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Health Effects of Subchronic Exposure to Low Levels of Wood Smoke in Rats

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Wood smoke is a significant source of air pollution in many parts of the United States, and epidemiological data suggest a causal relationship between elevated wood smoke levels and health effects. The present study was designed to provide information on the potential respiratory health responses to subchronic wood smoke exposures in a Native American community in New Mexico. Therefore, this study used the same type of wood under similar burning conditions and wood smoke particle concentrations to mimic the conditions observed in this community. Brown Norway rats were exposed 3 h/day, 5 days/week for 4 or 12 weeks to air as control, or to 1 or 10 mg/m^3 concentrations of wood smoke particles from *pinus edulis*. The wood smoke consisted of fine particles (< 1 μm) that formed larger chains and aggregates having a size distribution of 63–74% in the < 1-μm fraction and 26–37% in the > 1-μm fraction. The particle-bound material was primarily composed of carbon, and the majority of identified organic compounds consisted of sugar and lignin derivatives. Pulmonary function, specifically carbon monoxide-diffusing capacity and pulmonary resistance, was somewhat affected in the high-exposure group. Mild chronic inflammation and squamous metaplasia were observed in the larynx of the exposed groups. The severity of alveolar macrophage hyperplasia and pigmentation increased with smoke concentration and length of exposure, and the alveolar septae were slightly thickened. The content of mucous cells lining the airways changed from Periodic Acid Schiff- to Alcian Blue-positive material in the low-exposure group after 90 days. Together, these observations suggest that exposure to wood smoke caused minor but significant changes in Brown Norway rats. Further studies are needed to establish whether exposure to wood smoke exacerbates asthmalike symptoms that resemble those described for children living in homes using wood stoves for heating and cooking.

Key Words: **wood smoke; particles; chemical composition; rat; pulmonary function; histopathology; cytokines; lymphocytes.**

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Anthropological findings suggest that humans have used wood fire for cooking and heating since prehistoric times. Furthermore, forest fires cause environmental air pollution that can expose large populations to wood smoke. Although wood smoke levels in homes of developed countries have declined steadily over the years, combustion of biomass, particularly wood, can still be a major source of indoor and outdoor air pollution.

Epidemiologic studies have linked exposure to wood smoke with increased prevalence of respiratory illness in children and adults. Increased respiratory symptoms, decreased pulmonary function, and increased prevalence of chronic bronchitis have been associated with wood smoke exposure in Columbia (Dennis *et al*., 1996), Papua, New Guinea (Anderson, 1979), and Nepal (Pandey, 1984). Case-control studies showed that the risk of hospitalization with acute lower respiratory illnesses among Navajo children in Arizona living in households that cook and heat with wood was five times that for children living with families who cook with gas or electricity (Robin *et al*., 1996). Other studies of children in Michigan (Honicky *et al*., 1985), Idaho (Butterfield *et al*., 1989), Montana (Johnson *et al*., 1990), Colorado (Larson and Koenig, 1994), and Washington (Koenig *et al*., 1993) show associations between fine particulate matter, with wood smoke being a contributing source, and decreased pulmonary function, cough, wheeze, and upper and lower respiratory infections. These studies with children lack the confounders of cigarette smoking or occupational exposures and suggest a causal relationship between elevated wood smoke levels and adverse health outcomes.

Wood smoke can be an important contributor to particle and gaseous material in ambient air and can account for as much as 80% of the airborne particle concentrations during the winter heating season in some locations (Larson and Koenig, 1994). Indoor exposures to wood smoke can occur not only from infiltration of outdoor air, but also from non-airtight or improperly operated wood stoves and other wood-burning appliances. Some studies report wood smoke concentrations as total suspended particulate matter from 2.7 to 25 mg/m^3, much higher concentrations of particulate matter than those to which U.S.

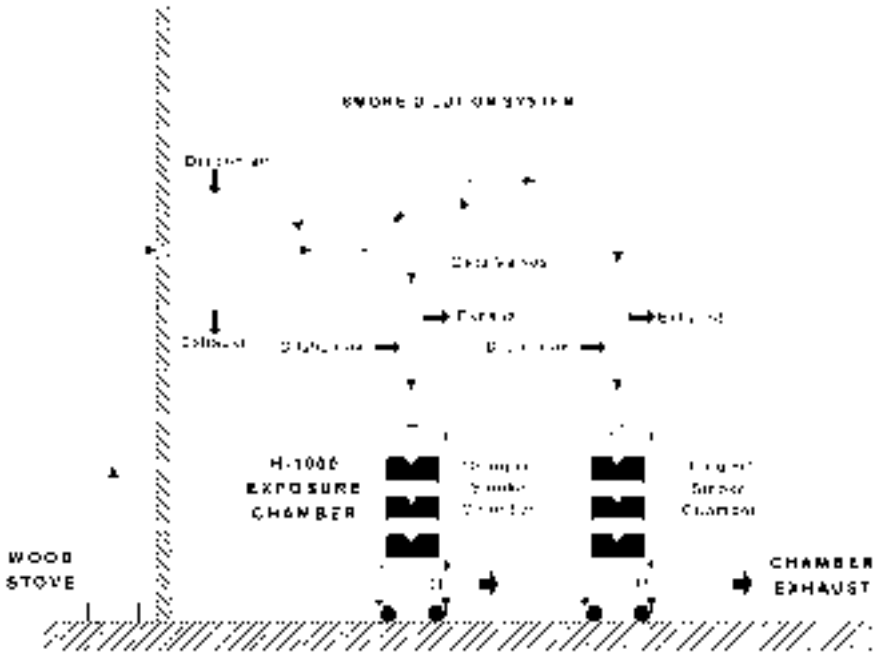

FIG. 1. Schematic presentation of the wood smoke exposure system.

populations are currently exposed (up to 1 mg/m^3 on a 24-h basis) (Larson and Koenig, 1994).

Although wood smoke is an important environmental air contaminant in several locations in the United States, the toxicological database on subchronic or chronic exposures to wood smoke is very small (Larson and Koenig, 1994). Currently, extrapolation of results from animal studies to human populations living in areas with elevated wood smoke concentration is difficult, because the existing studies used exposure concentrations higher than those relevant to human populations in the U.S. (Kewal Lal *et al*., 1993); (Liang *et al*., 1988) or short, acute exposures (Fick *et al*., 1984). Furthermore, the studies using subchronic exposures did not evaluate pulmonary function or symptoms of respiratory illness, key end points in epidemiological studies. The present study was designed to provide information on the potential respiratory health responses to subchronic wood smoke exposures in a Native American community in New Mexico. The incidence of childhood asthma in this community is about twice the national average (Clark *et al*., 1995), and exposure to particulate matter from combustion sources may exacerbate asthma (Koenig *et al*., 1993; Schwartz *et al*., 1993). Some homes in this community burn wood that is found in the surrounding areas for heating and cooking and use a type of primitive conventional wood stove without a catalyst. The intention of this study was to mimic conditions observed in this community by using the same type of wood under similar burning conditions and wood smoke particle concentrations.

The effects of wood smoke exposure on pulmonary function and the respiratory tract epithelia in Brown Norway rats are described. Brown Norway rats were chosen for this study because this animal model mimics features of human allergic asthma in several aspects. This strain exhibits high IgE levels, early- and late-phase bronchoconstrictions, and a helper T-cell type 2 response to allergic sensitization (McMenamin *et al*., 1995; Ohtsuka *et al*., 1997). These studies, therefore, will form the basis for further investigations on whether wood smoke exposure exacerbates asthma in rats sensitized or challenged to allergens.

MATERIALS AND METHODS

Animals. Brown Norway rats (6–7 weeks of age) were purchased from Charles River Laboratories (Wilmington, MA). Water and pelleted food (Certified Wayne Lab Blox, Allied Mills, Chicago, IL) were provided *ad libitum* outside of exposure hours. The inhalation exposure rooms were windowless but uniformly lighted with diffuse lighting from fluorescent lamps on a 12-h light/dark cycle.

After 2 weeks of quarantine, rats were randomly assigned by weight to three exposure groups (26 rats per group). At least 12 rats from each exposure group were removed for sacrifice after 30 or 90 days of exposure. Two additional rats were maintained in each group as backup in case of unexpected morbidity. All rats were given unique identification numbers by means of tail tattoo. Rats were maintained in the exposure chambers for 7 days for adaptation prior to their first wood smoke exposure. All animals were weighed not more than 1 week before exposures and at weekly intervals throughout the study. Clinical observation data were collected during each weighing session. Animals were checked once in the morning and once in the late afternoon at least 6 h apart 7 days a week for any clinical signs of abnormality, morbidity, or mortality. Food was removed from chambers before start of exposure, and then replaced when the day's exposure was complete.

Wood smoke exposure system. Wood smoke was diluted with air to either 1 or 10 mg particles/m^3 in multitiered, whole-body exposure chambers (H-1000, Lab Products, Inc., Aberdeen, MD) (Fig. 1). A third chamber was used for exposure to air as control. The wood stove (Vogelzang Boxwood Stove, Model BX-42E), similar to the type generally used in the homes of the Native

American population of interest, was purchased new from a local source. The wood stove was a simple box stove without a catalyst. It was set up in a separate room from the exposure chambers; thus, the rats were not subjected to the heat generated by the fire. The stove flue was connected to an exhaust plenum maintained at approximately 5 inches water-negative to ambient pressure. A fraction of the wood smoke was diverted from the flue, cooled, and diluted to the desired concentrations. Ambient air for smoke dilution and control exposure was drawn through HEPA filters, tempered, and humidified. The exposure chambers were maintained at a temperature of 24 ± 1°C and a relative humidity of between 40 and 70%. The chambers were operated at 12 ± 2 chamber air changes/ h (200 ± 30 l/min). A computer-based system provided 24-h monitoring and recording of airflow rate, temperature, humidity, and pressure. The uniform distribution of wood smoke in the chambers was verified during this study. Chamber exhaust air was routed through HEPA filters, then discharged into the atmosphere at a position remote to the facility air intakes.

Wood type and burning conditions. Wood was purchased from an individual who supplies wood to the Native American community of interest. The wood was *Pinus edulis*, the "common piñon" found in the northern two-thirds of New Mexico. After delivery, wood was stored outdoors under a rain shelter until burned in the stove. The wood was seasoned, but the water and bark content of the wood were not determined. The burning process was initiated prior to exposure by igniting 3 kg of wood with a propane torch. Smoke was diverted to the chambers 30 min after the fire was established. Every 15 min thereafter, 1 kg of wood was added to maintain continuity of the fire during the 3-h exposure period.

Exposure characterization. The particle concentration was determined using filter samples taken from a midchamber point. Filter holders were loaded with 25-mm diameter glass fiber filters, and flow was maintained at a constant volume of 2 l/min with a critical orifice. Filter samples were taken every 30 min throughout the 3-h exposure period from each chamber on each exposure day. Filters were weighed before and after the sampling period, and the weight differences were used to determine mass concentration.

The particle size distribution was determined using a micro-orifice uniform deposit impactor (MOUDI, MSP Corp., Minneapolis, MN) operated at a flow rate of 30 l/min. Probit analysis of the mass collected on the 10 stages and backup was used to determine the mass-median aerodynamic diameter (MMAD) and geometric standard deviation (SD) of the size distribution. A point-to-plane electrostatic precipitator was used to collect samples for electron micrographic analysis as described (Cheng *et al*., 1981). Electron micrographs were prepared using a Hitachi H7000 scanning/TEM (Hitachi Ltd., Tokyo, Japan).

Organic plus elemental carbon and speciated particle-bound organic compounds (Table 1) were analyzed at the Environmental Engineering Science Department, California Institute of Technology, Pasadena, CA (Cal-Tech). These samples were collected on seven separate occasions directly from the exposure chambers onto prebaked quartz fiber filters (Pall-Gellman; 47-mm Tissuequartz, QAO). Collected material was stored in baked glass containers at −80°C prior to shipment to Cal-Tech. A 1-cm^2 portion of each filter was analyzed for total organic and elemental carbon by the thermal analysis method described by Birch and Cary (1996). Results of the carbon analysis did not include the mass of oxygen, hydrogen, or other noncarbon atoms present in organic material, which can contribute substantially to the mass of material that would be weighed on a filter. To facilitate comparison of weighed mass to organic material, a factor of 1.4 was used to correct for the nonmeasured components of organic material, as previously described for wood smoke studies (Kleeman *et al*., 1999).

After carbon analysis, the remaining portion of each filter was composited and extracted for chemical analysis using methods described previously (Schauer *et al*., 2001). Briefly, filters were extracted by sequential additions of hexane (2 × 60 ml) and benzene/isoproponol (2:1) (3 × 60 ml) under ultrasonic agitation. Following filtration, each sample was reduced in volume by rotary evaporation and then by evaporation under nitrogen to a final volume of between 30 and 80 μl prior to gas chromatographic/mass spectrometric (GC/MS) analysis. To facilitate the analysis of polar organic compounds by gas chromatography, a separate aliquot of the sample was derivitized by diazomethane to convert organic acids to their nonpolar methylated analogues.

Gaseous nitrogen oxide concentrations were measured by extracting gas samples into Tedlar® bags. Bag samples were then analyzed for NO and NO_x

TABLE 1
Particle-Bound Organic Chemical Species Analyzed in Exposure Chambers

Chemical class	Number of species
n-Alkanes (C16–C32)	16
n-Alkenes (C19–C22)	4
n-Alkanoic acids (C8–C26)	18
Alkenoic acids (C16–C22+cis/trans C18)	8
Methyl alkanoates (C16–C24)	8
Methyl alkenoates (C18)	1
Guaiacols	16
Guaiacol, eugenol, cis-iso-eugenol, trans-iso-eugenol, 4-vinylguaiacol, 4-ethylguaiacol, 4-propylguaiacol, vanillic acid, methyl vanillate, homovanillic acidmethyl, homovanillate, vanillin, acetovanillone, propiovanillone, guaiacyl acetone, coniferyl aldehyde	
Syringol	1
Other benzenes/phenols	9
1,2-Benzenediol (pyrocatechol), methyl benzenediols, benzoic acid, hydroxybenzaldehydes, cinnamaldehyde, methyl hydroxybenzoates, hydroxyacetophenones, benzene acetic acid, benzene propanoic acid	
Dimers and lignans	2
Diguaiacyl ethanes (divanillyls), 2-deoxo-matairesinol	
Polycyclic aromatic hydrocarbons (PAH)	29
Phenanthrene, anthracene, 3 plus 2-methylphenanthrene, 2-methylanthracene, 9-methylphenanthrene, 1-methylphenanthrene, phenylnaphthalenes, dimethyl or ethyl 178 MW PAHs, fluoranthene, acephenanthrylene, pyrene, methyl 202 MW PAHs, retene, benzo[ghi]fluoranthene, cyclopenta[cd]pyrene, chrysene, benz[a]anthracene, methyl 226+228 MW PAHs, perylene, benzo[b+j+k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, indeno[1,2,3-cd]fluoranthene/pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene	
Oxygenated PAH	10
1+2-Naphthol, methylnapthols, methoxynaphthols, fluorenone, 1-H-phenalen-1-one, 9,10-anthracenedione, xanthone, benzanthrone	
Sugar derivatives	4
1,4:3,6-Dianhydro-a-D-glucopyranose, galactosan, mannosan, levoglucosan	
Coumarin	1
Furans	4
5-Hydroxymethyl-2-furaldehyde, 5-acetoxymethyl-2-furaldehyde, dibenzofuranols, benzonaphthofurans	
Resin acids	5
16,17-Bisnordehydroabietic acid, sandaracopimaric acid, dehydroabietic acid, 8,15-pimaradien-18-oic acid, isopimaric acid	
Diterpenoids	5
19+18-Norabieta-8,11,13-triene, dehydroabietane, manoyl oxide, methyl dehydroabietate	
Indanones	3
1-Indanone, methyl indanones	
Squalene	1

using a chemiluminescence analyzer (Model 200A, Advanced Pollution Instruments, San Diego, CA). CO was measured by extracting gas samples through filters directly into an electrochemical measuring transducer (Multiwarn II, Draeger Safety, Inc., Pittsburgh, PA). Gas from each chamber was analyzed for 5 days each during weeks 2, 6, and 9.

Respiratory function tests. The respiratory function of 8–10 rats per treatment group was measured by whole-body plethysmography using techniques described previously in detail (Mauderly *et al*., 1990). Rats were anesthetized with halothane, intubated orally with endotracheal and esophageal catheters, and measured in the prone position in a combination pressure and flow plethysmograph. During spontaneous breathing, respiratory pattern, dynamic lung compliance, and total pulmonary resistance were measured. Measurements during single-breath maneuvers induced by positive and negative airway pressures and during hyperventilation-induced apnea included quasistatic vital capacity and pressure-volume curves, functional residual capacity, single-breath N_2 washout, forced vital capacity and flow-volume curve, and single-breath, CO-diffusing capacity. Total lung capacity and residual volume were calculated from measured volumes.

Histopathologic examination. After 30 or 90 days of exposure, six rats from each group were killed for histopathologic examinations by intraperitoneal injection of 0.2 ml Euthasol (Delmarva Laboratories, Inc., Midlothian, VA) and exsanguination via the renal artery. The larynx and a part of the trachea were fixed in zinc formalin. The left lung was fixed in an expanded state by intratracheal instillation of zinc formalin at 25 cm of constant pressure for 6 h followed by immersion in the same fixative for 3–5 days. The fixed lungs were cut into five or six slices (each 5 mm thick) and embedded in paraffin. These slices were numbered from cephalad (slice 1) to caudad (slice 5 or 6).

To analyze the nasal epithelia, the head of each rat was removed from the carcass, as well as the lower jaw, skin, and musculature. Nasal passages were flushed retrograde through the nasopharyngeal orifice with zinc formalin, then immersed in a large volume of this fixative for at least 2 weeks. The noses were decalcified with 13% formic acid by immersion for 4 days. After decalcification, the nasal airways were transversely sectioned at four specific anatomic locations as described (Harkema and Hotchkiss, 1994). The following gross dental and palatine landmarks were used: immediately posterior to the upper incisor tooth (tissue block 1); at the incisive papilla (tissue block 2); at the second palatal ridge (tissue block 3); and in the middle of the front upper molar tooth (tissue block 4).

All tissues were fixed in zinc formalin for no less than 72 h. After fixation, tissue samples were embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin or Alcian Blue (pH2.5)/Periodic Acid-Schiff (AB/PAS) as described previously (Tesfaigzi *et al*., 2000).

Histopathologic changes in two sections of the trachea, one section from the larynx, four sections from the nose, and two sections from the left lung were examined by a pathologist in a random and blinded fashion. Microscopic observations were graded for severity. Tissue sections from the trachea and lung were stained with AB/PAS and analyzed for changes in mucous cell numbers by counting the AB/PAS-positive cells per millimeter basal using the NIH image analysis software Scion Image (written by Wayne Rasband, U.S. National Institutes of Health) on a Power Macintosh G4. Epithelia lining the intrapulmonary axial airways at generations 3 and 4, which are represented in lung slices 3 and 4, were quantified. Furthermore, epithelia from two regions of the trachea were subjected to morphometric analyses.

Analysis of bronchoalveolar lavage fluid. After 30 or 90 days of exposures, a set of six rats from each group was used for analysis of bronchoalveolar lavage (BALF). Rats were killed, thoracic contents exposed, and the lungs perfused through the pulmonary artery with saline. The larynx, trachea, and lungs were isolated. The entire lung was removed and lavaged through the trachea three times with 5 ml ice-cold phosphate-buffered saline without calcium and magnesium ions.

After removing the cells by centrifugation, levels of protein, lactate dehydrogenase (LDH), and β-glucuronidase in the BALF were determined, because these values are useful indicators of cell damage or death. The levels of several cytokines (IL-1β, IL-6, and TNF-α) in the BALF were determined by ELISA as described previously (Conn *et al*., 1995). These cytokines, which have generally been found to be present after an inflammatory process, were expected to be present after subchronic exposure to wood smoke. Cells recovered by BALF from the right lung lobes were enumerated using a hemocytometer. Cytological preparations were stained with Wright Giemsa, and differential counts were performed to determine the type of inflammation present in the BALF.

Exposures. Animals were exposed to air as control and 1 or 10 mg/m^3 wood smoke particle concentrations for 3 h/day, 5 days/week, for up to 90 days. The exposure concentrations were selected based on a pilot experiment, in which five rats each were exposed for 5 days to air as control, or to 2 or 20 mg particles of wood smoke/m^3. These concentrations were based on previous reports showing minor health effects in Wistar rats exposed to 14.99 mg/m^3 wood smoke concentrations for 19 months (Liang *et al*., 1988). The mean wood smoke concentrations for this pilot study were 2.1 (SD 0.948) and 20.5 (SD 3.407) mg particles/m^3. Analysis of the BALF showed no changes in β-glucuronidase activity. However, total protein and LDH levels were significantly elevated in the group exposed to 20 mg/m^3 compared to the air controls. No differences were noted in the numbers of macrophages, lymphocytes, eosinophils, and neutrophils. The number of macrophages with < 10 and > 10 carbonaceous particles, presumably smoke particles, increased from 0 to 24% in the group exposed to 2 mg/m^3. About 60% of macrophages contained > 10 particles in rats exposed to 20 mg/m^3. Based on our findings in these 5-day exposures, we anticipated that exposures for 30 and 90 days at 1 and 10 mg/m^3 wood smoke particle concentrations would produce measurable effects. Furthermore, concentrations of 1 mg/m^3 would more closely resemble those measured in the Native Americans homes this study was intended to mimic.

Lymphocyte proliferation assay. Lung-associated lymph nodes (LALNs) and spleens from six rats in each exposure group that were used to obtain lungs for lavage were removed at 30 or 90 days for lymphocyte proliferation assays. Immediately after removal of tissues, splenic and LALN cells were prepared, and their proliferation in response to Con A, a T-cell–specific mitogen, was analyzed as described (Singh *et al*., 2000).

Statistical analysis. Data are expressed as mean $\pm$ standard error of the mean (SEM). Differences among groups were examined by analysis of variance (ANOVA) and *t*-tests using SAS software (Cary, NC) and by application of Tukey's or Bonferonni correction for multiple comparisons as appropriate. Differences were considered significant at $p < 0.05$.

RESULTS

Particle concentration and size distribution. The average particle exposure concentrations were 0.021 (SD 0.037), 1.1 (SD 0.857), and 9.8 (SD 4.391) mg/m^3. The size distribution was bimodal, with 74% of the mass at 1 mg/m^3 particle concentration in the $<$ 1-μm fraction with a MMAD of 0.5 μm. The remaining 26% was $>$ 1 μm and had a MMAD of 6.7 μm. At the 10 mg/m^3 concentration, 63% of the mass was in the $<$ 1-μm fraction with a MMAD of 0.5 μm, and 37% was in the $>$ 1-μm fraction with a MMAD of 12 μm (Table 2). These findings were further confirmed by analyzing wood smoke samples with electron microscopy. The wood smoke particles were shown to consist of fine ($<$ 1 μm) particles forming larger ($>$ 1 μm) chains and aggregates (Fig. 2). Because it is generally accepted that particles of an aerodynamic diameter over 5 μm are not inhalable by rats (Miller, 2000), the concentrations of particles 5 μm in diameter or less estimated from the impactor data were 91% for the 1 mg/m^3 and 85% for the 10 mg/m^3 wood smoke concentrations. Based

TABLE 2
Size Distribution of Wood Smoke

Target concentration (mg/m^3)	Small size fraction (< 1 micron)			Large size fraction (> 1 micron)		
	MMAD (μm)	GSD	Mass (%)	MMAD (μm)	GSD	Mass (%)
10	0.496	1.73	62.6	11.7	2.65	37.4
1	0.405	1.87	73.8	6.7	2.23	26.2

on these percentages, concentrations that likely entered the rat airways were 0.91 mg/m^3 and 8.5 mg/m^3 at the low- and high-exposure levels, respectively.

Gaseous components. Carbon monoxide, total hydrocarbon levels, and NO_x levels increased with wood smoke particle concentrations (Table 3). The SD of the mean concentrations of the analyzed gaseous components indicates that all measured components varied greatly.

Chemical composition of particulate material. The particulate material consisted of 75% carbon, the majority (~ 80%) of which was composed of organic material, while the remainder was elemental carbon (Fig. 3, first bar). Fifteen percent of the organic material was characterized by GC/MS (Fig. 3, second bar). The remainder of the organic carbon was not speciated, either because the compounds were not targeted, or the material could not be extracted and eluted through a chromatographic column. Ten percent of the measured organic extract consisted of highly branched or cyclic organic compounds that clump together in the chromatographic analysis. These compounds, termed the unresolved complex mixture, can be quantified as a group of material but not segregated into individual compounds.

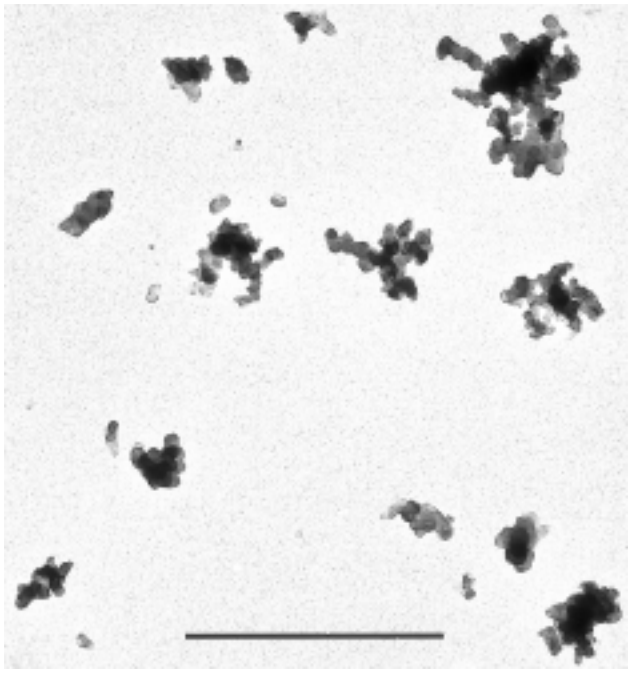

FIG. 2. Electron micrograph of wood smoke particles. Bar = 1 μm.

The third bar of Figure 3 shows the composition of the classes of individual measured compounds. The sugar derivatives and guaiacols composed the majority of the identified species and chemical classes measured, each accounting for approximately 2% of the organic carbon. Substituted benzenes/phenols, furans, alkanoic acids, polycyclic aromatic hydrocarbons (PAHs), and n-alkanes were the next most abundant classes of measured compounds. Other detectable but less prevalent compounds included the resin acids, diterpenoids, methylalkanoates, and coumarin.

Clinical observations and body weight. No clinical sign was noted on exposed rats, and there was no significant effect of exposure on body weight when exposed rats were compared with controls (Fig. 4).

Respiratory function tests. Respiratory function was only slightly affected by exposure, and differences between control and smoke-exposed groups were statistically significant for only a few parameters (Table 4). A modest (19%), but significant reduction of CO-diffusing capacity demonstrated an impairment of gas exchange in the high-exposure group. The reduction remained apparent after adjustment for differences in lung volume and body weight, indicating that it was most likely caused by reduced diffusion at the alveolar-capillary membrane.

An increase in resistance during spontaneous breathing was suggested by higher values for total pulmonary resistance for the exposed groups but not the control group; however, the difference was significant only f or the low-exposure group. No air flow decrement was evident during forced exhalation. Forced expiratory flows were slightly lower in the exposed groups, but the differences were not apparent after adjustment for differences in lung volume.

Dynamic lung compliance was higher in the exposed groups, and the difference was significant for the high-exposure group. In contrast, quasistatic compliance, a more specific measure of lung elastic recoil, was slightly (insignificantly) lower in the exposed groups. There was no significant difference in lung volumes between control and exposed groups, although vital capacity (both quasistatic and forced) was slightly smaller, and residual volume slightly larger, in the high-exposure group.

TABLE 3
Gaseous Components of Wood Smoke

	Control chamber		1 mg/m^3 chamber		10 mg/m^3 chamber	
	Mean	SD	Mean	SD	Mean	SD
CO, ppm	0.1	0.5	15	17	106.4	106.9
Total hydrocarbon, ppm	0.4	0.7	3.5	6.7	13.8	11.6
NO, ppm	<0.1	<0.1	2.2	1.1	18.9	9.3
NOx, ppm	0.1	<0.1	2.4	1.2	19.7	9.1

The 16% (nonsignificant) increase in the slope of phase III (alveolar plateau) of the single-breath N_2 washout in both exposed groups suggested that gas distribution in the lung may have been slightly less uniform. Dynamic lung compliance was higher in exposed groups, but quasistatic lung compliance in the lung volume range of spontaneous breathing was slightly (nonsignificantly) decreased, rather than increased.

Histopathology. At necropsy, the lungs of rats exposed to wood smoke were brownish to grayish in color, whereas the lungs of the air controls were pink. There was a minimal to mild chronic inflammation in the epiglottis of the larynx of several rats exposed to 1 or 10 mg/m^3 after the 30- or 90-day exposure. After 90 days, only two rats in the high-exposure group and one in the low-exposure group had a minimal to mild squamous metaplasia in the body of the larynx posterior to the ventral laryngeal pouch. Scant inflammation was associated with the metaplasia. No squamous lesions were obvious in the nose or trachea of any rat in these experiments. Morphometric analysis of the upper tracheal epithelium showed a 2-fold increase in the number of PAS-positive cells in rats exposed to 10 mg/m^3 for 30 days compared to the other groups ($p < 0.05$). However, after 90 days of exposure, the numbers decreased to levels observed in air-control rats. No changes in mucous cell metaplasia were noted in epithelia lining the larynx and trachea at the bifurcation after 30 or 90 days of exposure.

Increases in the number and pigmentation of alveolar macrophages (Figs. 5A and 5B) were related to increased wood smoke exposure. Alveolar macrophage numbers were increased in the high-exposure group after the 30-day sacrifice and were generally more severe after 90 days of exposure (data not shown). After 30 and 90 days of exposure, the severity of pigmentation was increased with exposure concentration. After 90 days, both exposure groups (Fig. 6B), but not the air controls (Fig. 6A), exhibited a slight thickening of the alveolar septae with hypertrophic alveolar epithelial cells and a few mononuclear cells in some alveoli that contained clumps of particle-laden macrophages. Some lungs from all groups had inflammatory lesions, which have been described by Charles River Laboratories as idiopathic granulomatous pneumonia of Brown Norway rats. These lesions were noted, but considered as background lesions.

Morphometric analyses of the epithelia of the intrapulmonary axial airways (at generations 3 and 4) of rats exposed for 30 days showed no difference in the number of AB- or PAS-positive mucous cells in any exposure group. However, after a 90-day exposure of rats at 1 mg/m^3, the numbers of AB- and

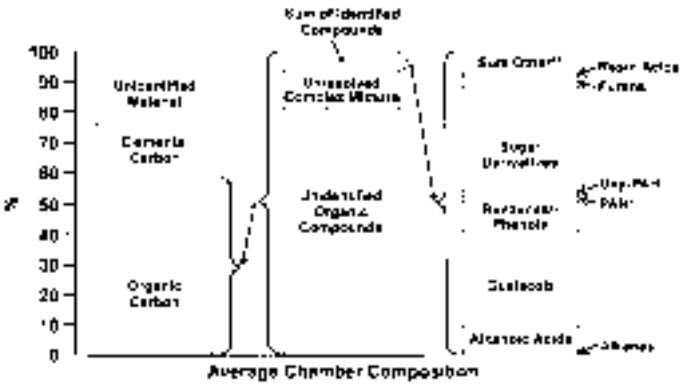

FIG. 3. Weight fraction of extractable organic matter in fine particle emissions from wood smoke samples in the exposure chambers.

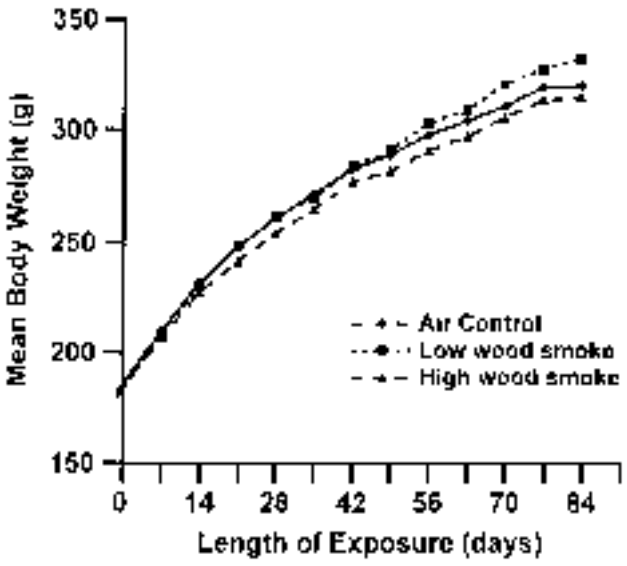

FIG. 4. Change in the body weight of rats during exposures to 1 or 10 mg/m^3 wood smoke or air as control.

TABLE 4
Respiratory Function Results

	Control ($n = 9$)		1 mg/m^3 ($n = 10$)		10 mg/m^3 ($n = 8$)	
	Mean	SD	Mean	SD	Mean	SD
Body weight (g)	320	12	330	20	310	18
DLCO (ml/min/mmHg)	0.36	0.04	0.35	0.06	0.29*	0.05
DLCO/lung volume (DLCO/ml)	0.021	0.002	0.021	0.002	0.017*	0.003
DLCO/body weight (DLCO/kg)	1.14	0.12	1.07	0.17	0.95*	0.16
Total pulmonary resistance (cm H_2O/ml s^{-1})	0.15	0.04	0.22*	0.06	0.20	0.05
Dynamic lung compliance (ml/cm H_2O)	0.50	0.11	0.60	0.16	0.71*	0.20
Quasistatic lung compliance (ml/cm H_2O)	1.00	0.08	0.98	0.10	0.94	0.17
Total lung capacity (ml)	18.7	1.3	18.7	1.4	17.8	2.5
Vital capacity (ml)	17.0	1.2	17.2	1.2	15.8	2.7
Functional residual capacity (ml)	4.9	0.9	4.3	0.9	5.0	1.4
Residual volume (ml)	1.6	0.7	1.5	0.7	2.0	1.2
Forced vital capacity (FVC) (ml)	16.2	1.2	16.6	1.2	15.5	2.5
Forced expired volume in 0.1 s (FEV 0.1)	56	2	54	3	54	7
Slope phase III of N_2 washout (N_2/ml [%])	0.44	0.09	0.51	0.15	0.51	0.09

Note. DLCO, CO-diffusing capacity.
* Indicates difference from control mean, significant at $p < 0.05$.

PAS-positive epithelial cells were increased ($p < 0.05$) compared with air-control rats. Rats exposed to 10 mg/m^3 showed no difference compared to the air-control group.

BALF Cell differentials. After 30 days of exposure, the numbers of lymphocytes and neutrophils were slightly but not significantly increased in the BALF of rats exposed to 10 mg/m^3 compared with the air control and 1 mg/m^3 groups (data not shown). No difference in lymphocyte and neutrophil numbers was observed between groups after the 90-day exposure. However, the number of macrophages in the BALF was decreased significantly by about half ($p < 0.05$) in the 10 mg/m^3 group compared to the 1 mg/m^3 group (Fig. 7).

Significant differences were readily observed in the number of macrophages containing < 10 and > 10 carbonaceous particles after 30 or 90 days of exposure. As expected, macrophages from the air-control group had no particles in alveolar macrophages. The percentage of macrophages with > 10 particles was higher in the 10 mg/m^3 group than in the 1 mg/m^3 group after both the 30- and 90-day exposures (Fig. 8).

Total protein, LDH, β-glucuronidase, and the cytokines TNF-α, IL-1β, and IL-6 in BALF. None of the groups differed in β-glucuronidase activity levels in the BALF. Although not significant, LDH levels appeared to increase in the 10-mg/m3 group, and protein levels appeared to decrease by exposure to 10 mg/m3 compared to the rats exposed to air or 1 mg/m3. The cytokines TNF-α, IL-1β, and IL-6 were below detectable levels (< 40 pg/ml).

Lymphocyte proliferation assay. T lymphocytes isolated from both the LALNs and the spleen were not affected in their proliferative response to Con A by any wood smoke exposure level or length of exposure.

DISCUSSION

Wood Smoke Characterization

The present study shows that burning piñon produced smoke that predominantly consisted of two-thirds submicron-size particles and one-third of larger aggregates of fine particles. Kleeman and colleagues (Kleeman *et al.*, 1999) showed that particles emitted from the combustion of pine, oak, and eucalyptus have mass distributions that peak at 0.1–0.3 μm particle diameter, and several investigators report similar findings (Dasch, 1982; Kamens *et al*., 1984). In these studies, samples were taken from a flue, whereas samples in this study were taken from inside the exposure chamber. The larger aggregates (> 1 μm) in our studies may have formed during the approximate 5-min residence time in the exposure chamber and may have been more representative of the particle-size distribution to which humans are exposed.

The carbonaceous component of wood smoke particles from the exposure chambers constituted about 75 % of the weighed mass, which is consistent with previous reports on the chemistry of wood smoke (Hildemann *et al*., 1991; McDonald *et al*. 2000; Rogge *et al*., 1998). More than 20% of the particulate mass was unaccounted for by chemical analysis in the present study. The difference between the measured chemical species and the weighed mass can be attributed at least in part to known wood smoke constituents (e.g., sulfates, potassium, chloride), which were not analyzed in our study. Although particle-bound water was not measured, it can contribute to the difference between the sum of chemical species and weighed mass (Van Loy *et al*., 2000). In addition, the weight of the

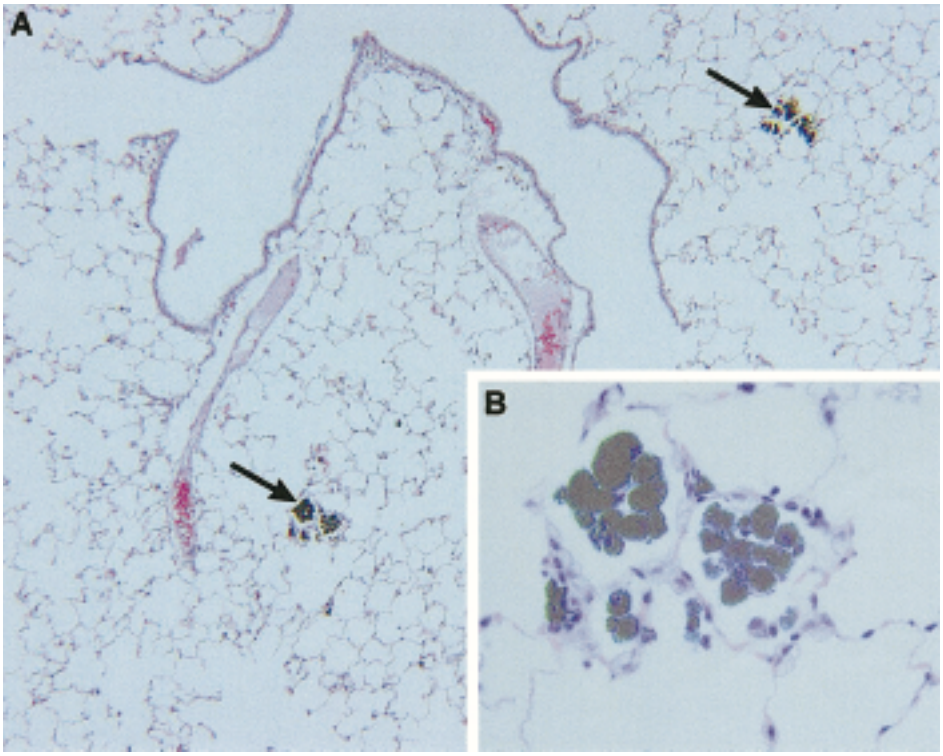

FIG. 5. (A) Distribution of particle-containing macrophages (see arrows) in the lung of a rat exposed to 10 mg/m^3 wood smoke for 90 days (30× magnification). (B) Higher magnification of the particle-containing macrophages (400× magnification).

organic material was estimated by applying a correction factor to the organic-carbon content measured by the thermal analysis. The thermal analysis measures only carbon in its elemental form, and without knowledge of the exact contribution of the noncarbon elements to the mass of organic material, the correction factor applied is only an estimate. Because organic carbon accounts for the majority of the particulate material, a small error in this estimate from the true value could significantly affect the total amount of organic carbon reported.

The measured chemical species in this study accounted for about 15% of the organic carbon present in wood smoke. The organic analysis employed here is comparable to contemporary comprehensive characterizations of both ambient air (Schauer and Cass, 2000) and air pollution sources such as diesel exhaust (Schauer *et al*., 1999) and wood combustion (Schauer *et al*., 2001). In general, characterizations of ambient and source-based organic material in the literature range from accounting for a fraction of a percent to as high as 50% of the total particle-bound organic material, because of limitation in current technology.

Most of the quantified chemical species in the wood smoke particles were composed of cellulose/hemicellulose- (sugar derivatives, furans) and lignin- (guaiacols, substituted benzenes/phenols) derived products. These findings reflect the structure and chemical composition of wood, which comprises approximately 65–75 % cellulose fibers bound together by lignin, an aromatic polymer making up 20–30 % of the overall mass (Rowell, 1984; Larson and Koenig, 1993). Many other compounds identified, such as the resin acids and diterpenoids, are also unique to wood smoke.

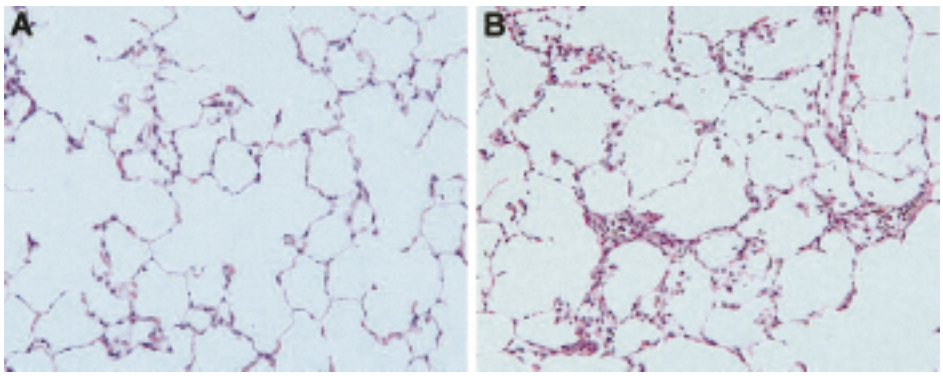

FIG. 6. A control lung (A) and a lung with alveolar epithelial cell hypertrophy and hyperplasia (B) adjacent to particle-containing macrophages in a rat exposed to 10 mg/m^3 wood smoke for 90 days (80× magnification).

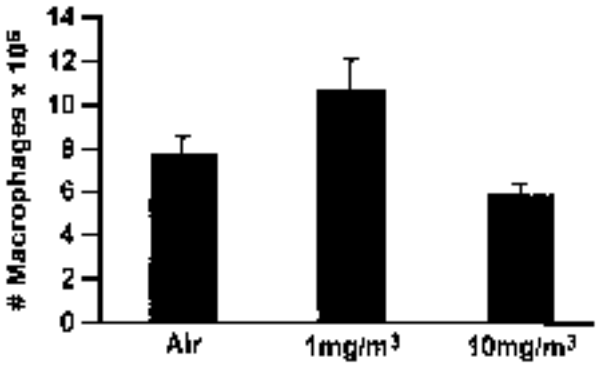

FIG. 7. Number of macrophages in the BALF of rats exposed to air, 1 mg/m^3, or 10 mg/m^3 wood smoke for 90 days. Group mean ± standard error from the mean was compared using a Poisson loglinear model. *Denotes significant difference from air control ($p < 0.05$).

Health Effects

Results of respiratory function tests indicated a small, exposure-related reduction in function consisting of reduced gas exchange at the alveolar membrane, and nonsignificant reductions in lung size and the uniformity of gas distribution within the lung. Overall, the impact was small and would be considered of little clinical importance. Only the reduction in gas exchange was consistent across multiple test parameters, and the difference from controls was less than 20%. This degree of reduction would be at the borderline of clinical significance in human subjects for whom test values are compared to normal ranges and thus, although statistically significant, is a modest effect. The slightly (insignificantly) smaller lungs of the high-exposure group, indicated by total lung capacity and vital capacity, and their slightly (insignificantly) larger residual volume may have been related to exposure. Because the lungs of rats are growing during the age range of this study (Mauderly and Gillett, 1992), the impact on lung size more likely reflects slower lung growth than an actual reduction in lung size. This conclusion is supported by the concurrence of slightly higher lung volume and body weight in the low-exposure group and slightly lower values in the high-exposure group than in controls, although neither the lung-volume nor body-weight differences were significant. The larger residual volume was consistent with earlier airway closure during exhalation, which in turn was consistent with the smaller quasistatic and forced vital capacity. It could be speculated that had the exposure continued, these differences might have progressed, consistent with changes observed in smokers.

The reason for the different effects on dynamic (increased) and quasistatic (slightly decreased) compliance cannot be determined from the measurements made in this study. Such an outcome might be possible if the increased resistance to airflow caused the exposed rats to breathe at a different set-point on the pressure-volume curve than the control rats. This explanation is unlikely, however, because functional residual capacity (volume at end expiration) was nearly identical in the control and high-exposure groups and represented very similar portions of total lung capacity (26% and 28%, respectively).

Although alveolar macrophage hyperplasia and pigmentation were increased with exposure concentration, decreased numbers of macrophages were found in the BALF in the high-exposure group. Significantly reduced numbers of macrophages were also found in the BALF of Fischer-344/Crl rats exposed for 12, 18, and 24 months to whole diesel exhaust diluted to a soot concentration of 3.5 mg/m^3 compared with air controls (Mauderly *et al*., 1989), although exposures to diesel exhaust particles at 1.5 mg/m^3 showed no difference compared to controls (Strom, 1984). These findings suggest that macrophages containing carbon particles remain in the lung tissue and may be harder to retrieve by bronchoalveolar lavage, possibly as a result of the observed clumping of particle-laden macrophages in alveolar spaces of exposed rats.

The mild mucous cell metaplasia observed in the upper trachea of rats exposed for 30 days was resolved after 90 days of exposure. However, the increase of the AB-positive mucous cells was caused by exposure to 1 mg/m^3 for 90 days. This change in staining pattern denotes a change in the content from neutral (PAS-positive) to acidic (AB-positive) glycoproteins (Samet and Cheng, 1994). Many glycoprotein-containing epithelial cells in rat airways are serous cells, which produce primarily neutral glycoproteins (Forrest and Lee, 1991), and they undergo a change to contain acidic glycoproteins in response to inhaled irritants (Jeffrey and Reid, 1977). It is possible that these changes occurred early in the high-exposure group and returned to control levels at 30 and 90 days of exposure.

After subchronic exposures to wood smoke, the cytokines TNF-α, IL-1β, and IL-6 were undetectable. Inflammatory cells, such as neutrophils, lymphocytes, or eosinophils, were not present in significantly increased numbers in the BALF. The absence of these indicators of early inflammation after subchronic exposures is not surprising; a reduction in inflam-

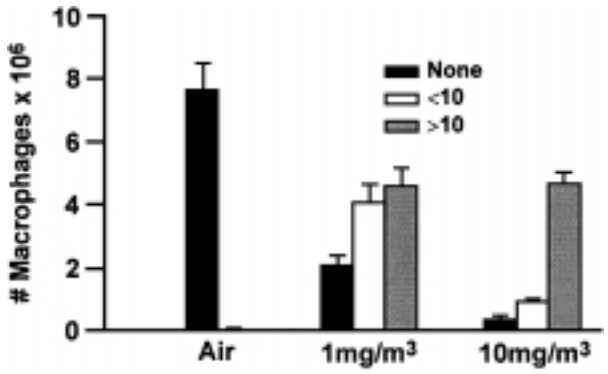

FIG. 8. Percentage of macrophages containing none, < 10, or >10 carbonaceous particles after 30 days of exposure to air or to wood smoke at 1 or 10 mg particles/m^3. Group mean ± standard error from the mean is shown. Similar numbers were obtained for rats exposed for 90 days.

mation with continued exposure has been reported for exposures to endotoxin (Shimada *et al*., 2000). However, the concentration levels of wood smoke may have been too low to elicit inflammation in the lung. These results and the lack of change in the proliferative response of T lymphocytes from the LALNs of exposed and nonexposed rats in response to Con A suggest that wood smoke alone may not affect the immune system.

In summary, our observations suggest that exposure to wood smoke caused minor, but significant effects on pulmonary function and on epithelia lining the nasal and tracheobronchial regions of Brown Norway rats. Future studies will use rats sensitized to allergens to determine whether wood smoke may affect a compromised immune system as fine particulate matter does in asthmatic children (Koenig *et al*., 1993).

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REFERENCES

Anderson, H. R. (1979). Respiratory abnormalities, smoking habits, and ventilatory capacity in a highland community in Papua New Guinea: Prevalence and effect on mortality. *Int. J Epidemiol.* **8,** 127–135.

Birch, M., and Cary, R. (1996). Elemental carbon-based method for monitoring occupational exposures to particulate diesel exhaust. *Aerosol Sci. Technol.* **25,** 221–224.

Butterfield, P., LaCava, G., Edmundson, E., and Penner, J. (1989). Woodstoves and indoor air. *J Environ. Health* **52,** 172–173.

Cheng, Y. S., Yeh, H. C., and Kanapilly, G. M. (1981). Collection efficiencies of a point-to-plane electrostatic precipitator. *Am. Ind. Hyg. Assoc.* **42,** 605–610.

Clark, D., Gollub, R., Green, W. F., Harvey, N., Murphy, S. J., and Samet, J. M. (1995). Asthma in Jemez Pueblo schoolchildren. *Am. J. Respir. Crit. Care Med.* **151,** 1625–1627.

Conn, C., McClellan, J., Maassab, H., Smitka, C., Majde, J., and Kluger, M. (1995). Cytokines and the acute phase response to influenza virus in mice. *Am. J. Physiol.* **268,** R78–R84.

Dasch, J. M. (1982). Particulate and gaseous emissions from wood-burning fireplaces. *Environ. Sci. Technol.* **16,** 639–645.

Dennis, R., Maldonado, D., Norman, S., Baena, E., Castano, H., Martinez, G., and Velez, J. (1996). Wood smoke exposure and risk for obstructive airways disease among women. *Chest* **109,** 55S–56S.

Fick, R. B., Jr., Paul, E. S., Merrill, W. W., Reynolds, H. Y., and Loke, J. S. (1984). Alterations in the antibacterial properties of rabbit pulmonary macrophages exposed to wood smoke. *Am. Rev. Respir. Dis.* **129,** 76–81.

Forrest, J. B., and Lee, R. M. K. W. (1991). The bronchial wall: Integrated form and function. In *The Lung: Scientific Foundations* (R. G. Crystel, Ed.), pp. 1081–1092. Raven Press, New York.

Harkema, J. R., and Hotchkiss, J. A. (1994). Ozone-induced proliferative and metaplastic lesions in nasal transitional and respiratory epithelium: Comparative pathology. *Inhal. Toxicol.* **6,** 187–204.

Hildemann, L. M., Markowski, G. R., Jones, M. C., and Cass, G. R. (1991). Submicrometer aerosol mass distributions of emissions from boilers, fireplaces, automobiles, diesel trucks, and meat-cooking operations. *Aerosol Sci. Technol.* **14,** 138–152.

Honicky, R. E., Osborne, J. S., 3rd, and Akpom, C. A. (1985). Symptoms of respiratory illness in young children and the use of wood-burning stoves for indoor heating. *Pediatrics* **75,** 587–593.

Jeffrey, P. K., and Reid, L. (1977). Ultrastructure of the airway epithelium and submucosal gland during development. In *Lung Biology in Health and Disease: Development of the Lung* (W. A. Hodson, Ed.), Vol. 6. Marcel Dekker, New York.

Johnson, K. G., Gideon, R. A., and Loftsgaarden, D. O. (1990). Montana air pollution study: Children's health effects. *J. Official Stat.* **5,** 391–408.

Kamens, R. M., Rives, G. D., Perry, J. M., Bell, D. A., Paylor, R. F., Jr., Goodman, R. G., and Claxton, L. D. (1984). Mutagenic changes in dilute wood smoke as it ages and reacts with ozone and nitrogen dioxide: An outdoor chamber study. *Environ. Sci. Technol.* **18,** 523–530.

Kewal Lal, K., Dutta, K., Vachhrajani, K. D., and Gupta, D. (1993). Histomorphological changes in lung of rats following exposure to wood smoke. *Ind. J. Exp. Biol.* **31,** 761–764.

Kleeman, M. J., Schauer, J. J., and Cass, G. R. (1999). Size and composition distribution of fine particulate matter emitted from wood burning, meat charbroiling and cigarettes. *Environ. Sci. Technol.* **33,** 3516–3523.

Koenig, J. Q., Larson, T. V., Hanley, Q. S., Rebolledo, V., Dumler, K., Checkoway, H., Wang, S. Z., Lin, D., and Pierson, W. E. (1993). Pulmonary function changes in children associated with fine particulate matter. *Environ. Res.* **63,** 26–38.

Larson, T. V., and Koenig, J. Q. (1993). A summary of the emissions characterization and noncancer respiratory effects of wood smoke. EPA-453/R93–036. U.S. Environmental Protection Agency.

Larson, T. V., and Koenig, J. Q. (1994). Wood smoke: emissions and noncancer respiratory effects. *Annu. Rev. Public Health* **15,** 133–156.

Liang, C. K., Quan, N. Y., Cao, S. R., He, X. Z., and Ma, F. (1988). Natural inhalation exposure to coal smoke and wood smoke induces lung cancer in mice and rats. *Biomed. Environ. Sci.* **1,** 42–50.

Mauderly, J. L., Bice, D. E., Cheng, Y. S., Gillett, N. A., Griffith, W. C., Henderson, R. F., Pickrell, J. A., and Wolff, R. K. (1990). Influence of preexisting pulmonary emphysema on susceptibility of rats to inhaled diesel exhaust. *Am. Rev. Respir. Dis.* **141,** 1333–1341.

Mauderly, J. L., Bice, D. E., Cheng, Y. S., Gillett, N. A., Henderson, R. F., Pickrell, J. A., and Wolff, R. K. (1989). Influence of experimental pulmonary emphysema on the toxicological effects from inhaled nitrogen dioxide and diesel exhaust. *Res. Rep. Health Eff. Inst.* **30,** 1–47.

Mauderly, J. L., and Gillett, N. A. (1992). Changes in respiratory function. In *Pathobiology of the Aging Rat,* Vol. 1, Blood and Lymphoid, Respiratory, Urinary, Cardiovascular, and Reproductive Systems (U. Mohr, D. L. Dungworth, and C. C. Capen, Eds.). ILSI Press, Washington, DC.

McDonald, J. D., Zielinska, B., Fujita, E. M., Sagebiel, J. C., Chow, J. C., and Watson, J. G. (2000). Fine particle and gaseous emission rates from residential wood combustion. *Environ. Sci. Technol.* **34,** 2080–2091.

McMenamin, C., McKersey, M., Kuhnlein, P., Hunig, T., and Holt, P. G. (1995). $\gamma\Delta$ T cells down-regulate primary IgE responses in rats to inhaled soluble protein antigens. *J. Immunol.* **154,** 4390–4394.

Miller, F. J. (2000). Dosimetry of particles in laboratory animals and humans in relationship to issues surrounding lung overload and human health risk assessment: a critical review. *Inhal. Toxicol.* **12,** 19–57.

Ohtsuka, R., Doi, K., and Itagaki, S. (1997). Histological characteristics of respiratory system in Brown Norway rat. *Exp. Anim.* **46,** 127–133.

Pandey, M. R. (1984). Domestic smoke pollution and chronic bronchitis in a rural community of the hill region of Nepal. *Thorax* **39,** 337–339.

Robin, L., Less, P., Winget, M., Steinhoff, M., Moulton, L., Santosham, M.,

and Correa, A. (1996). Wood-burning stoves and lower respiratory illnesses in Navajo children. *Pediatr. Infect. Dis. J.* **15,** 859–865.

Rogge, W. F., Hildemann, L. M., Mazurek, M. A., Cass, G. R., and Simoneit, B. (1998). Sources of fine organic aerosol. 9. Pine, oak, and synthetic log combustion in residential fireplaces. *Environ. Sci. Technol.* **32,** 13–22.

Rowell, R. (1984). *The Chemistry of Solid Wood.* Advances in Chemistry Series. American Chemical Society, Washington, DC.

Samet, J., and Cheng, P. (1994). The role of airway mucus in pulmonary toxicology. *Environ. Health Perspect.* **102** (Suppl.), 89–103.

Schauer, J. J., and Cass, G. R. (2000). Source apportionment of wintertime gas-phase and particle-phase air pollutants using organic compounds as tracers. *Environ. Sci. Technol.* **34,** 1821–1832.

Schauer, J. J., Kleeman, M. J., Cass, G. R., and Simoneit, B. R. T. (1999). Measurement of emissions from air pollution sources. 2. C1 through C30 organic compounds from medium duty diesel trucks. *Environ. Sci. Technol.* **33,** 1578–1587.

Schauer, J. J., Kleeman, M. J., Cass, G. R., and Simoneit, B. R. T. (2001). Measurement of emissions from air pollution sources. 3. C1–C29 organic compounds from fireplace combustion of wood. *Environ. Sci. Technol.* **35,** 1716–1728.

Schwartz, J., Slater, D., Larson, T. V., Pierson, W. E., and Koenig, J. Q. (1993). Particulate air pollution and hospital emergency room visits for asthma in Seattle. *Am. Rev. Respir. Dis.* **147,** 826–831.

Shimada, M., Tsukada, H., Ishizuka, O., Kon, Y., Hasegawa, T., Suzuki, E., and Gejyo, F. (2000). Lipopolysaccharide tolerance in relation to intrabronchial influx of neutrophils in the rat. *Lung* **178,** 235–248.

Singh, S. P., Kalra, R., Puttfarcken, P., Kozak, A., Tesfaigzi, J., and Sopori, M. L. (2000). Acute and chronic nicotine exposures modulate the immune system through different pathways. *Toxicol. Appl. Pharmacol.* **164,** 65–72.

Strom, K. A. (1984). Response of pulmonary cellular defenses to the inhalation of high concentrations of diesel exhaust. *J. Toxicol. Environ. Health* **13,** 919–944.

Tesfaigzi, Y., Fischer, M. J., Martin, A. J., and Seagrave, J. (2000). Bcl-2 in LPS- and allergen-induced hyperplastic mucous cells in airway epithelia of Brown Norway rats. *Am. J. Physiol. Lung Cell Mol. Physiol.* **279,** L1210–L1217.

Van Loy, M., Bahadori, T., Wyzga, R., Hartsell, B., and Edgerton, E. (2000). The aerosol research and inhalation epidemiology study (ARIES): PM2.5 mass and aerosol component concentrations and sampler intercomparisons. *J. Air Waste Manage. Assoc.* **50,** 1446–1458.