was measured at a near-by fixed monitoring site with Harvard impactor. The outdoor temperature was measured at the schoolyard during the tests.

In the baseline tests, the mean change in FEV1 was 2.8 %. According to the preliminary unadjusted results, there was no clear association between PM10 (lag 0) and mean additional change in FEV_1 outdoors (Table 1).

Table 1.

PM10, $\mu g/m^3$	5 - 11	12 - 19	20 - 29	30 - 135
Number of days	8	7	8	7
Number of tests	27	34	33	30
Temperature, °C				
Mean	-0.3	-1.3	-6.1	2.6
Range	-4.3 - 2.9	-9.9 12.5 10.6	-24.4 14.0	-8.8-
Mean additional change in FEV_1, %	-0.8	-1.2	-1.9	0.8

025 DIFFERENTIAL RESPIRATORY TRACT LINING FLUID (RTLF) REDUCED GLUTATHIONE (GSH) RESPONSES TO DIESEL EXHAUST IN HEALTHY AND MILD ASTHMATIC SUBJECTS.

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GSH has been proposed to play an important protective role in the lung, by limiting oxidative injury derived from inhaled particulate matter. In order to test this 25 healthy (9F, 16M; 24.5±4.3 years) and 15 mild asthmatic (5F, 10M; 30.3±8.4 years) subjects were exposed to diesel exhaust (DE-100mg/m³ particles, 0.6 ppm NO_2) and air (A) for 2h. All exposures were conducted in a randomised, single blinded, crossover control fashion, with successive exposures separated by >3 weeks. Nasal lavage (NL) was performed, pre- (*Pre-E*), 1h into- (*1h-E*), immediately post- (*0h-PE*) and at 5.5h post-exposure (*5.5h-PE*). Brochoscopy with bronchial wash (BW) and bronchoalveolar lavage (BAL) was performed 6h-post exposure (*6h-PE*) to obtain proximal and distal RTLF samples. DE exposure resulted in an increase in NL- and BW-fluid GSH concentrations at *5.5* and *6h-PE* respectively in healthy subjects: NL-fluid, 0.8 (0.5-1.6) after air vs. 1.4 (0.6-1.7) mmol/L after DE, $p<0.01$; and BW, 0.5 (0.4-0.6) after air vs. 0.8 (0.5-1.1) mmol/L after DE, $p<0.005$. A similar increase was not observed in BAL-fluid samples. In contrast increased RTLF GSH concentrations were not observed in any of the lavage fluid fractions obtained from the mild asthmatic subjects after DE. *Pre-E* GSH concentrations were also found to be significantly depressed ($p<0.05$) in all lavage fractions from the mild asthmatics compared with healthy subjects. If GSH is protective against inhaled particulate matter these data would suggest that healthy subjects would be more resistant (high GSH baseline concentrations, 'protective' adaptive responses) to the effects of DE than mild asthmatics. However in this study no differences were noted in lung function between the two groups and indeed airway inflammation was only pronounced in the healthy subjects. These data demonstrate therefore that GSH is not a good predictor of sensitivity to DE.

027 PATHOLOGY OF FATALITIES FROM FINE PARTICLE AIR POLLUTION (PM) IN THE GREAT LONDON SMOG OF 1952.

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Epidemiological studies from many diverse locations demonstrate similar dose-response relationships between airborne Particulate Matter (PM) air pollution and daily mortality. However, the absolute increases in human

mortality with current levels of PM are so low that collection of contemporary autopsy materials from deaths known to result from PM is not feasible. Therefore, we have investigated autopsies in archives from before, during and after During the Great London Smog of December 5-9, 1952 -- one of the sentinel events leading to recognition of the relationship of PM to daily mortality. During this episode London mortality increased dramatically from <250/day to >900/day, with >4000 estimated excess deaths recorded over a period of a several weeks. During the 1950 s and 60 s, the autopsy rate at the RLH was >60%, with >400 autopsies/year. We observed the total number of autopsies increasing with a monthly peak paralleling previously reported daily/weekly mortality from death certificates -- which have shown the major peak increases to be from pulmonary and cardiovascular deaths. Autopsies which listed Bronchitis and/or Emphysema (COPD) among major diagnoses showed the sharpest peak. The 1952-53 winter COPD autopsy peak lagged, and was much broader than the death certificate peak. This reflected deaths among people initially hospitalized during the Smog, or whose COPD worsened. Daily and weekly autopsy numbers were too few for analysis. Sudden deaths, included in the death certificate data, were less clearly reflected in hospital autopsies, and the peak in cardiovascular deaths was not as clear. Monthly autopsies several years before the great Smog, and several years after (during which stricter PM controls went into effect) showed smaller peaks for autopsies mentioning COPD. Analysis of the pathology and PM in these historic autopsy tissues allows comparison with contemporary PM studies.

028 ENDOTOXIN PRIMING AFFECTS THE LUNG RESPONSE TO ULTRAFINE PARTICLES AND OZONE IN YOUNG AND OLD RATS.

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Epidemiological studies have demonstrated a correlation between low levels of ambient ultrafine particles and morbidity in sensitive parts of the population, particularly the elderly with existing cardiopulmonary disease. Such correlations have been challenged due to doubts as to whether particles act alone to cause these detrimental effects. We hypothesized that the combination of ambient carbonaceous ultrafine particles (part of the urban fine particle mode) and ozone (O_3) would induce greater oxidative stress and lung injury than when administered alone and that their effects would be amplified in the compromised, aging lung. We exposed male F344 rats (10 wks, 20 mos) to ultrafine carbon particles (median diameter ~25 nm, ~100 mg/m^3, equivalent to ~50 mg/m^3 inhaled by humans) and to O_3 (1 ppm) alone and in combination for 6 hrs. Low-dose endotoxin (LPS) priming by inhalation (70 endotoxin units estimated alveolar deposited dose) was used as a model of respiratory tract infection. Inflammatory parameters in bronchoalveolar lavage (BAL) fluid and oxidant release from BAL cells were assessed 24 hrs after exposure. A significant main effect of carbon, O_3, and LPS on lung inflammation and a significant interaction between LPS and O_3 which resulted in lower inflammation was observed in young rats. In old rats, only LPS and O_3 had significant effects, but carbon and O_3 interacted and increased lung inflammation above the effect level for either component alone. In general, oxidant release by BAL cells corresponded with the PMN response. However, in young rats, the combination of LPS priming with carbon and O_3 exposure inhibited oxidant release. This inhibition of oxidant release was not observed in old rats. These results are consistent with our hypothesis that urban ultrafine carbonaceous particles are involved in increased morbidity in sensitive populations. In addition, the age of the organism and co-exposure to O_3 can significantly affect both lung inflammation and activation of inflammatory cells. In both age groups LPS enhanced the effects of the other components and allowed observation of effects that would otherwise be masked.

031 GENETIC LINKAGE ANALYSIS OF SUSCEPTIBILITY TO ACID-COATED PARTICLE EXPOSURE IN MICE.

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Particle-induced respiratory morbidity and mortality have been observed worldwide in industrialized cities, but the toxicologic mechanisms are not well known. Inhalation challenge with sulfate-associate carbon particles impairs murine alveolar macrophage (AM) immune function and enhances mortality associated with virus infection. Formal segregation and genome-wide linkage analyses were done to identify mechanisms of susceptibility to particle-induced AM dysfunction in susceptible C57BL/6J and resistant C3H/HeJ inbred mice and their progenitors. We phenotyped backcross [BX:B6 (N=112); BX:C3 (N=21)] and intercross [B6C3F_2 (N=105)] progeny for changes in AM phagocytic function 3 d following a 4 hr acid-coated particle (ACP) exposure. Segregation analyses with the software program (SAGE) Statistical Analysis for Genetic Epidemiology indicated that the two unlinked loci model best fit the segregation data. Selective genotyping of the F_2 cohort (50 meioses) was done for 156 simple sequence length polymorphic markers distributed throughout the genome. Linkage was assessed using Map Manager. Significant and suggestive quantitative trait loci (QTL) were identified on chromosomes 17 and 11, respectively. The QTLs were subsequently verified with the entire F_2 cohort. Both QTLs overlap previously identified QTLs for susceptibility to inflammation induced by another common pollutant, ozone. Results suggest genetic background is an important determinant of responsiveness to particle-induced morbidity, and have important implications for evaluating risk to environmental exposure.

032 EXPOSURE TO URBAN PARTICULATES INDUCES AIRWAY HYPERRESPONSIVENESS AND INFLAMMATION IN MURINE STRAINS.

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Epidemiological studies indicate that the incidence, as well as the morbidity/mortality of asthma has increased over the past 2 decades, particularly in urban environments. It has been hypothesized that exposure to particulate matter (PM) may be responsible for increases in the incidence of asthma . The goal of this study was to compare responses to urban PM in strains of mice that our laboratory has previously determined to be genetically susceptible (A/J) or resistant (C3H) to the development of asthma-like symptoms. We exposed A/J and C3H mice to 0.5 mg of either PM collected in urban Baltimore (AUB), coal fly ash from a standard reference source (NIST), or PBS (50 µl). Airway responsiveness (AHR) to i.v. acetylcholine was assessed and bronchoalveolar lavage (BAL) was conducted for determination of cell composition and cytokine measurement by ELISA at 24, 48, and 72 hrs after PM exposure. We found that AHR was increased at 24 and 48 hrs after AUB exposure compared to PBS controls, but returned to control values at 72 hrs in A/J mice. C3H mice also had increased AHR at 24 and 48 hrs which continued 72 hrs after AUB exposure. Exposure to NIST did not induce increases in AHR at any timepoint in either strain of mice. A significant pulmonary eosinophilia and neutrophilia that persisted through 72 hrs was induced by AUB exposure compared to PBS in both strains of mice. NIST exposure also induced increases in eosinophil and neutrophil numbers, but to a lesser degree than did AUB. Finally, at 48 and 72 hrs after AUB exposure, both strains of mice showed increased levels of the Th1 cytokine IFN-γ, while the Th2 cytokines IL-4 and IL-5 decreased. These results suggest that PM may play a role in inducing AHR in both genetically susceptible and non-susceptible individuals. Funded by grant numbers R826724-01 and P01ES09606-01

034 LONG-TERM MEAN CONCENTRATION OF AMBIENT FINE PARTICULATE MATTER ($PM_{2.5}$) AND INCIDENCE OF SMOKING-RELATED CANCERS AND ALL NONSKIN MALIGNANT CANCERS IN THE AHSMOG STUDY.

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Increasing evidence implicates the $PM_{2.5}$ component of particulate matter (PM) as having greater carcinogenic potential. Cancer incidence has been ascertained 1977-1992 in a cohort of 6338 nonsmoking participants in the Adventist Health and Smog (AHSMOG) study. In a previous report [Beeson et al., *Environ Health Perspec.* 1998;106(12):813-823] we observed an elevated risk of incident lung cancers in study participants associated with elevated long-term mean concentration of inhalable particles less than 10 microns in diameter (PM_{10}). Associations were stronger for males than females. Analyses pertaining to long-term concentration of $PM_{2.5}$ (1/1/66 - 4/1/77) are now available. Because of an insufficient number of incident lung cancer cases on the 64% of the AHSMOG cohort with $PM_{2.5}$ data, we combined sites known to be related to smoking under the hypothesis that respirable particulate matter could act like cigarette smoke in causing cancers at sites remote from the lung as well as for respiratory cancers. For smoking-related cancers (SRC) [26 for males, 34 for females], males demonstrated an increased risk (relative risk [RR]=2.03, 95% confidence interval [CI]=0.79-5.22) associated with an interquartile range (IQR) increase in mean concentration of $PM_{2.5}$ of 32 $\mu g/m^3$. This association was stronger than for PM_{10} in males. No associations with SRC were seen for females for either $PM_{2.5}$ or PM_{10}. For all incident nonskin malignant cancers (148 for males, 230 for females), the association with $PM_{2.5}$ was slightly larger in females (RR=1.08, 95% CI=0.80-1.47) than in males (RR=1.03, 95% CI=0.71-1.50). Other particulate and gaseous pollutants were also evaluated (PM_0 exceedance frequencies, mean concentrations of SO_2 and O_3). **Conclusion:** We observed gender differences in risk of incident cancer in association with long-term mean concentration of ambient respirable particulates. All analyses controlled for age, pack-years of past cigarette smoking, education, and alcohol consumption. Funding provided by the U.S. Environmental Protection Agency cooperative agreement #CR 819691and the American Cancer Society, grant # RD-397.

035 LONG-TERM ESTIMATED FINE PARTICLES LESS THAN 2.5 MICRONS IN DIAMETER ($PM_{2.5}$) AND MORTALITY IN CALIFORNIA NONSMOKERS.

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A cohort of 6338 nonsmoking Seventh-day Adventists has been followed since 1977 for the purpose of relating long-term concentrations of ambient air pollutants to health effects. Elevated long-term concentrations of PM_0 and other pollutants were found to be associated with increased risk of death from: all natural causes (ANC), and other specific causes through 1992 [*Am J Resp Crit Care Med*.1999; 159:000-000]. $PM_{2.5}$ data were not available for study. Analyses have now been extended to include $PM_{2.5}$ mean concentration estimated [*JEAEE* 1995;5(2):137-159] from site/seasonal-specific regression equations based on airport visibility data for 1966-1977. These data are available on 64% of the cohort. Death certificates were coded for cause of death by a certified nosologist blinded to air pollution data. A total of 781 (males: n=312; females: n=469) ANC deaths were identified in the $PM_{2.5}$ subcohort. Cox proportional hazards regressions were used to estimate relative risks (RRs) of death associated with an interquartile range (IQR) increase of mean concentration of PM2.5 of 32ug/m^3 adjusting for covariates. Covariates for ANC included age, years of education, pack-years of past smoking, years lived with a smoker, and total exercise level. The RRs of ANC were 1.26 (95% Confidence Interval [CI]=0.97-1.65) for males and 0.88 (95% CI=0.71-1.09) for females. Approximately 60% of ANC are cardiopulmonary (CP) deaths (males: 58.7%; females: 61.6%) in this study. The RR of CP was 1.09 (95% CI=0.77-1.55) for males and 0.79 (95% CI=0.60-1.04) for females. We also investigated deaths with any mention of nonmalignant respiratory disease as underlying or contributing causes (CRC). For both genders combined, the RR was 1.35 (95% CI=0.93-1.95) with the RR being higher in males (RR=1.69, 95% CI=0.94-3.05) than in females (RR=1.15, 95% CI=0.72-1.85). Only 22 deaths were attributed to lung cancer [males: n=13; females: n=9] as the underlying cause in the $PM_{2.5}$ subcohort. Increased RR of lung cancer death associated with one IQR increase in mean concentration $PM_{2.5}$ was only observed in males (RR=3.57, 95% CI=0.78-16.29). Increased RRs of death associated with elevated $PM_{2.5}$ were observed for ANC, CP, CRC and lung cancer for males in this cohort but not for females except for CRC. Funded by: US-EPA #CR 819691.

038 CARDIOPULMONARY AND THERMOREGULATORY RESPONSES TO EXPOSURE TO INSTILLED AND INHALED PARTICULATE MATTER IN HEALTHY AND COMPROMISED RATS.

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Recent epidemiological studies link exposure to ambient-derived particulate matter (PM) with cardiopulmonary-related morbidity and mortality. To test this association in animals, we conducted a series of studies to investigate the cardiopulmonary and thermoregulatory toxicity of residual oil fly ash (ROFA) PM in conscious, unrestrained rodents. All animals (n=16/group/model studied) were implanted with radiotelemetry transmitters to monitor changes in electrocardiogram (ECG), heart rate (HR), and core body temperature (T_{co}) throughout the experimental time period. The first study examined the effects of intratracheally *instilled* ROFA (0, 0.25, 1.0, 2.5 mg) in healthy and cardiopulmonary-compromised Sprague-Dawley rats. Rodent models used to simulate cardiopulmonary stress/disease included: a) rats maintained at an ambient temperature of 10°C, b) rats pretreated with ozone (1 ppm × 6 h) to induce pulmonary inflammation, and c) rats pretreated with monocrotaline (MCT; 60 mg/kg, *ip*) to induce pulmonary vasculitis and hypertension. Healthy rats demonstrated biphasic dose-dependent decreases in HR and T_{co} following instillation of ROFA and these effects were exacerbated in compromised animals. ROFA-induced increases in ECG abnormalities (skipped beats, premature contractions) were directly correlated with the severity of cardiopulmonary dysfunction. More detailed analyses of ECG waveform changes suggest that ROFA primarily affects the repolarization of the myocardial tissue. Mortality (n=6) following ROFA exposure occurred only in MCT-treated animals. The second study investigated the effects of *inhaled* ROFA (15 mg/m^3 × 6 h/d × 3 d) in rodent models of both pulmonary (MCT-treated Sprague-Dawley rats) and systemic (Spontaneously Hypertensive rats) hypertension. These animals demonstrated similar HR and T_{co} responses as above but of lesser magnitude. While there was a moderate ROFA-induced potentiation of adverse ECG events (arrhythmias, ST-segment depression), there were no lethalities following inhalation of ROFA. The results of these studies demonstrate substantial cardiotoxicity in rats after exposure to ROFA and support previous epidemiological studies that report an association between preexisting cardiopulmonary disease and potentiation of adverse health effects following exposure to airborne particulates. (This abstract has been internally cleared and does not reflect EPA Policy.)

039 CARDIOPULMONARY AND THERMOREGULATORY EFFECTS OF EXPOSURE TO METALLIC CONSTITUENTS OF RESIDUAL OIL FLY ASH PARTICLES IN HEALTHY AND COMPROMISED RATS.

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Growing epidemiological evidence suggests a direct relationship between exposure to ambient particulate matter (PM) and the incidence of cardiopulmonary-related morbidity and mortality. We have previously shown that intratracheal instillation (IT) of residual oil fly ash (ROFA) PM in rats produces a biphasic response consisting of an immediate (0–6 h) and delayed (12–72 h) hypothermia accompanied by profound bradycardia, arrhythmias, pulmonary inflammation, and lethality. It has been proposed that specific metallic constituents of ROFA (e.g., Fe, V, and Ni) may play a role in the observed cardiac toxicity. The present studies examined the effects of these transition metals on indices of cardiopulmonary and thermoregulatory function in a rodent model of pulmonary hypertension (PHT). Male Sprague-Dawley rats (60 d) were implanted with radiotelemetry transmitters capable of monitoring electrocardiogram (ECG), heart rate (HR), and core body temperature (T_{co}). At 7 d postsurgery, rats were injected with monocrotaline (MCT; 60 mg/kg, *ip*) and allowed 14 d to initiate the development of PHT. In the first study, animals (n=16) were divided into four equal groups and rats within each group were instilled with either saline vehicle (SAL; 0.3 ml; pH=2) or Fe (105 μg $Fe_2(SO_4)_3$), Ni (262.5 μg $NiSO_2$), or V (245 μg VSO_4) in SAL. Fe-treated rats showed a mild cardiac and hypothermic response that did not differ significantly from that of control animals. Rats exposed to V exhibited significant acute (0–4 h) decreases in HR ($_{max}$60 bpm) and T_{co} ($_{max}$2.1 C), while exposure to Ni induced delayed (18–72 h) decreases in HR (110 bpm) and T_{co} (2.4 C). Arrhythmogenesis (skipped beats, premature contractions) was most pronounced in V-treated rats on post-IT Day 1 and in Ni-treated animals thereafter. Mortality (n=2) was observed only in the Ni-treated group. The second study, while otherwise identical, exposed rats to all possible metal combinations to test for interactions. Telemetry results were similar to those in the first study, however, there was an increase in mortality (n=6) among the exposed rats, primarily the Ni- and V-treated animals. These studies demonstrate substantial cardiopulmonary toxicity in rats after IT exposure to metals contained in ROFA and suggest that the adverse effects observed following ROFA instillation may be the result of complex interactions among these metallic constituents. (This abstract has been internally cleared and does not reflect EPA Policy.)

040 LONG-TERM INHALATION EXPOSURE OF NORMOTENSIVE AND SYSTEMICALLY HYPERTENSIVE RATS TO PM: OXIDATIVE BURDEN AND CARDIOPULMONARY INJURY.

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Hypertension is a significant health problem worldwide and may be a risk factor in particle (PM)-associated cardiopulmonary injury. In a series of studies, we have previously shown that a rat model of systemic hypertension (spontaneously hypertensive; SH) exhibits greater pulmonary vascular leakage, oxidative burden, and ECG abnormalities from acute intratracheal or inhalation exposure to residual oil fly ash (ROFA) PM when compared to normotensive Wistar Kyoto (WKY) rats. The purpose of this study was to correlate systemic changes in fibrinogen, platelets and red blood cell glutathione recycling enzymes to cardiopulmonary injury following short and long-term inhalation exposure to ROFA PM. SH or WKY rats (11-13 wks) were exposed to either air or ROFA containing soluble iron, vanadium, and nickel by nose-only inhalation (15 mg/m^{3}x6 h/dx3 d) for 1 or 4 (WKY) or 1, 2, or 4 wks (SH) and the responses were analyzed (1 or 4 d post-inhalation). Control SH, in general, had higher fibrinogen, platelet, and RBC counts, hematocrit, hemoglobin (Hb), and heart weights when compared to control WKY rats. No rat strain or ROFA exposure-related effects on white blood cells or RBC counts were apparent. Plasma fibrinogen levels increased only in SH rats following 1 wk of exposure but not at 2 or 4 wks post. This increase in fibrinogen was associated with a slight increase in blood platelet counts. Red blood cell glutathione reductase (following 1 wk) and peroxidase activities (following 4 wks) were increased only in SH rats exposed to ROFA. ROFA-induced lung injury, as determined by bronchoalveolar lavage fluid (BALF) analysis for protein, albumin, and presence of red blood cells (RBC), was more severe in SH when compared to WKY rats. The increase in BALF neutrophils was similar in SH and WKY rats. BALF of control SH rats consistently exhibited higher protein and albumin levels, and neutrophilic inflammation than WKY rats. In both strains, increases in BALF protein, albumin, lactate dehydrogenase activity, and neutrophilic inflammation were progressive over 4 wks of exposure whereas BALF glutathione was increased only in WKY rats (2-2.5 fold) following 1 wk of exposure. These results suggest a potential link between systemic effects and pulmonary injury from PM in hypertensive rats. The episodic long-term exposure used in this study was associated with progressive pulmonary injury in both rat strains. Reversible effects were only observed in fibrinogen levels of SH rats and glutathione levels of WKY rats. This study implicates systemic hypertension as a potential risk factor in long-term PM-induced cardiopulmonary and systemic effects and emphasizes the usefulness of SH rats as a susceptible model. (Does not reflect EPA policy).

042 RESIDUAL OIL FLY ASH (ROFA) ENHANCES SENSITIZATION TO HOUSE DUST MITE (HDM) IN BROWN NORWAY RATS.

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Epidemiological studies have shown an association between elevated levels of particulate matter (PM) air pollution and increased morbidity and hospital visits in asthmatics. Residual oil fly ash (ROFA) is a primary combustion particle containing sulfate and metals such as vanadium, nickel, and iron. In this study the effect of ROFA on sensitization to house dust mite (HDM) was examined in a Brown Norway rat model of pulmonary allergy. Rats were instilled via the trachea with 200 or 1000 μg ROFA 3 days prior to local sensitization with 10 μg HDM, and challenged with 10 μg HDM 14 days later. Immunological endpoints were examined at 2, 7, and 14 days after sensitization, and at 2 and 7 days after challenge (16 and 21 days post-sensitization, respectively). Antigen-specific immunoglobulin (Ig) E and associated immediate bronchoconstriction responses to antigen challenge were increased in the ROFA-treated groups compared with the HDM control group. Lymphocyte proliferation to antigen was enhanced at days 7 and 21 in the bronchial lymphocytes of ROFA-treated groups. Bronchoalveolar lavage fluid (BALF) eosinophil numbers and lactate dehydrogenase were significantly increased in the1000 μg ROFA group at days 2 and 16. BALF total proteins were elevated at days 2 and 7 in both ROFA-treated groups, and BALF interleukin (IL)-10 and TNFα were elevated in the 1000 μg ROFA group at days 2 and 16, respectively. In addition, IL-5 and IL-10 mRNA was upregulated in the lung tissue of the 1000 μg ROFA group at day 7. These results suggest that exposure to ROFA, a PM2.5 particle, has an adjuvant effect on sensitization to HDM. This abstract does not reflect EPA policy.

059 AIR POLLUTION AND INCIDENCES OF CARDIAC ARRHYTHMIA

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Elevated levels of particulate air pollution have been associated with an increase in hospital admissions and mortality for cardiovascular diseases. This study tests the hypothesis that patients with implanted cardioverter

defibrillators (ICD) experience potentially life-threatening arrhythmias associated with particulate air pollution episodes. Tachycardia and ventricular fibrillation were identified from records of 100 patients with ICD devices at the Beth Israel-Deaconess Hospital Cardiac Device Clinic for the years 1995 through1997. During the same period, 24 hour mean concentrations of particulate matter (PM_{10} and $PM_{2.5}$), Black Carbon and gaseous air pollutants were measured in the Boston area. Air pollution concentrations were moderate during this period. However, an increase of 26 ppb NO_2 was associated with increased tachycardia and ventricular fibrillation two days later, odds ratio of 1.8 (95% confidence interval: 1.1 to 2.9). Patients with repeated events (10 or more events during a three year follow-up) were especially at risk of experiencing arrhythmia in association with $PM_{2.5}$ (odds ratio: 1.6 for an increase of 22 $\mu g/m^3$ (95% confidence interval: 1.0 to 2.6)) and NO_2 (odds ratio: 2.8 for an increase of 26 ppb (95% confidence interval: 1.5 to 5.1)). These results suggest that elevated levels air pollutants are associated with potentially life-threatening arrhythmia leading to therapeutic interventions by an implanted cardioverter defibrillator.

060 ACTIVATION OF THE AUTONOMIC NERVOUS SYSTEM AND BLOOD COAGULABILITY IN ASSOCIATION WITH AN AIR POLLUTION EPISODE.

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Air pollution episodes have been consistently associated with increased mortality and hospital admissions due to cardiovascular disease while the biological mechanism remains to be elucidated. Mean heart rates during 20 second 12 lead EKG recording was measured in a cohort of 2681 men and women aged 25-64 years who participated in the MONICA Augsburg cohort during the winter 1984/85 and the winter 1987/88. Elevated concentrations of sulfur dioxide and total suspended particles (TSP) were measured during the air pollution episode in January 1985. Linear regression analyses were conducted using generalized estimating equation and adjusting for cardiovascular risk factors and meteorology. Increases in heart rate were observed during the air pollution episode (mean increase: 1.8 beats per minute (95% confidence interval (CI): 0.7 to 2.9 beats per minute)). A stratified analyses for persons with elevated plasma viscosity (> 1.35 mPa s in men and > 1.33 mPa s in women) during the first examination showed a mean increase of 5.1 beats per minute (95% CI: 2.1 to 8.2 beats per minute) comparing episode to non-episode days. A mean increase of 1.4 beats per minute (95% CI: 0.3 to 2.5 beats per minute) was observed in persons with normal plasma viscosity measurements. Persons with increased coagulability of the blood might be an important subgroup susceptible to an activation of the autonomic nervous system in response to air pollution.

066 EFFECT OF CCONCENTRATED $PM_{2.5}$ ON PULMONARY RESISTANCE AND COMPLIANCE IN ASTHMATIC RATS

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Epidemiological studies on the health effects of PM have identified people with a pulmonary disease like asthma as a group at risk. Asthma is characterized by airway hyperresponsiveness. The health effects of the complex mixture of ambient particulate matter (PM) can best be studied by using the mixture itself. To increase ambient concentrations an ambient fine particulate concentrator (AFPC) in combination with a $PM_{2.5}$ size selective inlet was applied. The levels of concentrated particles in the range of 0.1 2.5 µm (CAPs) are roughly 20 times higher than ambient levels, whereas the concentrations of smaller particles and the gaseous components are unaffected. To study the effects of PM on the severity of asthma-like effects, two independent experiments were performed in which BN rats were sensitized (day 1-5) and challenged (day 21-25) intranasally with timothy grass pollen *(Phleum pratense)* (as a model for atopic asthma) and subsequently exposed to CAPs for 4 hours per day during 4 consecutive days. Mean (and maximum) CAPs levels in these two experiments were 189 (390 ug/m^3) and 178 ug/m^3 (393 ug/m^3), respectively. Rats were anesthetized 4-5 days after the last exposure and the pulmonary resistance (R_L), dynamic compliance (C_{dyn}) and airway responsiveness to inhaled aerosolized methacholine (0.5 - 128 mg/ml for 30 seconds using a ultrasonic nebulizer) was measured.A small but statistically significant increase in both R_L and airway responsiveness and a decrease of C_{dyn} was observed. Remarkably, while mass concentrations of CAPs in both studies were comparable, these effects could not be related to the mass concentrations of CAPs. In addition, this study shows that CAPs can result in adverse effects in compromised airways of an animal model mimicking a human PM risk group.

075 VANILLOID (CAPSAICIN) AND ACID SENSITIVE IRRITANT RECEPTORS UNDERLIE MOUSE STRAIN-SENSITIVITY TO PARTICULATE MATTER.

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The causes of strain-species differences in response to PM exposure remain unresolved. BALB/c and C57BL/6 (B6) mice, exposed intratracheally to residual oil fly ash (ROFA, 3 mg/kg), were examined 24 hr later for signs of airway inflammation. BALB/c showed significantly higher numbers of neutrophils and increases in airway hyper-responsiveness to iv methacholine, whereas B6 mice showed no change in either endpoint. Neurons dissociated from dorsal root ganglia (DRG) which enervate the upper and lower pulmonary airways, were cultured from BALB/c and B6 fetal mice. Cultures were exposed (4 hr) to various urban and industrial PM (50 mM), neuropeptides, pH 5.0, 6.5 buffers, or capsaicin (1-3 mM). In all instances, DRG neurons from BALB/c mice released significantly higher levels of the proinflammatory cytokine IL-6 into their nutrient media compared to B6 mice. Single cell calcium recordings were taken from Fluo-3 labeled DRG cultures in response to acidity (pH 5.0, 6.5) and capsaicin. Significantly higher numbers of BALB/c neurons responded with increases in intracellular calcium compared to B6 neurons to these exposures. However, qualitatively the responses (i.e., peak amplitudes) were equivalent between strains. Together these data suggest that irritant receptors (i.e., capsaicin, acid sensitive) located on sensory fiber terminals subserve airway inflammation to PM and that these receptors are quantitatively different in responsive and non-responsive mouse strains. (*This abstract does not reflect EPA policy.*)

098 HAEMATOLOGICAL CHANGES ASSOCIATED WITH EXPOSURE TO PARTICULATE AIR POLLUTION: RESULTS OF A PANEL STUDY.

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Particulate air pollution has been associated with excess mortality and morbidity from cardiovascular disease. We have investigated whether this might be a consequence of alterations in the blood by repeatedly measuring haematological factors in elderly people and relating them to measurements of PM_{10}. One hundred and twelve subjects aged $\geq$ 60 years, resident in Edinburgh and Belfast, provided repeated blood samples over 18 months. Estimates of individual exposure to PM_{10} were made for each 3-day period prior to blood sampling. Relationships between blood values, personal exposure to PM_{10} and city centre measurements of PM_{10} were investigated by analysis of covariance, adjusting for city, season, temperature and repeated individual measurements. Estimated personal PM_{10} exposure negatively correlated with haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cell count (RCC) ($p<0.001$) and with platelets and factor VII ($p<0.05$). City centre PM_{10} measurements negatively correlated with Hb, RCC, PCV and fibrinogen and positively correlated with C-reactive protein. The estimated changes in Hb

associated with an alteration in PM_{10} of 100μg/m³ were 0.44g/dl(95% CI 0.62-0.26) for personal PM_{10} and 0.73g/dl (1.11-0.36) for city centre PM_{10}. The mechanism of these effects is unknown but may relate to peripheral sequestration of red cells which could be associated with cardiovascular effects. This study was funded by UK Department of Health.

108 AGE-RELATED RESPONSES TO CONCENTRATED URBAN AIR PARTICLES (CAPs).

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Epidemiological studies have reported that elderly individuals have a higher risk of detrimental responses following exposure to elevated levels of ambient particulate matter. To investigate this finding in a toxicological model, aged Fisher rats were exposed for three days to concentrated urban air particles (CAPs) from Boston. Based on results from previous work, we tested the hypothesis that older animals would exhibit more severe pulmonary inflammation and hematological changes following the CAPs exposure when compared to young, normal animals (Nadziejko et al, 1996, *AJRCCM* 155:A247; Clarke et al, 1997, *AJRCCM* 157:A151). Aged Fisher rats (>14 months) and juvenile Fisher rats (4-6 weeks) were obtained and observed in a virus-antigen free facility for 3 months prior to exposure. Following observation, animals (10 rats/group X 4 groups total = 40 rats) were exposed to CAPs or filtered air (FA) for 5 hours/day for three consecutive days. Daily integrated CAPs concentrations were 80, 170, and 50μg/m³ on day 1, 2, and 3, respectively. None of the animals died throughout the duration of exposure. Twenty-four hours following the last day of exposure, blood was collected by cardiac puncture, and bronchoalveolar lavage (BAL) was performed. Old rats exhibited a small, but significant, increase in BAL polymorphonuclear leukocytes (PMN) following exposure to CAPs ($0.58 \pm 0.16 \times 10^6$ cells (FA) versus $1.47 \pm 0.39 \times 10^6$ cells (CAPs)). There were no significant changes due to CAPs in total BAL cell counts, BAL lactate dehydrogenase, total white blood cell (WBC) counts, or the percent of WBC PMN, lymphocytes, and monocytes. However, when comparing all groups of old versus young, advanced age caused significant decrements in the total BAL cell counts, total WBC counts, percent of blood lymphocytes, and blood hemoglobin; a significant increase in the percent of blood PMN was also observed. Young rats exhibited significantly higher total BAL cell counts marked by a significant increase in BAL PMN following CAPs exposure ($0.29 \pm 0.20 \times 10^6$ cells (FA) versus $5.69 \pm 1.52 \times 10^6$ cells (CAPs)). The above results suggest 1) young Fisher rats may represent a sensitive model for the examination of pulmonary inflammatory responses following CAPs exposure and 2) the lack of an inflammatory response in the aged rats, despite the presence of higher numbers of circulating neutrophils, may reflect decreased sensitivity to inhaled particles and pathogens. Funded by: ES00002, ES08129, HL05947, and HL54958

113 RESPONSE TO WINTER AIR POLLUTION IS LARGER IN ALLERGIC SYMPTOMATIC CHILDREN THAN IN NON-ALLERGIC, NON-SYMPTOMATIC CHILDREN

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Studies in various locations have found effects of daily variations of ambient air pollution on acute respiratory symptoms and Peak Expiratory Flow (PEF). Some studies have reported that subjects with questionnaire reported chronic respiratory symptoms had a stronger response than asymptomatic subjects. Little is known about which objective, medical characteristics of subjects predict an increased response to air pollution. We therefore studied whether the response to air pollution differed across subgroups defined by both baseline questionnaire and objective medical characteristics. In a large panel study in the Netherlands we performed a detailed medical characterization of the more than 1000 participants. With a screening questionnaire we selected panels of symptomatic and non-symptomatic subjects. Medical characterization included skin prick testing for major allergens, total serum IgE, bronchial hyperresponsiveness and serum eosinophils. Participants reported PEF and occurrence of acute respiratory symptoms in a diary. Daily measurements of PM10, Black Smoke and gaseous pollutants were performed at fixed sites. Separate logistic regression analyses adjusting for confounders were conducted in four different subgroups for each medical characteristic e.g. symptomatic with/without atopy and asymptomatic with/without atopy. The most consistent associations were found in the children, which reported chronic respiratory symptoms and had either a positive skin prick test or increased total serum IgE. This pattern was less clear for eosinophily and bronchial hyperresponsiveness (BHR). In conclusion, subjects who reported chronic respiratory symptoms and were allergic had the strongest association with outdoor air pollution.

114 CARDIOPULMONARY EFFECTS OF PARTICULATE MATTER (PM) EXPOSURE IN COMPROMISED RATS DISPLAYING COMPENSATED CARDIAC HYPERTROPHY (CCH)

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Supportive experimental evidence is needed to provide biological plausibility to epidemiological studies suggesting that PM exposure represents a public health risk to susceptible subpopulations. An animal disease model of CCH [spontaneously hypertensive rats (SHRs), males, 15 months old] was employed to examine the cardiopulmonary effects produced by PM derived from various sources. SHRs were exposed to either: residual oil fly ash (ROFA; 1.28mg/kg BW; total metal (TM) 133μg/kg BW); fine ($PM_{0.4-3.3}$) Ottawa ambient air PM (Ott; 6.60mg/kg BW; TM 111μg/kg BW); or Mt. St. Helen's volcanic ash (MSH; 6.60mg/kg BW; TM 8.4μg/kg BW) by intratracheal-instillation (IT). Control SHRs were intubated but not instilled with any material. Cardiac and thermoregulatory parameters [electrocardiogram (ECG), heart rate (HR) and core body temperature (T_{co})] were monitored using radiotelemetry. Lung function measurements were obtained using a whole body plethysmograph. Lung injury and extra-pulmonary effects were determined by biochemical and cellular analyses performed on bronchoalveolar fluid and plasma recovered from SHRs at 24h and 96h post-IT. SHRs with CCH displayed a higher baseline permeability to plasma protein in the lung and were more sensitive to PM-induced lung injury when compared to young (65-70 days old) normotensive Sprague-Dawley rats. At the doses employed, the ability of each PM sample to induce lung injury in SHRs with CCH was found to be Ott≥ROFA>MSH. Both Ott and ROFA induced immediate but distinctive biphasic hypothermic (1.7-2.5°C ↓) and bradycardic (80-100 bpm↓) responses in SHRs with CCH; analogous end points in MSH-exposed rats did not differ from those of control animals. A similar order of effect (Ott>ROFA>MSH=Control) was observed for these PM samples on thermoregulatory and cardiac function, as measured by changes in T_{co}, HR, ECG, and arrhythmia incidence. Elevated creatine kinase and α-hydroxybutyrate dehydrogenase levels were present in plasma recovered at 24h and 96h from Ott and ROFA exposed SHRs suggesting cardiac injury. Preliminary results indicate the ability of different fractions of Ott PM to induce cardiac effects and alter pulmonary function were: Ott suspension > Ott leachate; similar responses to washed Ott particles did not differ from those of control animals. These results suggest SHRs with CCH are more sensitive to adverse health effects of PM derived from anthropogenic sources and may represent a model to study cardiac-mediated susceptibility to PM exposure.(This abstract does not necessarily reflect EPA policy)

120 DAILY RESPIRATORY MORTALITY AND AIR POLLUTION IN MEXICO CITY: IMPORTANCE OF CONSIDERING PLACE OF DEATH. MM Tellez-Rojo[1], I Romieu[2], S Ruiz-Velasco[3], F Meneses[1], MA Lezana[4], M Hernández-Avila[1]. [1]Instituto Nacional de Salud Pública, Cue., Mor. México; [2] Pan American Health Organization; [3] UNAM, DF, México; [4] Secretaría de Salud, México

We studied the association between PM10 and ozone ambient concentrations and the daily number of death from respiratory causes among elderly residing of Mexico City. Ambient air pollution data were provided by the monitoring network of the metropolitan area of Mexico City that is composed of 33 stations. During the study period (1994) PM10 daily average levels ranged from 23.4 to 175.3 $\mu g/m^3$ and ozone daily 1-h maximum ranged from 39.4 to 216.7 ppb. We compiled information on primary causes of death as well as secondary causes that may have lead to death. Death were classified as total respiratory mortality, mortality for chronic obstructive pulmonary diseases (COPD) and mortality for low respiratory infections. The analysis was conducted separately according to place of death (within and outside hospital units) using time-series methodology including Poisson regression and IWFLS. Total respiratory mortality and mortality for COPD were significantly related to PM10 over different lags. An increase of 30 $\mu g/m^3$ (interquartile rage) was related to a 8.7% (2.7% to 15.1%) increase in respiratory mortality and to 12.6% (95% CI 3.9% to 22.1%) increase in mortality for COPD with a 3-day lag when death occurred out of medical units. For deaths occurring within medical units, we observed a longer lag (5 days) and a smaller risk estimate. Ozone ambient levels were also related to respiratory mortality, but results were inconsistent. However, we observed and interactive effect between PM10 and ozone on respiratory mortality. Our results suggest that focusing on sensitive population and taking into account primary and secondary cause of death as well as considering place of death may reduce missclassification in epidemiological studies and provide more accurate effects of the adverse impact of PM10 on mortality.

124 DIFFERENTIAL AIRWAY INFLAMMATORY RESPONSES TO DIESEL EXHAUST IN HEALTHY VS. ASTHMATIC SUBJECTS

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Diesel exhaust (DE) is a major contributor to particulate matter (PM) pollution. Recently, it was shown that exposure to DE (PM_{10} 300 mg/m^3) induced a pronounced airway inflammation in healthy subjects (Salvi *et al. Am J Respir Crit Care Med,* 1999). In order investigate a lower concentration of DE and to compare the airway inflammatory responses to DE between healthy subjects and mild asthmatics, 25 healthy and 15 asthmatic subjects were exposed to DE (PM_{10} 100 mg/m^3) and air for two hours on two separate occasions. Bronchoscopy with bronchial wash (BW), bronchoalveolar lavage (BAL) and biopsy sampling was performed six hours after exposure. In the healthy subjects, exposure to DE induced increases in IL-6 ($p<0.05$) and IL-8 ($p<0.05$) and a trend towards a significant increase in neutrophil numbers ($p=0.07$) in BW, whereas lymphocyte numbers increased in BAL ($p<0.05$). Immunohistochemistry on endobronchial biopsies revealed the upregulation of the expression of the adhesion molecules P-selectin ($p<0.01$) and VCAM-1 ($p<0.05$) in the vascular endothelium and a decrease in epithelial CD3+ lymphocytes ($p<0.05$). In the mild asthmatic subjects, the only significant change after exposure to DE was a decrease in eosinophil numbers in BW ($p<0.05$). The acute cytokine response with IL-6 and IL-8, together with enhanced expression of the vascular adhesion molecules in the biopsies seen in healthy subjects, suggest an early stage in the recruitment of inflammatory cells. Interestingly, asthmatics were found to differ from healthy subjects in the airway response to DE. The mechanisms behind these differences in response need to be clarified.

129 DOUGLAS ASTHMA STUDY: BASELINE & FOLLOW-UP OF FIFTH GRADERS

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A study of respiratory prevalences & acute responses was conducted in 5th grade school children in the border city of Douglas in 1997-98, part of ongoing US-Mexico studies of such disease and the impact of air pollution. The primarily Hispanic population of 213 students completed baseline questionnaires, as did their parents, and daily symptom & peak flow diaries for 11 weeks. Particulate air pollution (PM_{10} & $PM_{2.5}$) was monitored simultaneously. The prevalence rates were: 10.8% current asthma, 31.2% of other serious respiratory symptomatology, and 40.7% with mild symptoms. The odds ratios for respiratory conditions associated with smoking in the home were 1.7 for current asthma, and 4.1 & 2.2 for serious & mild symptoms, respectively. Regression analyses showed that daily peak flow was negatively associated, and daily symptoms were positively associated with both PM_{10} & $PM_{2.5}$ levels, after adjusting for temperature. (Supported by the ALA of Arizona).

131 MULTIPLE RESPIRATAORY HOSPITAL ADMISSIONS AND PARTICULATE MATTER: SHORT-TERM EFFECTS FOR THE ELDERLY IN DETROIT, MI.

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Unlike daily mortality, a person can contribute multiple counts to daily emergency hospital admissions. While this phenomenon does not negate the adversity of air pollution effects, its extent may alter the interpretation of the estimated relative risks obtained from aggregate time-series analyses. We investigated this issue by constructing separate time-series for those who had only one hospital admission over the study period and for those who had multiple admissions, using unscheduled hospital admissions in the elderly for pneumonia and COPD from the Detroit-Ann Arbor-Flint Metropolitan area during 1991-1994. The effects of Particulate Matter less than 10μm (PM10) were estimated using Poisson regressions adjusting for weather, seasonal cycles, and day-of-week. For pneumonia admissions, the extent of multiple admissions was not substantial, with average admissions per individual of 1.17; and the PM relative risk estimates per inter-quartile-range (26μg/m3) for the populations with multiple admissions (RR=1.045; 95%CI=1.012-1.080) and single admissions (RR=1.041; 95%CI=1.021-1.062) were comparable. For COPD, however, the extent of multiple admissions was substantial (average admissions per person = 1.58); and the PM relative risk estimate for the population with multiple admissions (RR=1.013; 95%CI=0.986-1.040) was smaller than that for the population with single admissions (RR=1.039; 95%CI=1.009-1.071). These results suggest that the estimated PM relatives risks for the entire population are not biased upward by the effects in the sub-group with multiple admissions

139 SHORT-TERM, LOW-DOSE INHALATION OF AMBIENT PARTICULATE MATTER EXACERBATES ONGOING PNEUMOCOCCAL INFECTIONS IN STREPTOCOCCUS PNEUMONIAE-INFECTED RATS.

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Infection, specifically pneumonia, contributes substantially to the increased mortality among elderly individuals following exposure to particulate matter (PM). Studies were performed to test the hypothesis that alterations in pulmonary immunologic mechanisms important for resistance against infectious pneumonia contribute to the observed increase in PM-induced mortality in exposed-individuals. For these studies, the effects of inhaled concentrated ambient $PM_{2.5}$ from New York City air upon rats already-infected with *Streptococcus pneumoniae* 48 hr prior to exposure were examined. The results indicated that a single 5 hr exposure to PM induced time-dependent increases in pulmonary bacterial burdens and numbers of

circulating white blood cells, and decreases in levels of lavageable neutrophils/cytokines and bronchus-associated lymphoid tissue (BALT) compared to that in the infected air-exposed control rats. Moreover, exposure of infected rats to $PM_{2.5}$ increased the extent of pneumonia-associated lung lesions, as well as the incidence of septicemia in rats for up to 18 hr following PM exposure. Taken together, these findings suggest that exposure of rats with fulminating *S. pneumoniae* infections to concentrations at or somewhat greater than the promulgated 24 h NAAQS of 65 μg $PM_{2.5}/m^3$ can exacerbate the disease process and compromise host ability to adequately "handle" an ongoing pneumococcal infection. These changes, in turn, could contribute to the increased incidence of pneumonia-related deaths observed in PM-exposed elderly populations. Supported by HEI #94-03A.

140 SHORT-TERM EXPOSURE TO AIR POLLUTION IN A ROAD TUNNEL ENHANCES THE ASTHMATIC RESPONSE TO ALLERGEN

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Twenty volunteers with mild allergic asthma were exposed for 30 minutes in a Stockholm city road tunnel. The median NO_2 level during exposure was 313 (range: 203-462) μg/m³. Median PM_{10} and $PM_{2.5}$ were 170 (103-613) and 95 (61-218) μg/m³. For control, the subjects were exposed to much lower pollution levels in a suburban area. Four hours after the exposure, the persons inhaled a low dose of allergen. Asthmatic reaction during early phase (EP) was measured as the increase in specific airway resistance 15 minutes after allergen inhalation and during late phase (LP) as decrease in lung function (FEV_1) 3-10 hours after allergen inhalation. Asthma symptoms and drug use were monitored up to 18 hours after allergen inhalation. Subjective symptoms during the tunnel exposure were not pronounced. However, subjects exposed to tunnel NO_2 levels of ³300 μg/m³ had significantly greater EP reaction, following allergen exposure, as well as lower lung function and more asthma symptoms during LP, compared to control. Also, subjects with $PM_{4.5}$ exposure ³100 μg/m³ had a slightly increased EP compared to control. We conclude that exposure to air pollution levels occurring in road tunnels may significantly enhance asthmatic reactions induced four hours later by allergen inhalation.

143 THE ASSOCIATION BETWEEN AIRBORNE PARTICLES AND DAILY DEATH RATE: AN HYPOTHESIS.

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We propose a unifying hypothesis to explain the association between daily fluctuations in ambient particles and death rate, which can be tested experimentally. Epidemiologic findings: the association has been demonstrated in cities with differing climates, and sources of pollution; elderly persons with advanced chronic diseases have been chiefly at risk. Implications of these findings: No specific property of ambient particles (or air pollution in general) is an essential proximate cause of death; a number of such properties may be sufficient causes; other forms of environmental stress, organism's capacity to maintain an optimal steady state, is eroded by disease and unsuccessful aging. With progressive loss of homeostasis, the risks of dying increases; the number and variety of stresses capable of disrupting an already unstable steady state and acting as the proximal cause of death grows, while the level of any particular stress sufficient to cause death diminishes. To model homeostatic decay for predictive purposes, one has recourse to three attributes of biological regulatory systems; these attributes normally maintain stability ("set-point" concept), oscillate rhythmically (circadian), and respond proportionately to a variety of signals. A more inclusive model Support: US Dept of Energy.

146 EFFECT OF CONCENTRATED AMBIENT PM ON EKG WAVEFORMS IN NORMAL AND COMPROMISED RATS.

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Previous work from our laboratory has shown that increases in heart rate occur in rats after exposure to concentrated ambient PM on some, but not all, exposure days. The present study examined the effect of filtered air or concentrated ambient PM on EKG waveforms in healthy and monocrotaline-treated rats. Normal and monocrotaline-treated rats were exposed for 3 hours to 184 and 339 μg/m³ concentrated PM2.5, respectively. EKG waveforms and heart rate were acquired for a 1 hour baseline period before and for 18 hours after exposure. EKG waveform analysis was performed using software developed by Sean Dowd and Penn Watkinson (USEPA). In the monocrotaline-treated rats, there was no major increase in heart rate, but there were significant changes in waveform intervals. An increase in the length of the P-R segment, suggestive of a slowing in atrial conduction, persisted from the fourth hour post-exposure until the end of the monitoring period. The R-T interval was significantly shorter in PM-exposed rats and this was largely a result of a decrease in the duration of the T wave. While these particular parameters were not changed in the normal rats exposed to concentrated ambient PM, there were other changes in the EKG waveform as well as an increase in heart rate during the first 3 hours postexposure. This research was funded in part by HEI.

153 DAILY MORTALITY BY CAUSE AND SOCIO-ECONOMIC STATUS IN SANTIAGO, CHILE.

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Daily counts of cause-specific deaths for nine years from 1988 to 1996 were regressed using Poisson regression on respirable (PM_{10}) and fine suspended particles ($PM_{2.5}$), SO2, CO and ozone concentrations, on the same day and from lag 1 to 9, controlling for seasonal and meteorological conditions. A significant positive association was found between total mortality and both particles ($p<0.000$) and carbon monoxide level ($p< 0.000$). The association remained significant for up to 9 days for fine suspended particles ($PM_{2.5}$) and 5 days for CO. The strongest effect for particulate matter was found for $PM_{2.5}$ two days before the date of death, and for CO for the mean of the same and previous four days. The effect of particulate matter and carbon monoxide was not confounded by SO_2 nor ozone, which were both non significant. The analysis by socio-economic status (as measured by educational level) shows that people with lower level are more sensitive to air pollution effects, even when controlling for the age composition of the sample. Our results are in agreement with previously reported associations and add to the body of evidence showing that particulate and CO pollution are strongly associated with increased daily mortality. The effect increases with age and lower socio-economic level and is higher for respiratory and cardiovascular diseases. The strength of association between fine suspended particles and respiratory diseases is higher than any other previously reported.

166 STATISTICAL ANALYSIS OF THE SIX CITY STUDY - REGENERATION, RE-ANALYSIS AND RE-EXAMINATION OF THE PARTICULATE/MORTALITY ASSOCIATION

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The details on the consolidation of source data and on the method to augment the particulate measurements using two-day averages are presented. These procedures describe the re-generation of the database used in the Six City

Study paper published in JAWMA (October 1996). We used the specific Generalized Additive Model (GAM) proposed in the JAWMA paper to re-calculate the linear association of $PM_{2.5}$ and daily mortality. Site-specific and combined estimates are reviewed. Additional models and diagnostics are presented to support another interpretation of the GAM results for the site specific and combined estimates. Additional analyses are used to explore the robustness of the estimate of linear association and to understand the accuracy of the standard error used in hypothesis tests. In light of these additional analyses, alternative conclusions are proposed about the appropriateness of the use of linearity to extrapolate mortality estimates for higher particulate exposure levels. Finally, decomposition of the data set into interesting subgroups (by season, by age, by chronic illness and by particulate levels) produces insights into the consistency of mortality estimates at higher $PM_{2.5}$ exposure levels for the linear dose response predictor. Graphic representations of the dose response curve provide an understanding of the behavior of the dose response relationship and suggest other methods for extrapolation.

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