

World Trade Center Fine Particulate Matter Causes Respiratory Tract Hyperresponsiveness in Mice

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Pollutants originating from the destruction of the World Trade Center (WTC) in New York City on 11 September 2001 have been reported to cause adverse respiratory responses in rescue workers and nearby residents. We examined whether WTC-derived fine particulate matter [particulate matter with a mass median aerodynamic diameter < 2.5 μm ($\text{PM}_{2.5}$)] has detrimental respiratory effects in mice to contribute to the risk assessment of WTC-derived pollutants. Samples of WTC $\text{PM}_{2.5}$ were derived from settled dust collected at several locations around Ground Zero on 12 and 13 September 2001. Aspirated samples of WTC $\text{PM}_{2.5}$ induced mild to moderate degrees of pulmonary inflammation 1 day after exposure but only at a relatively high dose (100 μg). This response was not as great as that caused by 100 μg $\text{PM}_{2.5}$ derived from residual oil fly ash (ROFA) or Washington, DC, ambient air PM [National Institute of Standards and Technology, Standard Reference Material (SRM) 1649a]. However, this same dose of WTC $\text{PM}_{2.5}$ caused airway hyperresponsiveness to methacholine aerosol comparable to that from SRM 1649a and to a greater degree than that from ROFA. Mice exposed to lower doses by aspiration or inhalation exposure did not develop significant inflammation or hyperresponsiveness. These results show that exposure to high levels of WTC $\text{PM}_{2.5}$ can promote mechanisms of airflow obstruction in mice. Airborne concentrations of WTC $\text{PM}_{2.5}$ that would cause comparable doses in people are high (~ 425 $\mu\text{g}/\text{m}^3$ for 8 hr) but conceivable in the aftermath of the collapse of the towers when rescue and salvage efforts were in effect. We conclude that a high-level exposure to WTC $\text{PM}_{2.5}$ could cause pulmonary inflammation and airway hyperresponsiveness in people. The effects of chronic exposures to lower levels of WTC $\text{PM}_{2.5}$, the persistence of any respiratory effects, and the effects of coarser WTC PM are unknown and were not examined in these studies. Degree of exposure and respiratory protection, individual differences in sensitivity to WTC $\text{PM}_{2.5}$, and species differences in responses must be considered in assessing the risks of exposure to WTC $\text{PM}_{2.5}$. **Key words:** airway hyperresponsiveness, inflammation, neutrophil, nose-only inhalation, oropharyngeal aspiration, risk assessment. *Environ Health Perspect* 111:981–991 (2003). doi:10.1289/ehp.5931 available via <http://dx.doi.org/> [Online 20 November 2002]

The World Trade Center (WTC) disaster in New York City on 11 September 2001 sparked enormous concern about the quality of the environment in the surrounding neighborhoods. One of the immediate concerns was the effect of dust from the collapse and burning of the towers on breathing, especially in more susceptible individuals. Those returning to their homes as well as those who work in the area have reported throat irritation, cough, and other indications of mucous tissue sensory irritation (Haughney 2002; Kelley 2001). Nose and throat irritation may be caused by particulate matter (PM) that deposits in the nasal passages and upper airways and stimulates sensory nerve reflexes (Costa and Schelegle 1999). Exposure to airborne dust may also cause inflammation, mucus production, coughing, and sneezing in an effort to clear the lung of particles (Raabe 1999). However, inflammation, mucus production, and airway hyperresponsiveness may all contribute to airway obstruction. As asthma is characterized by all of these cardinal features (Sears 1997), it is reasonable to

suspect that asthmatic individuals may be more sensitive to agents that further promote airway obstruction.

Our objective in this series of studies was to evaluate the potential health effects of respirable $\text{PM}_{2.5}$ (particulate matter with a mass median aerodynamic diameter < 2.5 μm) derived from the collapsed towers of the WTC. Toxicologic assessment of PM dispersed in the areas surrounding the WTC will provide basic hazard identification information from which a broad health assessment may be derived to address public health concerns. To this end a team of scientists from New York University collected bulk samples of settled dust from several sites within 0.5 miles of Ground Zero on 12 and 13 September 2001. The bulk samples of dust were size fractionated to obtain fine $\text{PM}_{2.5}$ (in addition to coarser size fractions), which can be readily inhaled and deposited in the respiratory tract and is therefore relevant for study of toxicologic effects. The collection, size fractionation, and chemical analysis of these samples are described in a companion paper (McGee et al. 2003).

These studies compared the toxicity of samples of size-fractionated WTC $\text{PM}_{2.5}$ with previously tested $\text{PM}_{2.5}$ samples in mice. The use of mice offers a number of advantages for toxicity studies: *a*) less sample is needed to assess toxicity; *b*) the biology of the mouse has been intensively studied in the scientific literature; *c*) a wide array of mouse-specific analytical reagents is available; and *d*) we have extensive experience in assessing physiologic responses, inflammation, and respiratory tract injury in mice exposed to other samples of air pollutants. A dose–response study in mice was conducted comparing aspirated WTC $\text{PM}_{2.5}$ (pooled from seven different locations near the WTC site) with low- and high-toxicity $\text{PM}_{2.5}$ control samples. An acute inhalation exposure study was conducted on one WTC $\text{PM}_{2.5}$ sample, as upper airways irritation is a primary complaint of those living and working in the WTC area. Finally, a short-term time-course study was conducted comparing aspirated samples from seven different locations with each other and with a standard $\text{PM}_{2.5}$ sample.

This article is part of the mini-monograph “World Trade Center Fine Particulate Matter—Chemistry and Toxic Respiratory Effects.”

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Several methods were common to all three of these experiments to determine the toxicologic effects of WTC PM_{2.5}. The ability of these PM_{2.5} samples to affect respiratory tract responsiveness to aerosolized methacholine (Mch) was determined. Because this chemical triggers airway narrowing, the test is appropriate to determine sensitivity to agents that induce airway obstruction. Bronchoalveolar lavage (BAL) is a standard technique that quantifies numbers of inflammatory cells and concentrations of proteins and enzymes indicative of lung injury. Lung pathologic effects were assessed in a semiquantitative fashion in all studies, and pathologic effects in the nasal region were determined in the inhalation study. Comparing the toxicologic effects of dust derived from the destruction of the WTC with reference PM_{2.5} samples that have been extensively characterized will be beneficial and relevant to the overall assessment of health consequences of environmental pollutants related to this disaster.

Detailed experimental findings of these studies are available in a U.S. EPA report (2002).

Materials and Methods

Particulate matter samples. We used WTC and reference PM samples, which were described and analyzed in a companion article (McGee et al. 2003). Fallen dust samples were collected on 12 and 13 September within 0.5 miles of the WTC site. Because the fallen dust contained large amounts of very coarse PM, we extracted the PM_{2.5} by a size-separation procedure that included sieving, aerosolization, collection of the PM_{2.5} fraction on Teflon filters, and removal of the PM_{2.5} from the filters (McGee et al. 2003). The tested samples included PM_{2.5} from seven individual collection sites (WTC8, WTC11, WTC13, WTCB, WTCC, WTCE, and WTCF), a PM_{2.5} sample pooled from these seven locations (WTCX), and a sieved sample (< 53 μm) from an eighth location that was size-separated to PM_{2.5} during nose-only aerosol exposure (WTC3). The sites were located east (WTC11: 0.1 miles; WTC8: 0.4 miles; WTC3: 0.3 miles), southeast (WTC13: 0.1 miles; WTCF: 0.25 miles), south (WTCB: 0.25 miles), west-northwest (WTCC: 0.2 miles), and north-northeast (WTCE: 0.25 miles) from the center point of Ground Zero. The reference PM_{2.5} samples were derived from Mt. St. Helens dust (MSH) (Graham et al. 1985), residual oil fly ash [ROFA; sample 3 from Kodavanti et al. (1998)], and Standard Reference Material (SRM) 1649a (urban PM from Washington, DC) from the National Institute of Standards and Technology (NIST).

Experimental animals and weight randomization. Young adult (7-week-old) female outbred CD-1 mice were obtained from Charles River Breeding Laboratory

[CrI:CD-1 (ICR) BR] in Raleigh, North Carolina, or Portage, Michigan. Mice were housed in plastic cages on beta-chip bedding in groups of four per cage, maintained on a 12-hr light/dark cycle at approximately 22°C and 50% relative humidity in our Association for Assessment and Accreditation of Laboratory Animal Care–approved facility, and held for a minimum of 5 days before treatment. Food (Prolab RMH 3000; PMI Nutrition International, St. Louis, MO) and water were provided *ad libitum*. In all experiments, we used a validated weight randomization program to assign mice to exposure groups.

Experimental design. Experiment A was designed to study the dose–response characteristics of the pooled sample of WTC PM_{2.5} (WTCX); experiment B was designed to study upper respiratory tract responses associated with nose-only inhalation exposure of WTC3 PM_{2.5}; and experiment C was designed to examine toxic responses of WTC PM_{2.5} from individual collection sites. We emphasized experiments using oropharyngeal aspiration (A and C) over inhalation experiments (B) because *a*) sample quantities were limited; *b*) we could deliver a precise quantity of PM to the lung at a specific time point, whereas inhaled dose is more difficult to predict or quantify; and *c*) intratracheal instillation (equivalent to oropharyngeal aspiration) is specifically recommended in evaluation of panels of test materials for their relative potential to produce toxicity (Driscoll et al. 2000).

In experiment A, groups of female CD-1 mice were exposed to pooled WTCX (10, 31.6, or 100 μg), MSH (100 μg), ROFA (10 or 100 μg), or saline vehicle control by oropharyngeal aspiration on day 0. In experiment A1, conducted in three replicates (total *n* = 12 mice per group), we assessed airway responses to aspiration of the PM samples by comparing breathing parameters just before and after aspiration. On day 1, diffusing capacity of the lung for carbon monoxide (DLCO) was assessed, and BAL fluid cells, proteins, and enzymes were recovered and quantified to assess lung injury and inflammation. In experiment A2, conducted in two replicates (total *n* = 8 mice per group), we determined airway responsiveness to Mch aerosol 1 day after exposure, and then removed the lungs for histopathologic assessment.

Because oropharyngeal aspiration of PM bypasses the nose, potentially relevant upper airways responses may go undetected. We designed experiment B to test whether such responses are significant. We exposed two groups of mice (*n* = 48 per exposure group) in nose-only inhalation exposure tubes to aerosolized PM_{2.5} sample (WTC3) or air only for 5 hr. Breathing parameters were compared just before and after inhalation exposure in 12 mice from each group. On days 1, 3, and 6 postexposure, mice from each group were

assessed for BAL parameters (*n* = 8) or responsiveness to Mch aerosol, followed by assessment of lung and nasal histopathology (*n* = 8).

As effects of the pooled WTCX sample in experiment A may have been dominated by one or more site samples that were toxic compared with other site samples, we designed experiment C to examine the variability of pulmonary responses associated with WTC PM_{2.5} samples collected from different geographic locations, as well as responses at two different time points. In two subexperiments of experiment C, mice were exposed by oropharyngeal aspiration to 100 μg PM_{2.5} from one of seven individual WTC sample sites, to 100 μg SRM 1649a (referred to as SRM hereafter), or to saline vehicle only. In experiment C1, mice were exposed to WTC8, WTC13, WTCF, SRM, or saline. In experiment C2, mice were exposed to WTC11, WTCB, WTCC, WTCE, or saline. On days 1 and 3, mice were assessed for responsiveness to Mch aerosol, BAL parameters, and lung histopathology (*n* = 8 per group per time point, except *n* = 4 for the saline group in experiment C2). Statistical analysis of the data was performed within each subexperiment.

Oropharyngeal aspiration of particulate matter samples. PM_{2.5} samples were weighed and resuspended in sterile saline (Sigma, St. Louis, MO) at a concentration of 2 mg/mL. Samples were vortexed and used undiluted (100-μg dose in an aspiration volume of 50 μL) or diluted with saline to 0.632 mg/mL (31.6-μg dose) or 0.2 mg/mL (10-μg dose). All samples were sonicated for 2–4 min at 22°C prior to oropharyngeal aspiration. Mice were anesthetized in a Plexiglas chamber with methoxyflurane (Metofane; Mallinckrodt, Mundelein, IL). Fifty microliters of PM_{2.5} suspension or saline alone was pipetted in the back of the oropharynx, and the tongue was held until the animal was forced to aspirate the sample. This technique is equivalent to intratracheal instillation in deposition efficiency (Foster et al. 2001) and has been used successfully (e.g., Gavett et al. 1999; Kodavanti et al. 1998).

Nose-only inhalation exposure. Mice were exposed to WTC3 or air only in two separate nose-only inhalation exposure chambers. We conducted the exposures for 5 hr in 52-port nose-only flow-by inhalation chambers (Lab Products, Seaford, DE). The sieved (< 53 μm) WTC3 sample was desiccated at room temperature prior to use. The aerosol exposure system consumes low amounts of sample and is described in a previous reference (Ledbetter et al. 1998). Particles are carried through a particle charge neutralizer and 2.5-μm cut-point cyclone to remove particles larger than PM_{2.5} and finally enter the inlet of the nose-only chamber. Mice were randomized into exposure groups, and 49 in each group were placed in nose-only exposure tubes (one extra mouse per group). Mice were not acclimated to the tubes

prior to exposure, as stress may be an important component of the response to WTC PM. Dust concentration was determined gravimetrically on Teflon filters (45 mm diameter with 1- μ m pore size), and real-time PM concentration was estimated with an aerosol monitor (Dust Track; TSI Inc., St. Paul, MN) on the chamber exhaust. The particle size was determined gravimetrically using a Mercer Cascade Impactor (Intox Products, Albuquerque, NM).

Respiratory responses assessed by whole-body plethysmography. We examined whether exposure to PM_{2.5} results in immediate changes in breathing parameters in unanesthetized, unrestrained mice in a 12-chamber whole-body plethysmograph system (Buxco Electronics, Sharon, CT). Pressure signals generated by breathing are used to compute respiratory rate [frequency (f) of breaths per minute] and other parameters including enhanced pause (PenH) every 6 sec. PenH was automatically calculated by the software (BioSystem XA, version 2.5; Buxco) (and confirmed by examination of random data) using expiration time (Te), relaxation time (RT), and peak expiratory and inspiratory flows (PEF, PIF) according to the expression: $\text{PenH} = [(Te - RT)/RT] \times [PEF/PIF]$. Although PenH is at best an indirect measure of flow resistance, it does correlate well with lung resistance and reflects changes occurring during bronchoconstriction (Hamelmann et al. 1997), although other responses such as mucus hypersecretion may increase PenH. We determined baseline measurements in mice for 10 min, paused for oropharyngeal aspiration (or stopped for inhalation exposure), and then resumed recording measurements for 1 hr. We found that using the first 10–15 min of data after exposure was not more sensitive in detecting changes in respiratory parameters than the entire hour of post-exposure monitoring, so responses over the whole postexposure hour were used and averaged. The percent change in f and PenH after exposure to PM was expressed as $[(\text{postvalue} - \text{prevalue})/\text{prevalue}] \times 100\%$.

We measured respiratory responsiveness of mice to increasing concentrations of aerosolized Mch in the system described above. After measuring baseline parameters for 5 min, an aerosol of saline or Mch in increasing concentrations (4, 8, 16, 32, and 64 mg/mL) was nebulized through an inlet of the chamber. The response to saline or Mch was measured over the aerosolization period (1 min), an aerosol drying step (2 min), and an additional 1-, 2-, 3-, 4-, 8-, or 12-min period (after exposure to 0, 4, 8, 16, 32, or 64 mg/mL Mch, respectively). After subtracting baseline values from responses to saline or Mch, the area under the curve (PenH AUC; $\text{PenH} - \text{sec}$) for these recording intervals was calculated using a trapezoidal method.

Diffusing capacity of the lung for carbon monoxide. The diffusing capacity of the lung

for carbon monoxide is a useful test of the integrity of the alveolar–capillary membrane (Levitzky 1995). To determine DLCO rapidly with increased sensitivity, four mice were placed together in a single 7.8-L bell jar associated with a gas uptake system (consisting of an oxygen monitor, flow meter, pump, pressure gauge and transducer, mass flow controller, and computerized data collection and control system). Approximately 6.6 mL research-grade CO (99.99%) was injected into the system. The initial concentration of CO in the chamber was approximately 700 ± 10 ppm. CO concentrations were taken every 15 sec (Bendix model 8501-5CA CO analyzer; ABB Process Analytics, Lewisburg, WV) and continued for approximately 10 min. The DLCO is expressed as the slope of the fitted line of [CO] versus time (ppm/min).

Bronchoalveolar lavage. Mice were anesthetized with urethane (1.5 g/kg ip) and killed by exsanguination via severing the renal artery. The trachea and lungs were exposed and a 20-gauge catheter was sutured into the trachea. Mice were lavaged with two aliquots of Ca^{2+} , Mg^{2+} , and phenol red-free Hank's balanced salt solution (HBSS, 35 mL/kg; Life Technologies, Bethesda, MD). Approximately 85% of the total instilled volume was recovered in all treatment groups. The BAL fluid was maintained on ice and centrifuged at $360 \times g$ for 10 min at 4°C. BAL cells were resuspended in 1 mL HBSS and counted (Z1; Coulter, Hialeah, FL). Cytospin preparations of BAL cell samples were made and stained with Wright–Giemsa using an automated slide stainer (Hematek 2000; Miles Inc., Elkhart, IN). Cell differentials were performed by one person (SHG) counting 500 cells per slide. Assays for total protein, albumin, lactate dehydrogenase (LDH), and *N*-acetyl- β -D-glucosaminidase (NAG) were carried out on an aliquot of BAL supernatant as previously described using a Cobas Fara II centrifugal spectrophotometer (Hoffman-LaRoche, Branchburg, NJ) (Gavett et al. 1997). Total protein and albumin are increased after damage to the alveolar epithelial barrier (Henderson et al. 1985). LDH is a cytoplasmic enzyme released by dead or dying cells, whereas NAG is indicative of lysosomal enzyme release (Henderson et al. 1985).

Histopathology. Mice were anesthetized with urethane and killed as described above for BAL. Lungs were removed and fixed by tracheal perfusion with ice-cold 4% paraformaldehyde at 25 cm pressure for 15 min. The trachea was tied off and placed in 4% paraformaldehyde at 4°C. After 24 hr the lungs were placed in phosphate-buffered saline (PBS) at 4°C. Fixed lungs were processed to paraffin blocks, sectioned at an approximate thickness of 5 μ m, placed on glass slides, and stained with hematoxylin and eosin. Longitudinal coronal sections were cut

on a plane to include mainstem bronchi for viewing a maximal amount of lung area. Histopathologic observations for individual animals in each experiment were tabulated, and the degree of severity of inflammatory changes and the presence of PM-related pigment were graded on a scale of 1 to 5 (1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately severe, 5 = severe/high). The pathologist knew which animals were included in a group, the control group, the day after treatment, and the doses given to the experimental groups but did not know the identities of the PM samples.

We examined nasal histopathology in mice from the nose-only inhalation exposure. Both nasal passages were fixed by slowly flushing 1–2 mL ice-cold 4% paraformaldehyde retrograde through the nasopharynx. The nasal cavities were immersed in the fixative at 4°C for at least 24 hr, then placed in 0.1 M PBS (pH 7.2). Nasal cavities were decalcified in 13% formic acid for 5 days, then rinsed in distilled water for 1 hr. Three transverse tissue blocks, cut perpendicular to the hard palate, were selected for analysis. The first block was sectioned from the proximal aspect of the nasal cavity immediately posterior to the upper incisor tooth (T1). The second block was taken at the level of the incisive papilla (T2), and the third and most distal block was taken at the level of the second palatal ridge (T3). Tissue blocks were embedded in paraffin, and 6 μ m-thick sections were cut from the anterior surface and stained with hematoxylin and eosin. Nasal tissues (three sections/mouse) from a total of 48 mice tested for Mch responsiveness (8 mice/exposure group/time point) were examined by light microscopy, and lesions were graded on the following scale: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked inflammation.

Statistical analysis. We used SAS procedures (version 8.2; SAS, Inc., Cary, NC) to analyze data from all experiments. We used replicated completely randomized designs for experiment A, and crossed designs for experiments B and C involving treatments (TRT) and days (DAY). Randomized block designs were used to assess DLCO. Responses to increasing concentrations of Mch were generally linear or log-linear. Consequently, we used regression techniques [analysis of covariance (COV)] rather than analysis of responses at individual doses of Mch to analyze respiratory responsiveness. Regression analysis incorporates the entire dose–response data set, and insight is gained through smoothing in the regression process. Techniques similar to ordinary stepwise regression were used in COV analyses. An overall test of parallelism of the regression lines was performed first. We then tested whether subgroups of the TRT and DAY combinations had common slopes. If the subgroups had a

common slope, we then used individual contrast tests to determine whether a single line could be used to simultaneously fit the subgroups, or whether separate lines were necessary. When initial multivariate repeated measures analysis of variance (MANOVA) tests showed significant interactions between dose of Mch and TRT or DAY in airway responsiveness studies, we used univariate linear regression in subsequent tests.

For other end points, we determined whether the variances of each TRT and DAY combination with a univariate response could be considered homogeneous. If the variance ratios were greater than 10-fold, then all the

responses were ranked from smallest to largest across all TRT and DAY combinations, and ranks replaced the original responses for the univariate analysis of variance (ANOVA). When interactions between TRT and DAY occurred, these were pointed out, and in some cases further ANOVA tests were performed for each DAY. When ranks were used for the response, the ranks were regenerated for each day separately. When the effect of TRT was significant, follow-up comparisons of means were performed using Tukey's multiple comparison tests. Group differences were considered significant if the test statistical type I error $p < 0.05$.

Table 1. Experiment A: immediate airway responses^a and DLCO.^b

Group	Breathing frequency			PenH			DLCO (ppm/min)
	Pre-exposure (breaths per min)	Post-exposure (breaths per min)	Change (%)	Pre-exposure (unitless)	Post-exposure (unitless)	Change (%)	
Saline	492.3	348.1	-29.7	0.73	0.96	23.9	-3.865
	11.5	25.6	4.2	0.08	0.17	8.3	0.325
MSH-100	474.0	320.6	-31.7	0.92	1.25	40.5	-4.005
	13.5	23.0	5.2	0.13	0.18	14.8	0.177
ROFA-10	492.0	343.5	-29.7	0.74	1.05	42.3	-3.810
	14.3	18.6	4.1	0.11	0.17	13.5	0.107
ROFA-100	461.0	307.0	-33.4	0.88	1.51	76.4*	-3.799
	11.9	18.7	3.7	0.10	0.20	18.6	0.364
WTCX-10	467.2	322.4	-31.1	0.85	1.14	52.4	-3.624
	14.7	22.5	4.1	0.15	0.18	21.8	0.455
WTCX-31.6	476.8	348.5	-26.3	0.86	1.14	26.8	-3.801
	15.5	18.9	4.3	0.18	0.34	15.6	0.232
WTCX-100	486.8	325.3	-33.1	0.79	1.11	40.7	-4.094
	14.1	26.6	5.1	0.08	0.16	11.8	0.275

^aValues shown are means (shaded) and SEM immediately below means ($n = 12$ per group). Respiratory parameters were measured immediately before (Pre-) and after (Post-) oropharyngeal aspiration of PM samples or saline on day 0. ^bDiffusing capacity of the lung for carbon monoxide was determined 1 day after exposure on four mice from each treatment group, placed together in a single chamber. Values shown are average slopes (shaded) of chamber [CO] versus time (ppm/min), after subtraction of value from empty chamber, and SEM immediately below means ($n = 3$ replicate experiments). *Percent increase in PenH was significantly greater in ROFA-100-treated mice versus saline-treated mice ($p < 0.05$).

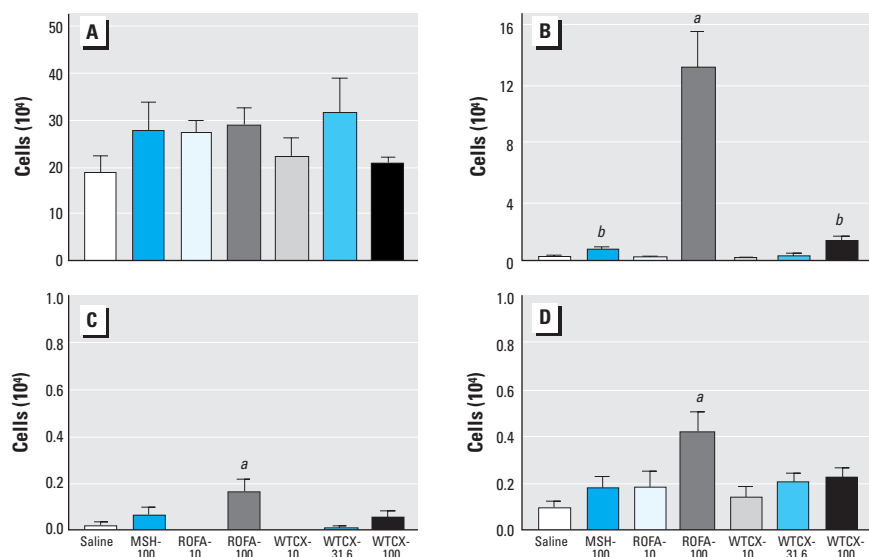


Figure 1. Experiment A: BAL cell numbers recovered from mice 1 day after aspiration of PM samples in saline or saline vehicle alone. Values shown are means and SEM ($n = 12$ per group). Cell types shown are (A) macrophages, (B) neutrophils, (C) eosinophils, and (D) lymphocytes.

^a $p < 0.05$ versus saline group. ^b $p < 0.05$ versus saline group (comparison of rank values) after exclusion of ROFA-100 data with larger variances than other groups.

Results

Experiment A: dose-response relationships of World Trade Center PM_{2.5}. Immediate airway responses, gas exchange, and bronchoalveolar lavage parameters. In experiment A1, mice were exposed by oropharyngeal aspiration to various doses of PM_{2.5} samples or saline on day 0, and ventilatory parameters were assessed immediately before and after exposure. There were no differences among groups in breathing frequency, but mice exposed to the 100- μ g dose of ROFA (ROFA-100) had a significant 76% increase in PenH immediately after exposure compared with that in saline control mice (Table 1), indicating airway obstruction. There were no significant changes in immediate responses in mice exposed to any dose of WTCX. One day after exposure, no differences were found in the DLCO, indicating that none of the PM_{2.5} samples caused injury severe enough to significantly reduce gas exchange at the alveolar-capillary barrier (Table 1).

Bronchoalveolar lavage parameters were determined immediately after testing for DLCO. Because of the high variance of data in the ROFA-100 group, we judged it necessary to compare ROFA-100 data alone versus saline control data. Other comparisons were made between the saline control group and the other groups after excluding ROFA-100 data. Significant increases in neutrophils (31% of total cells), eosinophils, and lymphocytes were found in ROFA-100 mice compared with those in saline control mice (Figure 1). Significant differences in neutrophil numbers were found between the saline control group and both the MSH-100 group and the WTCX-100 group ($p < 0.05$). Neutrophils comprised about 7% of total BAL cells in the WTCX-100 group but only about 1% or less in the WTCX-31.6 and WTCX-10 groups. Proteins and enzymes in BAL supernatant indicative of lung damage were all significantly increased in the ROFA-100 group 2- to 4-fold compared with those in saline control mice (Table 2). No significant changes in BAL proteins and enzymes were found in any of the other PM exposure groups relative to saline controls.

Responsiveness to methacholine aerosol.

In experiment A2, with the same exposure protocol as in experiment A1, responsiveness to increasing concentrations of Mch aerosol was assessed 1 day after exposure. To assess overall responsiveness and account for variability, power function equations were fit to the PenH AUC versus [Mch] data for each group (Figure 2). The analysis showed that the saline, MSH, ROFA-10, WTCX-10, and WTCX-31.6 groups could all be modeled with a common power function exponent. Among these five groups, ROFA-10 mice had a small but significant increase in the coefficient of the equation versus the saline group

($p = 0.03$). The ROFA-100 and WTCX-100 groups could be modeled with a power function with a significantly different exponent (1.471; $p = 0.001$) versus the common exponent of the other five groups, indicating that these two groups are hyperresponsive compared with the other five groups. In addition, the coefficient for the WTCX-100 group was significantly different from and greater than that of the ROFA-100 group ($p = 0.0001$), showing that mice exposed to the 100- μg dose of WTCX were significantly more reactive to Mch than the ROFA-100 group.

Lung histopathology. After tests for airway responsiveness to Mch aerosol, mice were killed and assessed for pathologic changes in the lungs (Table 3). In both the MSH-100 and ROFA-100 groups, focal acute bronchiolar inflammation was found at similar incidences and average severity, which was minimal (average score: MSH-100 = 0.8; ROFA-100 = 1.0). Although one mouse in the WTCX-10 group had a finding of minimal focal acute bronchiolar inflammation, for an average group score of 0.1, this lesion was not found in any of the mice in the WTCX-31.6 or WTCX-100 groups (Figure 3), suggesting that the lesion in the one WTCX-10 mouse was not treatment related. Free bronchiolar pigment (outside macrophages and presumably corresponding to PM) was identified in all ROFA-100 mice at an average severity of 1.5 (Table 3), and in six of eight mice in the ROFA-10 group at an average severity of 0.8. One mouse in the WTCX-31.6 group (but none in the WTCX-100 group) had minimal free bronchiolar pigment, again suggesting that this finding is not treatment dependent. These findings indicate that both ROFA-100 and MSH-100, but not the pooled WTCX-100 or any lower dose, caused minimal focal acute bronchiolar inflammation.

Table 2. Experiment A: BAL supernatant biochemical indicators of lung injury.^a

Group	Protein ($\mu\text{g/mL}$)	LDH (U/L)	Albumin ($\mu\text{g/mL}$)	NAG (U/L)
Saline	155.2	29.8	21.8	2.2
	6.8	2.1	1.2	0.4
MSH-100	168.8	27.8	22.3	2.0
	8.8	1.7	1.2	0.4
ROFA-10	157.9	32.3	20.8	3.1
	5.3	1.2	0.9	0.4
ROFA-100	279.5*	93.2*	39.2*	7.9*
	16.8	10.3	2.8	1.2
WTCX-10	153.7	30.4	20.8	1.9
	4.3	1.8	0.8	0.3
WTCX-31.6	160.2	33.6	21.7	1.8
	6.3	1.6	1.3	0.2
WTCX-100	161.4	33.7	21.3	2.3
	4.8	2.1	1.0	0.3

^aValues shown are means (shaded) and SEM immediately below means ($n = 12$ per group). BAL fluid was recovered 1 day after exposure. Total protein, LDH, albumin, and NAG were measured in BAL fluid supernatant. *Significantly greater values in ROFA-100-treated mice versus saline-treated mice ($p < 0.05$).

Experiment B: effects of nose-only inhalation exposure. Exposure results and immediate airway responses. We exposed mice to the WTC3 PM_{2.5} sample at an average gravimetric concentration of $10.64 \pm 3.10 \text{ mg/m}^3$. The mass median aerodynamic diameter (MMAD) was $1.05 \mu\text{m}$, and the geometric standard deviation (σg) was 2.67. Chamber temperature and relative humidity were 23.3°C and 11% in the control chamber and 23.9°C and 11% in the WTC3 chamber. Ventilatory parameters were measured in 12 mice from each group before and after the nose-only exposure. Ventilatory rate decreased after exposure in both groups (air: $-35 \pm 4\%$; WTC 3: $-30 \pm 4\%$), but there was no significant difference between them. PenH was increased by an average of $30 \pm 14\%$ after exposure to air and by an average of $60 \pm 18\%$ after exposure to WTC3. Although this difference was not significant ($p = 0.20$), we found that PenH increased in all 12 mice exposed to WTC3 but in only 8 of 12 mice exposed to air (Figure 4). Furthermore, some of the increases in WTC3-exposed mice were

quite large. These data suggest the possibility that individual mice in this outbred strain may be susceptible to bronchoconstrictive effects of WTC PM.

Responsiveness to methacholine aerosol. Unlike experiment A, responsiveness to Mch aerosol in experiment B could be modeled with linear equations. Analysis of the data showed that there were significant interactions of treatment, day, and Mch concentration ($p = 0.01$), implying that the results depended on a combination of these factors (data not shown). Although the data indicated that the air day 6 and WTC3 day 1 groups were less responsive to Mch aerosol challenge than the other four groups, the biologic significance of this finding is unclear. Close examination of the Mch responsiveness data from experiment B showed they were more variable than that from experiment A.

Bronchoalveolar lavage parameters. We quantified numbers of BAL cells 1, 3, and 6 days after nose-only exposure to air or WTC3 (Table 4). Mice exposed to WTC3

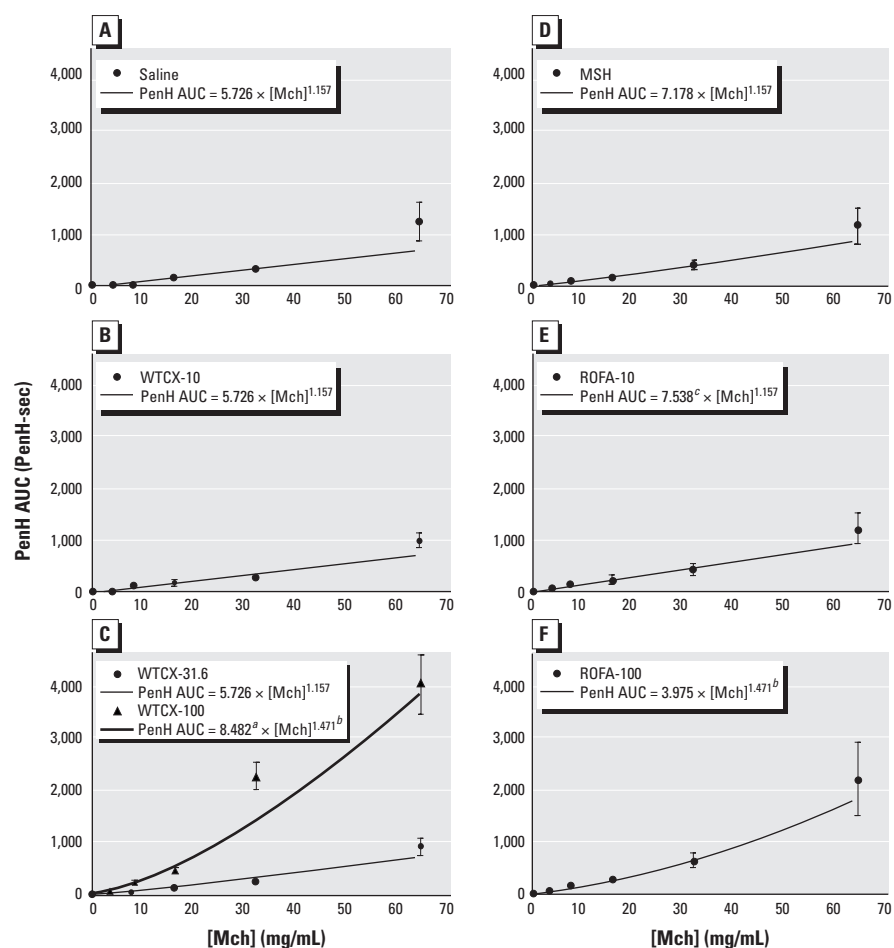


Figure 2. Experiment A: airway responsiveness to Mch aerosol in mice exposed to PM samples or saline vehicle and tested 1 day later ($n = 8$ /group; data shown are mean \pm SEM). (A) Saline, (B) WTCX-10, (C) WTCX-100 and WTCX-100, (D) MSH, (E) ROFA-10, (F) ROFA-100. Power function equations were fit to the data.

^aSignificantly different coefficient versus ROFA-100 coefficient ($p = 0.0001$). ^bSignificantly different exponent versus common saline, MSH, ROFA-10, WTCX-10, and WTCX-31.6 exponent ($p = 0.001$). ^cSignificantly different coefficient versus saline coefficient ($p = 0.03$).

Table 3. Experiment A: Summary of treatment-related histopathologic findings in mice 1 day after oropharyngeal aspiration of PM samples.^a

Treatment group	Bronchiole inflammation, (acute, focal)		Bronchiole pigment (free, focal)		Bronchiole pigmented macrophage (focal)		Peribronchiolar inflammation (acute, focal)	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Saline	0/8	0.0	0/8	0.0	0/8	0.0	0/8	0.0
MSH-100	6/8	0.8	0/8	0.0	2/8	0.3	0/8	0.0
ROFA-10	2/8	0.3	6/8	0.8	0/8	0.0	1/8	0.1
ROFA-100	6/8	1.0	8/8	1.5	0/8	0.0	0/8	0.0
WTCX-10	1/8	0.1	0/8	0.0	0/8	0.0	0/8	0.0
WTCX-31.6	0/8	0.0	1/8	0.1	0/8	0.0	0/8	0.0
WTCX-100	0/8	0.0	0/8	0.0	0/8	0.0	0/8	0.0

^aIncidence denotes number of mice in group with finding/total number of mice examined. Average severity score for the group is shown based on the following scoring system: 0 = not present, 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately severe, 5 = severe/high.

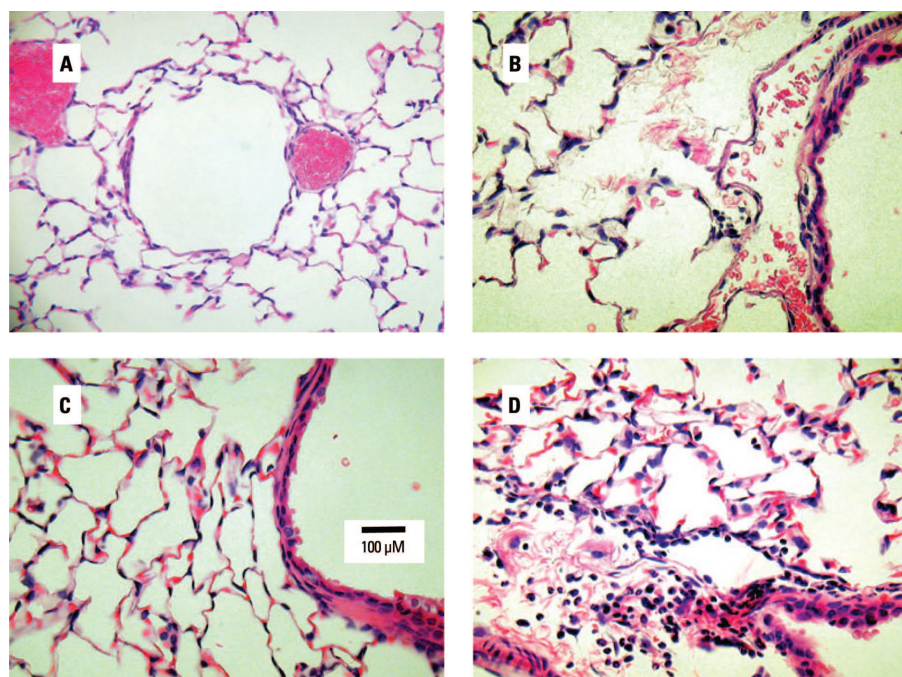


Figure 3. Experiment A: representative lung micrographs 1 day after aspiration of PM samples or saline vehicle (all panels: bar = 100 µm). (A) Saline-exposed control mouse with no remarkable findings. (B) Mouse exposed to 100 µg MSH showing minimal degree of focal acute bronchiolar inflammation. (C) Mouse exposed to 100 µg pooled WTCX sample with no remarkable findings. (D) Mouse exposed to 100 µg ROFA showing slight/mild degree of focal acute bronchiolar inflammation.

Table 4. Experiment B: BAL parameters after nose-only inhalation exposure.^a

Group	Day	BAL cell number ($\times 10^{-4}$)				Protein ($\mu\text{g}/\text{mL}$)	Albumin ($\mu\text{g}/\text{mL}$)	LDH (U/L)	NAG (U/L)
		Mac	Neut	Eos	Lym				
Air	1	14.80	0.012	0.003	0.046	165.2	21.0	29.0	1.5
		3.11	0.004	0.002	0.010	6.1	1.1	3.4	0.1
WTC3	1	17.48	0.006	0.000	0.067	147.1***	16.9***	23.9	1.6
		3.13	0.003	0.000	0.013	6.7	1.2	3.3	0.1
Air	3	16.56	0.008	0.000	0.125	136.6	16.2	33.0	1.8
		1.13	0.004	0.000	0.041	10.4	1.2	6.4	0.0
WTC3	3	26.72	0.034	0.016	0.197**	138.1***	15.8***	28.9	1.6
		3.11	0.017	0.009	0.036	7.8	1.5	3.4	0.1
Air	6	22.24	0.000	0.000	0.140	172.6	22.4	30.2	1.4
		1.13	0.000	0.000	0.026	8.5	1.3	2.4	0.2
WTC3	6	29.86*	0.019	0.005	0.281**	146.5***	17.5***	27.1	1.4
		2.58	0.008	0.004	0.056	6.8	1.1	3.1	0.1

Abbreviations: Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; Neut, neutrophils.

^aValues shown are means (shaded) and SEM immediately below means ($n = 8$ per group). *Significant difference ($p = 0.01$) between air and WTC3, day 6 different from day 1. **Significant difference ($p = 0.02$) between air and WTC3; day 3 and day 6 both different from day 1. ***Significant overall treatment effect (WTC3 < air; no significant day effect); $p = 0.05$ (protein) or $p = 0.007$ (albumin).

had significantly greater numbers of macrophages ($p = 0.01$) on day 6 versus day 1 and greater numbers of lymphocytes ($p = 0.02$) on both days 3 and 6 versus day 1 compared with those in air-exposed mice. Macrophages still comprised about 99% of all recovered cells in both groups at all time points, indicating that WTC3 did not induce a significant acute inflammatory reaction. The small increases in macrophages and lymphocytes are probably a nonspecific reaction to inhalation of PM, which induces macrophage recruitment for phagocytosis and clearance of the particles (Adamson and Bowden 1981). Surprisingly, we found higher levels of total protein ($p = 0.05$) and albumin ($p = 0.007$) in the air group, without any significant effect of day after treatment (Table 4). However, the overall levels of proteins and enzymes were low in both groups and at all time points compared with values from experiment A. The results indicate that at this exposure concentration and duration, WTC3 PM_{2.5} does not induce acute lung injury.

Nasal and lung histopathology. The only nasal alteration observed by light microscopic examination was minimal to mild acute, focal inflammation (rhinitis) in four of the eight mice exposed to WTC3 PM_{2.5} and killed 24 hr postexposure. This minimal inflammatory response was bilateral and restricted to the most proximal tissue section examined (T1). It was characterized by a slight increase in the number of neutrophils in the mucosal tissues lining the lateral meatus, especially in the ventral lateral meatus, the dorsomedial aspect of the proximal maxilloturbinate, and the ventral aspect of the proximal nasoturbinate in both nasal passages. It must be emphasized, however, that the severity of this focal rhinitis was minimal to mild (i.e., severity score of 1 or 2 out of 4). In addition, there were no associated histologic alterations in the surface epithelium or in the subepithelial tissues in the affected areas. This inflammation did not result in any apparent epithelial cell injury often observed with many inhaled agents. No nasal lesions were observed in mice exposed to WTC3 and killed 3 or 6 days postexposure, suggesting that any dust-induced acute inflammation quickly resolved and did not result in any persistent injury to the nasal mucosa detectable by light microscopy.

No remarkable findings were observed in the lungs of any of the mice exposed to air or to WTC3 at any time point. Because nasal lesions as described above were restricted to the proximal T1 region and were not found in the more distal T2 and T3 regions, the lack of any findings in the lung suggests that the proximal region of the nose effectively scrubbed out enough of the PM during the exposure to WTC3 to limit deposition further down the respiratory tract.

Experiment C: effect of geographical location of World Trade Center particulate matter samples on responses. Responsiveness to methacholine aerosol. Analysis of the responsiveness to Mch in both subexperiments showed that linear regression equations could be fit to the PenH AUC versus [Mch] data (Figure 5). In both subexperiments, tests for equal slopes on days 1 and 3 after exposure showed that day was not a significant factor, so a single equation was fit to the data for both days in each group. In experiment C1, the WTC8, WTCF, and SRM groups could be described with a common slope and intercept. The common slope of these three groups was significantly different from and greater than that of the WTC13 or saline C1 groups ($p < 0.0005$), indicating that WTC8, WTCF, and SRM caused hyperresponsiveness to Mch aerosol. The slope of the WTC13 group was significantly greater than that of the saline C1 group, showing that WTC13 mice were hyperresponsive compared with control mice,

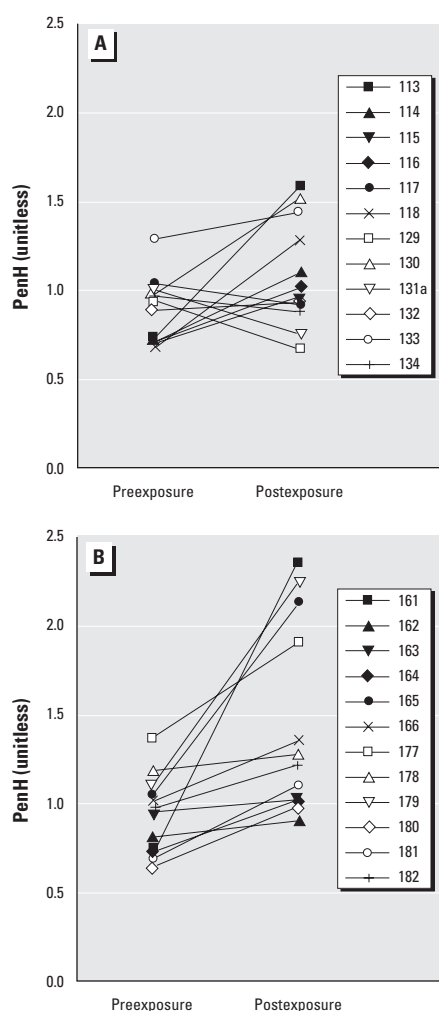


Figure 4. Experiment B: PenH values measured immediately before and after nose-only exposure to (A) air only or (B) WTC 3 PM_{2.5}. Legends refer to individual mouse numbers.

though less so than WTC8, WTCF, and SRM mice. In experiment C2, the WTC11, WTCB, WTCF, WTCC, and WTCE groups could all be described with a common slope and intercept, which was similar to that found for WTC8, WTCF, and SRM groups in experiment C1. The common slope of the four WTC groups was significantly different from and greater

than that of the saline C2 group ($p = 0.001$), indicating that WTC11, WTCB, WTCC, and WTCE also caused airway hyperresponsiveness. These results are generally consistent with those from experiment A, where the 100- μ g dose of pooled WTCX sample induced significant hyperresponsiveness to Mch aerosol compared with control PM samples and saline. All

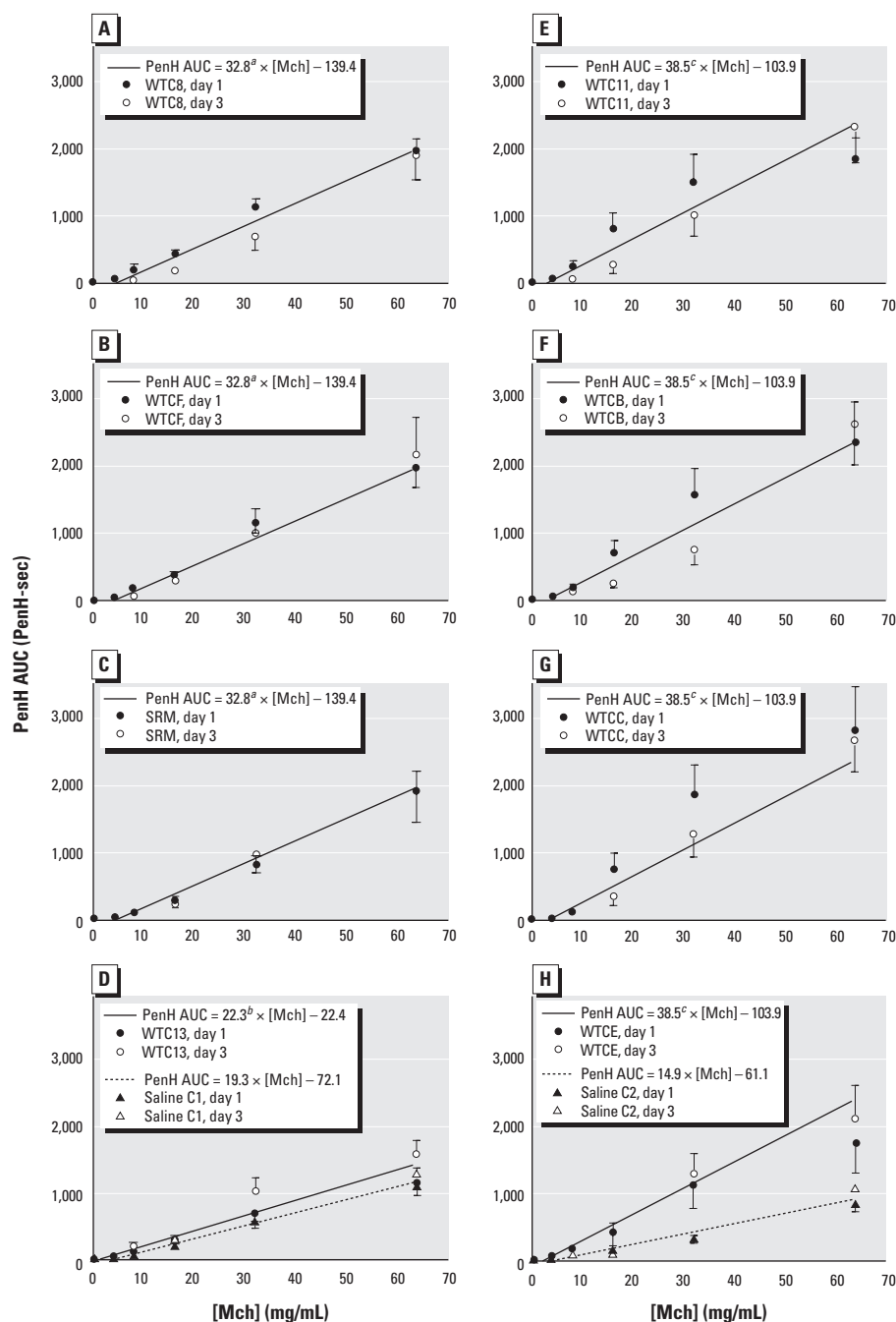


Figure 5. Experiment C: airway responsiveness to Mch aerosol 1 or 3 days after aspiration of saline vehicle, SRM 1649a, or WTC PM samples from individual collection sites ($n = 8$ per group except saline experiment C2: $n = 4$). A single regression equation was fit to the data for both days in each group.

^aIn experiment C1 (A, B, C, D), a common equation was fit to the (A) WTC8, (B) WTCF, and (C) SRM 1649a data, and the slope of the line was significantly different from and greater than the slopes of the (D) WTC13 and saline C1 lines. ^bIn experiment C1, the slope of the line for the (D) WTC13 group was significantly different from and greater than the slope for the saline C1 group. ^cIn experiment C2 (E, F, G, H), a common equation could be fit to the (E) WTC11, (F) WTCB, (G) WTCC, and (H) WTCE data, and the slope of the line was significantly different from and greater than the slope of the saline C2 line.

but one of the WTC PM samples, as well as the SRM control PM, appeared to cause similar degrees of hyperresponsiveness. However, the WTC13 sample, located just 0.1 miles southeast of Ground Zero, caused a lower degree of hyperresponsiveness.

Bronchoalveolar lavage parameters. In experiment C1, we found significant increases in numbers of neutrophils on day 1 in all PM-exposed groups compared with saline C1 mice (Figure 6). An average of 14.7×10^4 neutrophils was recovered from SRM mice (45% of total BAL cells). Significantly lower numbers of neutrophils were found in WTC13 (6.1×10^4) and WTCF (6.9×10^4) mice, while numbers of neutrophils were lower still in WTC8

mice (3.2×10^4). The neutrophilic response abated by day 3, and there were no significant differences among the five groups. Numbers of lymphocytes were significantly increased in WTC8, WTC13, WTCF, and SRM mice compared with saline C1 mice on both days ($p = 0.0001$) and increased from day 1 to day 3 ($p = 0.0001$). No biologically significant differences in eosinophil or macrophage numbers could be discerned. In experiment C2, significant increases in neutrophils and eosinophils were found in WTC11 and WTCE mice compared with saline C2 mice, and again, neutrophil numbers declined from day 1 to day 3 ($p = 0.0001$). The average number of neutrophils in these two WTC groups was comparable

to those found in the WTC13 and WTCF groups in experiment C1. These results differ substantially from those found in experiment A, where $100 \mu\text{g}$ of pooled WTCX induced only a mild neutrophilic response in the lung 1 day after aspiration (average 1.43×10^4). Some WTC individual site samples (WTCF, WTC13, WTC11, WTCE) caused about 4 times the amount of neutrophil recruitment as WTCX, whereas the others (WTC8, WTCB, WTC) caused about twice as much recruitment. It is not clear how the individual site samples could all cause more lung inflammation than the pooled WTCX sample, which was composed of the individual site samples, but cohort variability in this outbred strain could be substantial. To adequately address this question, pooled WTCX and individual site samples need to be tested together in the same experiment. In general, responsiveness to Mch aerosol and pulmonary inflammation were not well correlated. Mice in the WTC13 group had one of the largest neutrophilic and eosinophilic responses, yet had a significantly lower degree of Mch responsiveness. Mice in the WTCC group had one of the largest responses to Mch challenge, yet their neutrophil and eosinophil responses were low relative to those of the other WTC groups.

In experiment C1, we found that BAL total protein levels were significantly increased in the SRM group compared with the WTC8 group ($p = 0.05$; data not shown). In experiment C2, there were no treatment-related effects on protein levels. The results for the individual site WTC PM samples are comparable to those found with the pooled WTCX sample in experiment A, where no differences from control saline mice were found.

Lung histopathology. Although the lungs of all mice in experiment C were lavaged before fixation (they were not lavaged in experiments A or B), the pattern and the morphology of the PM-induced findings were relatively consistent among all treated groups. Focal acute bronchiolar inflammation and focal bronchiolar pigmented macrophages (presumably PM) were consistently observed in all groups of mice dosed with each of the different PM samples (Table 5). Some groups also had findings of focal free bronchiolar pigment, consistent with the pigment in macrophages. No remarkable findings were observed in the lungs of the saline control group (Figure 7A). The degree of focal acute bronchiolar inflammation was greatest in the SRM (Figure 7C), WTCF, and WTC13 (Figure 7D) groups on day 1 (average severity scores of 1.9, 2.0, and 2.1, respectively). The scores in the WTCC (Figure 7B), WTCB, WTC8, WTCF, and WTC11 groups were lower (average severity scores of 0.8, 1.1, 1.1, 1.3, and 1.3, respectively). By day 3, the focal acute bronchiolar inflammation was greatest in the SRM group (average severity score 2.1;

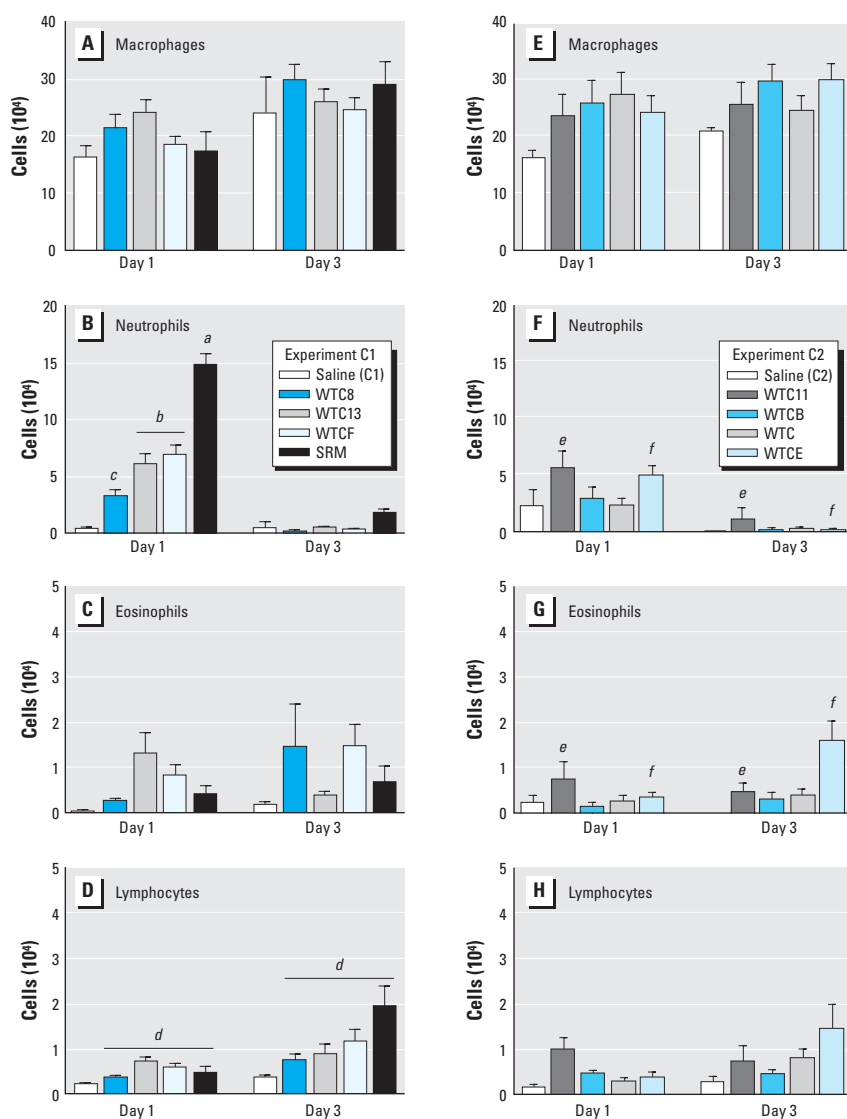


Figure 6. Experiment C: BAL cell numbers recovered 1 or 3 days after aspiration of saline vehicle, SRM 1649a, or WTC PM samples from individual collection sites ($n = 8$ per group except saline experiment C2: $n = 4$).

^aIn experiment C1 (A,B,C,D), neutrophil numbers (B) were significantly greater in SRM group than in all other groups. ^bWTC13 and WTCF significantly greater than WTC8 and saline C1 groups. ^cWTC8 significantly greater than saline C1 group. ^dLymphocyte numbers (D) significantly greater in WTC8, WTC13, WTCF, and SRM groups compared with saline C1 group. ^eIn experiment C2 (E,F,G,H), there were significantly greater numbers of neutrophils (F) and eosinophils (G) in WTC11 group compared with saline C2 group. ^fSignificantly greater numbers of neutrophils (F) and eosinophils (G) in WTCE group versus WTCB and saline C2 groups.

Figure 7E), whereas the scores were reduced in all of the WTC PM groups relative to their scores on day 1 (Figure 7F). The histopathologic scoring system is semiquantitative, and much larger numbers of mice per group would be necessary to determine statistically significant differences among groups. Nevertheless, these results show differences from those found in experiment A with the pooled WTCX sample. Oropharyngeal aspiration of 100 µg WTCX did not cause any treatment-related histopathologic findings. In contrast, all individual site samples of WTC PM induced at least minimal focal acute bronchiolar inflammation, and some samples caused slight/mild and even moderate degrees of inflammation. The findings of pulmonary inflammation in WTC PM groups by histopathologic examination are consistent with the results from the quantification of BAL cell numbers.

Discussion

We investigated the effects of exposure to samples of WTC PM_{2.5} on respiratory parameters, pulmonary inflammation, and lung injury in young adult female CD-1 mice, an outbred strain expected to have significant variability in biologic responses, in three separate experiments. A pooled sample of PM_{2.5} composed of roughly equivalent amounts of samples from seven different locations around the WTC site caused a mild degree of pulmonary inflammation (7% neutrophils in BAL fluid) and had no effect on parameters of acute lung injury at a dose of 100 µg aspirated directly into the lungs. ROFA, a toxic, positive-control, fine PM sample, caused a much higher degree of lung inflammation and lung injury at the same dose. However, mice exposed to 100 µg pooled WTC PM_{2.5} had highly significant increases in airway responsiveness to Mch aerosol challenge that were significantly greater than those for ROFA. The airway hyperresponsiveness induced by WTC PM_{2.5} implies that components of the dust can promote airflow obstruction. Mice exposed to lower doses of pooled WTC PM_{2.5} (10 and 31.6 µg) and mice exposed by nose-only inhalation did not have any biologically significant responses. We estimated the total dose deposited in the respiratory tract by inhalation exposure: 18.8 mL/min (ventilation based on weight; Costa et al. 1992) × 300 min × 10.64 mg/m³ × 0.23 (total respiratory tract deposition efficiency estimate using the rat as a substitute animal model; Freijer et al. 1999) ≈ 14 µg. This dose is considerably less than the 100-µg aspirated dose and probably accounts for the lack of pulmonary effects following nose-only inhalation exposure. Whereas mice are obligate nose breathers, humans have significant oral breathing, and significantly more PM can bypass the nasal passages (Schlesinger 1985). Studies have shown considerably less deposition efficiency

in the alveolar region of rodents compared with humans (Asgharian et al. 1995).

Mice exposed to samples of WTC PM_{2.5} from the seven individual sites around Ground Zero had greater lung inflammation (2- to 4-fold) than mice exposed to the WTC PM_{2.5} sample pooled from these sites. These findings occurred in separate experiments and would need to be confirmed by a direct comparison, but nonetheless, all groups of mice exposed to the individual site samples developed hyperresponsiveness to Mch aerosol challenge, similar to mice exposed to the pooled sample. No particular pattern of responses was found corresponding to the geographic location where the samples were taken. The one group that had lower Mch responsiveness (WTC13) was centrally located only 0.1 mile southeast of the center of Ground Zero. The responses to the WTCF sample, which was blown indoors at 120 Broadway (McGee et al. 2003), were similar to those caused by the other WTC PM samples collected outdoors. Airway neutrophils in mice exposed to individual site WTC PM_{2.5} samples diminished from 1 day to 3 days after exposure, although airway hyperresponsiveness did not diminish significantly. Further experiments are necessary to determine the persistence of pulmonary responses in mice, which may lead to insights into whether WTC PM-associated effects in people are persistent.

The results of these studies should be examined in the context of previous studies on the effects of environmentally relevant PM samples in rodents. Rats were intratracheally instilled with 2.5 mg (~8.3 mg/kg) of various emission source and urban ambient air PM

samples (Costa and Dreher 1997), a dose about twice, based on body weight, the 100 µg WTC PM_{2.5} dose in mice (~4 mg/kg). Oil fly ashes and urban ambient air PM samples (including a ROFA similar to the one used in the present study and SRM 1649a) induced strong neutrophilic responses 24 hr after exposure, whereas biochemical markers of lung injury were lower in the urban air PM samples compared with the oil fly ash samples. ROFA at this dose induced airway hyperresponsiveness in rats that persisted at least 4 days and was greater than that observed in an urban ambient air PM sample (Pritchard et al. 1996). The fact that WTC PM_{2.5} induced a significantly greater degree of airway hyperresponsiveness in mice than ROFA, which is used as a toxic positive control particle in many studies, suggests a very significant respiratory effect of a relatively high-dose exposure to WTC PM_{2.5}.

Some people were exposed acutely to high concentrations of dust in the WTC disaster and subsequently developed wheezing or symptoms of sensory irritation such as cough and irritation of the nose and throat. These effects resemble, in some respects, the reactive airways dysfunction syndrome (RADS). RADS can occur after single or multiple high-level occupational exposures to an irritating vapor, fume, or smoke (Gautrin et al. 1999). Effects can occur within minutes or hours after exposure and include cough, dyspnea, and wheezing. Clinical tests can show airways obstruction, persistent airway hyperresponsiveness, and inflammation. The recovery process appears to be dependent on the initial degree of injury. A recent study has

Table 5. Experiment C: summary of treatment-related histopathologic findings in mice 1 or 3 days after oropharyngeal aspiration of particulate matter samples.^a

Treatment group	Sub-experiment	Day	Bronchiole inflammation (acute, focal)		Bronchiole pigmented macrophage (focal)		Bronchiole pigment, (free, focal)	
			Incidence	Severity	Incidence	Severity	Incidence	Severity
WTC13	C1	1	8/8	2.1	8/8	2.0	4/8	0.6
WTCE	C2	1	8/8	2.0	8/8	1.9	2/8	0.3
SRM 1649a	C1	1	8/8	1.9	7/8	2.0	7/8	0.9
WTC11	C2	1	8/8	1.3	7/8	0.9	0/8	0.0
WTCF	C1	1	8/8	1.3	6/8	0.8	0/8	0.0
WTC8	C1	1	6/8	1.1	6/8	0.8	0/8	0.0
WTCB	C2	1	6/8	1.1	6/8	0.8	0/8	0.0
WTCC	C2	1	6/8	0.8	4/8	0.5	0/8	0.0
Saline	C1	1	1/8	0.1	0/8	0.0	0/8	0.0
SRM 1649a	C1	3	8/8	2.1	8/8	2.0	0/8	0.0
WTC11	C2	3	6/8	1.1	2/8	0.3	0/8	0.0
WTCE	C2	3	6/8	0.8	6/8	0.8	0/8	0.0
WTC8	C1	3	4/8	0.8	1/8	0.1	0/8	0.0
WTC13	C1	3	4/8	0.6	3/8	0.4	0/8	0.0
WTCB	C2	3	3/8	0.4	2/8	0.3	0/8	0.0
WTCF	C1	3	3/8	0.4	1/8	0.1	0/8	0.0
WTCC	C2	3	2/8	0.3	1/8	0.1	0/8	0.0
Saline	C1	3	0/7	0.0	0/7	0.0	0/7	0.0

^aSaline-treated control mice in subexperiment C2 were not examined. Incidence denotes number of mice in group with finding/total number of mice examined. Average severity score for the group is shown based on the following scoring system: 0 = not present, 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately severe, 5 = severe/high. Groups are arranged in descending order of severity within each postexposure day, first by severity of focal acute bronchiolar inflammation, and then by severity of focal bronchiolar pigmented macrophages.

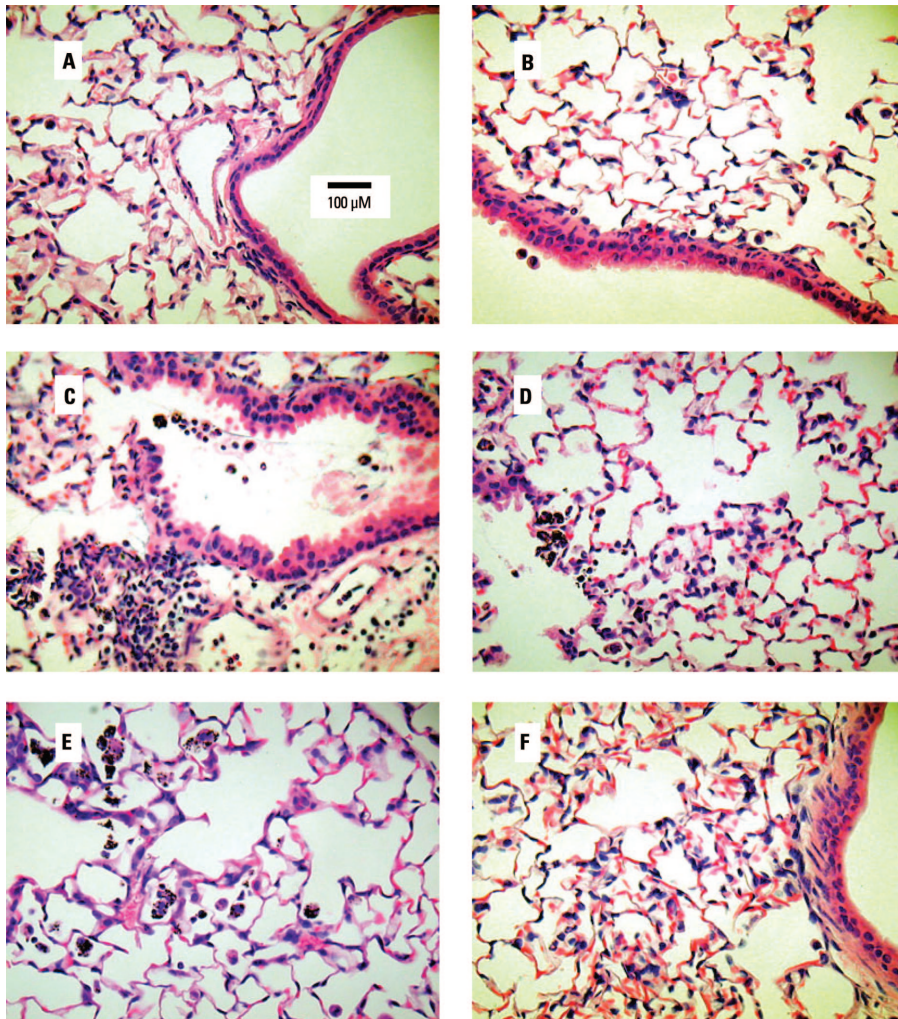


Figure 7. Experiment C: representative lung micrographs 1 or 3 days after aspiration of 100 µg PM sample or saline vehicle (all panels: bar = 100 µm). (A) Saline-exposed control mouse, day 1, with no remarkable findings. (B) Mouse exposed to WTCC, day 1, showing minimal degree of focal acute bronchiolar inflammation (FABI). (C) Mouse exposed to SRM 1649a, day 1, with moderate degree of FABI. (D) Mouse exposed to WTC13, day 1, with moderate degree of FABI. (E) Mouse exposed to SRM, day 3, with slight/mild degree of FABI. (F) Mouse exposed to WTC13, day 3, with minimal degree of FABI.

Table 6. Estimation of WTC PM_{2.5} concentrations required to produce human doses equivalent to mouse doses used in study.

Assumptions, intermediate steps, and final solution	Dose deposited in mouse tracheobronchial and pulmonary regions (µg)		
	10	31.6	100
Mouse alveolar pulmonary surface area (m ²) ^a	0.103	0.103	0.103
Mouse dose per tracheobronchial (TB) or pulmonary surface area (µg/m ²) ^b	97	307	973
Human TB surface area (m ²) ^c	0.415	0.415	0.415
Total human TB dose equivalent to mouse TB dose (µg/m ² × m ²) ^d	40	128	404
Deposition fraction in human TB region ^e	0.066	0.066	0.066
Total inhaled dose in micrograms (total human TB dose/TB deposition fraction)	612	1,932	6,115
Quantity of air breathed in 8-hr work shift at ventilation of 30 L/min (m ³) ^f	14.4	14.4	14.4
WTC PM _{2.5} concentrations required to produce human doses equivalent to mouse doses used in study (µg/m ³)	42	134	425

^aFrom Jones and Longworth (1992)—calculated allometric equation: mammalian alveolar pulmonary surface area in m² = 3.36 × (weight, kg)^{0.935}, where weight = 0.024 kg (average mouse weight in all studies). TB surface area is minimal compared with alveolar surface area and can be ignored in calculation (Overton et al. 2001). ^bAssumes dose bypasses nose and spreads out evenly over TB and pulmonary alveolar regions. ^cBased on 5'10" male 30 years of age with functional residual capacity (FRC) of 3,300 mL (Overton et al. 2001). ^dCalculations assume no clearance of particles after deposition in human respiratory tract during an 8-hr work shift. ^eCalculations made with Multiple Path Particle Deposition model, version 1.11 (Freijer et al. 1999), which assume human Yeh-Schum 5-lobe model, FRC = 3,300 mL (appropriate for 5'10" male 30 years of age), upper respiratory tract volume = 50 mL, density of particles = 1 g/cc, diameter = 1 µm MMAD, inhalability adjustment on, $\alpha_g = 2.5$, breathing frequency = 15 min⁻¹, tidal volume = 2,000 mL, minute volume = 30 L/min, inspiratory:expiratory ratio = 1, and oronasal mouth breathing. ^fEstimate of minute ventilation during moderate to heavy sustained work (Åstrand and Rodahl 1986).

shown that firefighters at the WTC site who had high exposure (defined as present at the scene of the WTC collapse) had persistent respiratory symptoms such as cough, wheezing, bronchial hyperresponsiveness, nasal congestion, and gastroesophageal reflux disease (Prezant et al. 2002). Although it is too soon to determine whether these effects will prove to be persistent, resulting in RADS, the possibility was specifically noted by the authors. The effects of a high-dose exposure to WTC PM_{2.5} in mice (100 µg) appear to mimic some of these responses, especially the significant increase in airway hyperresponsiveness to Mch, although pulmonary inflammation was not as robust as one might expect in a realistic animal model of RADS. The persistence of WTC PM-induced airway hyperresponsiveness in mice and its similarity to RADS remain to be determined.

Close examination of the data suggested that individual mice within the outbred CD-1 strain vary in sensitivity to the effects of WTC PM_{2.5} (Figure 4). Some people may also have particular susceptibility to the hazards posed by exposure to WTC PM_{2.5}. Asthmatics may be hyperresponsive to nonspecific irritants such as cold dry air (Anderson and Daviskas 2000) or cigarette smoke (Bonham et al. 2001). This subpopulation is likely to be at high risk for development of dust-induced airways obstruction (Donaldson et al. 2000; Nel et al. 2001; Peden 2001). Aqueous solutions of WTC PM_{2.5} are alkaline (pH 8.88–10.00; McGee et al. 2003). Although few studies have been published regarding the effects of alkaline aerosols on pulmonary function in asthma, one study reported that inhalation of high concentrations of an alkaline aerosol (pH 9.8–10.3) had no significant effect on irritant symptoms or specific airways resistance in mild asthmatic patients (Eschenbacher 1991). This aerosol was composed of a simple mixture of sodium carbonate, sodium bicarbonate, and sodium hydroxide. The chemical composition of WTC PM_{2.5} is much more complex (McGee et al. 2003), and interactions of numerous chemical species may be associated with development of airway hyperresponsiveness.

How does the dose of 100 µg WTC PM_{2.5}, which caused bronchiolar inflammation and airway hyperresponsiveness in mice, relate to exposure of people at the WTC site? Because inflammation was observed mainly in the airways, and airway hyperresponsiveness is mainly due to dysfunction of airway smooth muscle (Fredberg 2000), the dose metric that probably is most relevant is dose per surface area of the tracheobronchial (TB) region of the respiratory tract. The TB region is defined as the region from the trachea down to the terminal bronchioles (Overton et al. 2001). The concentrations of WTC PM_{2.5} in air that could produce doses per TB surface area in humans equivalent to those in mice may be estimated using the

assumptions detailed in Table 6. The calculations show that concentrations of 42, 134, and 425 $\mu\text{g}/\text{m}^3$ WTC $\text{PM}_{2.5}$ inhaled over an 8-hr period would produce human doses per TB surface area equivalent to the mouse-aspirated doses of 10, 31.6, and 100 μg , respectively. Obviously, many factors may cause wide variation in the calculation of dose, and extrapolation of responses from the mouse to the human involves another dimension of uncertainty. Nevertheless, it is reasonable to conclude that a healthy worker breathing heavily in the dusty environment generated after the collapse of the towers could have inhaled $\text{PM}_{2.5}$ equivalent to the 100- μg dose in the mouse. Therefore, inhalation of very high concentrations of WTC $\text{PM}_{2.5}$ ($\sim 425 \mu\text{g}/\text{m}^3$ or greater) over a short period (8 hr) may contribute to development of pulmonary inflammation, airway hyperresponsiveness, and manifestations of sensory irritation such as cough. Individuals especially sensitive to inhalation of dusts, such as asthmatics, may experience these effects at lower doses of inhaled WTC $\text{PM}_{2.5}$. However, most healthy people would not be expected to respond to moderately high WTC $\text{PM}_{2.5}$ levels (130 $\mu\text{g}/\text{m}^3$ or less for 8 hr) with any adverse respiratory responses. The effects of chronic or repeated exposures to lower levels of WTC $\text{PM}_{2.5}$ or the persistence of any respiratory effects are unknown and were not components of this study.

It is important to consider several limitations of these studies. First, most of the experiments used oropharyngeal aspiration to deliver PM samples to the respiratory tract rather than more physiologically relevant inhalation exposure methodology. We believe that using aspiration, as described in the experimental design, had many advantages and was necessary in these circumstances. However, future studies may be needed to more closely examine bronchoconstriction and sensory irritation during inhalation exposure to WTC PM in mice and in guinea pigs, a species known to be especially sensitive to sensory irritants (Costa and Schelegle 1999). Second, these studies evaluated only short-term toxicologic effects after acute exposure, and no direct information is provided on the long-term effects of acute or chronic exposures to WTC $\text{PM}_{2.5}$. Third, these studies examined only the fine fraction of PM, whereas the toxicity of coarse and larger size PM fractions was not investigated. However, it is important to remember that the size-fractionation techniques employed in this report are not absolute, and significant quantities of $\text{PM} > 2.5 \mu\text{m}$ are present in the samples. Furthermore, analysis of WTC $\text{PM}_{2.5}$ and $\text{PM} < 53 \mu\text{m}$ showed that they were similar in chemical composition (McGee et al. 2003), suggesting that only differences in respiratory tract deposition patterns of fine and coarse WTC PM would affect biologic responses.

Coarse PM may be more relevant for upper airways sensory irritation because larger particles will deposit mainly in the upper airways where sensory innervations are predominant (Costa and Schelegle 1999). However, chronic effects of fine PM may be greater than coarse PM, as it can be inhaled more deeply and deposit in peripheral regions of the lungs and is more slowly cleared. Coarse PM is much less inhalable in small rodents than in humans, and less is deposited in the respiratory tract (Menache et al. 1995). Consequently, interpretation of results derived from exposure of mice to coarse PM is problematic, and small rodents are probably not the ideal species for the study of the effects of coarse PM. Nevertheless, because upper airways irritant responses seem to be so important in people exposed to WTC-derived dust, future studies should examine the specific toxicity of coarse WTC PM on respiratory responses in appropriate animal models.

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