UC Irvine

Title

Effects of sulfate aerosols in combination with ozone on elimination of tracer particles inhaled by rats

Permalink

https://escholarship.org/uc/item/4nd2r8g3

Journal

ISSN

Authors

Phalen, Robert F
Kenoyer, Judson L
Crocker, T Timothy
et al.

Publication Date

1980-07-01

DOI

Copyright Information

Peer reviewed

EFFECTS OF SULFATE AEROSOLS IN COMBINATION WITH OZONE ON ELIMINATION OF TRACER PARTICLES INHALED BY RATS

Robert F. Phalen, Judson L. Kenoyer,
T. Timothy Crocker, Thomas R. McClure

Air Pollution Health Effects Laboratory,
Department of Community and Environmental Medicine,
University of California, Irvine, California

Several inhaled atmospheres were tested for effects on the rat respiratory defense system. Materials studied included ozone and aerosols of ammonium sulfate, ferric sulfate, and sulfuric acid; relative humidity was also a controlled experimental variable. Each sulfate was studied alone as a submicrometer aerosol at a concentration of 3.5 mg/m³ in air and combined with ozone at 0.8 ppm. Results were compared with those for sham-exposed animals and for rats exposed to ozone alone.

Air pollutant exposures, inside stainless steel chambers, were one time only for 4 h. The end points for evaluation of effects were measurements of early and late rates of clearance of radiolabeled insoluble tracer particles. Tracer particles were inhaled before air pollutant exposures and particle clearance was followed for about 2 wk.

Ozone alone slowed the early (0–50 h after exposure) particle clearance and stimulated clearance during the later phase (2–17 d). High humidity usually amplified these effects of ozone as well as many of the other atmospheres studied. Sulfate aerosols alone tended to produce relatively small effects on early or late clearance. Combinations of ozone and aerosols resulted in effects that were similar to those of ozone alone. The data do not support the hypotheses that sulfate aerosols synergize with ozone in altering respiratory tract clearance, sulfuric acid being a probable exception. These data alone cannot be used to predict the overall health effects of the materials studied.

INTRODUCTION

One of the first phases of inquiry about a possibly hazardous environmental air pollutant is to learn how acutely toxic it is when inhaled. This may involve exposing animals to a high airborne concen-

tration for a period of time that will resemble a natural episodic exposure. In modern cities sulfate aerosols are often found in combination with a variety of other airborne pollutants, including ozone, a known toxic gaseous photochemical air pollutant. This animal research was performed to explore the toxicities of selected sulfate salts alone and in the presence of ozone. The implications for public health will lead to questions such as whether air pollution alerts should be called on the basis of combined ozone and sulfate levels, and how much expense is justified for control of sulfate pollutants in relation to control of other pollutants. These and similar questions can be resolved only after sufficient health-related data are acquired by research on both humans and laboratory animals. Exposures of laboratory animals to high concentrations of previously unstudied materials or mixtures often precede human studies, for obvious ethical reasons.

The objectives of this research were to obtain evidence for or against synergism of ozone and sulfate aerosols, and to collect data on the toxicity of several sulfate aerosols alone at mass concentrations exceeding the ambient concentration by about 50 times or more. The results were expected to lead to selection of one or more sulfates or sulfate-ozone combinations for future tests (1) in animals under long-term exposure for analysis of disease-producing potential and (2) in human subjects to test whether the animal data revealed a risk to humans.

Levels of inhaled pollutants that cause significant effects are different in different species. Much of this variation is explained by differences in the amount of air breathed per unit of body weight per unit of time (greater in smaller animals) and by possible differences in the filtering efficiency of the nose. Our studies have involved the rat, whose respiratory tract characteristics are such that we do not necessarily expect the responses to quantitatively resemble those of humans.

Levels of ozone and sulfate in urban air have been repeatedly measured and reported in the literature (Appel et al., 1978; Gloria et al., 1974; Kinosian and Duckworth, 1973; National Academy of Sciences, 1977; U.S. Environmental Protection Agency, 1976). Perhaps the most consistently high levels of ozone in the United States occur in the west, specifically in the Los Angeles air basin, where maximum hourly average concentrations have exceeded 0.5 ppm (National Academy of Sciences, 1977). In the same geographic area sulfate particles of mass median aerodynamic diameter (MMAD) near 0.3–0.4 μm have been measured in 2-h average samples at concentrations up to 70 μg per cubic meter of air, the most probable forms being ammonium salts (Appel et al., 1978).

Mammals depend on several respiratory tract defense mechanisms, including mechanical clearance, for maintenance of health in the face of a variety of inhaled materials. To quantitate the mechanical removal rates of deposited material in the upper and lower respiratory tract, a study design was used that involved the deposition of small amounts of radiolabeled,

chemically inert tracer particles prior to exposure to the air pollutant atmospheres. Data were obtained for each rat by (1) collection of excreta during the first 2 d after exposure and (2) *in vivo* radiation measurements over the upper body during the first 17 d after exposure. These data were analyzed for each rat to provide early and late clearance half-times for the tracer particles. Group sizes of about 30 were used for statistical comparison of pollutant- and sham-exposed animals. Such clearance data can be used in models for estimating the residence times of inhaled materials deposited by inhalation in the respiratory system. Also, interference with particle clearance from the lung is presumed to be an important health-related event that might predispose the animal to developing lung disease from any of several causative agents.

The effects of ozone on the respiratory system, including production of focal lesions and increased susceptibility to infection, have been well documented (National Academy of Sciences, 1977) and will not be described in detail here. Less is known about the effects of sulfates on the respiratory tract, but studies have shown that various forms are capable of inducing bronchial constriction and possibly a secondary alveolitis in guinea pigs (Amdur et al., 1978; Cavender et al., 1977a). Rats appear to be less susceptible than guinea pigs, and pulmonary function studies on humans, sheep, and dogs tend to show little or no effect of inhaled sulfate particles alone (Hackney et al., 1978; Sackner et al., 1978).

When combined, ozone and sulfate particles have been found by some investigators to have effects primarily attributable to ozone alone (Hackney et al., 1978; Juhos et al., 1978). Others have reported that production of mucus glycoprotein in tracheal explants is stimulated by ozone and H_2SO_4 in a greater than additive fashion (Last and Cross, 1978). In one study of mouse infectivity the authors concluded that increased lethality occurred only when ozone immediately preceded H_2SO_4, but not vice versa (Gardner et al., 1977). One must conclude that more information is needed to evaluate the effects of ozone and sulfate aerosols in combination.

Tests in the laboratory to evaluate respiratory tract clearance mechanisms have been used in toxicological studies for some years. Several materials are known to alter particle deposition or clearance, or killing or inactivation of microorganisms. Cigarette smoke, a toxic mixture and frequent coinsult in human inhalation exposure situations, has been relatively well studied. Albert et al. (1969, 1970, 1974) found effects in humans and donkeys that depend on dose level and duration of exposure. Low single doses or a few repeated exposures to smoke were associated with accleration of clearance rates in the tracheobronchial tree in both species. Heavier doses and long-term repeated exposures were associated with sporadic clearance, intervals of clearance stasis, and even apparent retrograde movement of deposited particles (again in both species). Cigarette smoke exposures have also been shown to increase the survival of

inhaled viable bacteria in hamsters; excess deaths due to bacterial infections were seen in animals exposed to cigarette smoke for 2 h at a concentration of "3% v/v" (Henry et al., 1970).

Preexisting influenza infection has been shown to impair both upper and lower respiratory tract clearance. Studies by Green (1965) with P-8 virus-infected mice that were exposed to viable staphylococcus bacteria showed that infected animals did not effectively kill the bacteria. Similarly, Creasia et al. (1973) found that P-8 virus-infected mice had drastically impaired clearance of radioactive "insoluble" particles. In humans, Camner (1973) found that influenza infection could impair tracheobronchial clearance, even up to 1 mo after disappearance of the familiar clinical symptoms.

Goldstein et al. (1971) reported work in which mice were challenged with radiolabeled viable staphylococcus both before and after exposures to relatively low levels of ozone and NO_2. Prior exposure to ozone (0.6–2 ppm, 17 h) or ozone plus NO_2 (0.1–0.2 and 1.5–4.2 ppm, 17 h) led to (1) decreased overall deposition of bacteria and (2) impaired killing of deposited bacteria. In the same series of studies, exposures to ozone plus NO_2 (0.4 and 4–6.8 ppm, 4 h) after inhalation of bacteria led to increased survival of the bacteria. Ozone alone at 2 ppm (4 h) led to increased survival and increased clearance of the inhaled bacteria.

Sulfur dioxide (1 ppm, 7 h and 5–25 d) was shown by Ferin and Leach (1973) to diminish the clearance of inert particles in both deep lung and tracheobronchial tree in the rat. A similar effect on bronchial clearance in donkeys was seen after brief exposure (300 ppm SO_2, 30 min) by Spiegelman et al. (1968).

Sulfuric acid mist exposures by Fairchild et al. (1975) at 3 mg/m^3 (1.8 μm count median diameter) in guinea pigs caused increased total deposition of inhaled streptococcus. Presumably, bronchial constriction or altered breathing patterns were mechanisms. The effect of sulfuric acid aerosols on tracheobronchial clearance of tracer particles in the donkey was studied by Schlesinger et al. Single, 1-h exposures to acid particles near 0.5 μm aerodynamic median diameter at concentrations in air up to 2 mg/m^3 were associated with slowing of clearance in 3 of 4 animals (Schlesinger et al., 1978). Repeated exposures to acid particles at 0.1 mg/m^3 for 1 h/d, 5 d/wk, for 6 mo were associated with erratic, usually slowed, clearance (Schlesinger et al., 1979).

The usefulness of particle deposition and clearance studies as tests of toxic effect has been reviewed (Phalen et al., 1975), as has the comparative use of various mammalian species in such studies (Phalen et al., 1977). Clearance kinetics of inhaled particles have been reviewed by Morrow (1977).

METHODS

Since the toxicity of ozone and sulfates combined was to be studied, a decision was made to use a high, but not totally unrealistic, ozone level of

0.8 ppm, and the maximum concentration of sulfate that could be maintained given a particle diameter near 0.5 μm (MMAD). The maximum aerosol concentration was thus limited by the agglomeration rates of small particles, and the concentration selected was 3-4 mg per cubic meter of air. With such a level, animal responses should be exaggerated over those expected in humans under probable brief environmental exposure.

Since ammonia is also present in urban air, ammonium sulfate was selected for study. Interest in the potential effects of catalyst-equipped automobiles led to the selection of sulfuric acid mist as a material for study. Also, since published data indicate that ferric sulfate has a greater irritant potency than ammonium sulfate (Amdur et al., 1978), ferric sulfate was selected.

Studies of the effects of SO_2 and salt particles on bronchial constriction in guinea pigs has shown that elevated relative humidity was associated with a greater response (McJilton et al., 1976). Therefore all our exposures were performed at both low (30-40%) and high (greater than 80%) relative humidity.

Thus, 16 atmospheres were examined: clean air, ozone alone, ammonium sulfate alone, ferric sulfate alone, sulfuric acid mist alone, each sulfate combined with ozone, and each of these 8 atmospheres at low and high relative humidity. To protect against unreproducible responses by a unique batch of animals each animal experiment was repeated; a total of 32 groups of rats were evaluated. These studies were part of a larger effort in which a variety of biological end points were used. The data reported here are limited to particle clearance studies.

Animals

Specific-pathogen-free Sprague-Dawley rats were procured from Hilltop Lab Animals, Inc. (Chatsworth, Calif.). Male rats weighing about 200 g were brought directly to the laboratory in specially filtered shipping boxes. Newly arrived animals were housed in a laminar-air barrier caging system in stainless steel hanging cages for about 1 wk before being randomly placed in an experimental exposure protocol.

Besides the resident pure-air system to protect the animals' health, the caging was cleaned three times a week and the room disinfected once a week, with a rotational change to sterilized caging once every 3 wk. Conventional bedding in the trays beneath the wire cage bottoms was replaced by rock salt, which mixes with excreted urine to create a hypertonic environment unfavorable to bacterial growth. About 500 rats housed in this system have been examined histopathologically over the past 3 yr. The incidence of even mild morphologically recognizable lung involvement is less than 5% when the animals are held for less than 2 wk on the laminar air isolator system. Widespread interstitial inflammatory disease has never been seen in our laboratory.

Exposures

Rats in each purchased batch were exposed by inhalation to radio-active tracer particles and then randomly divided into experimental and control groups. One hour after a 20-min nose-only exposure to tracer particles, animals were placed in individual compartments in open-mesh stainless steel exposure cages. Cages were placed on one level only of a University of Rochester type 1 m^3 stainless steel chamber for a 4-h exposure to either purified or intentionally polluted air.

The tracer particles, which we tagged with tightly bound [51]Cr (Hinrichs et al., 1978), were monodisperse polystyrene latex (PSL) spheres (Dow Chemical Co., Indianapolis, Ind.) having geometric diameters near 1.4 μm as determined by electron microscopy (Fig. 1). Aerosols were produced from an aqueous suspension of 0.1% solids (by volume), using a Lovelace-type laboratory compressed-air nebulizer (Aries Inc., Davis, Calif.). The MMAD of the aerosol particles, as determined with a multistage laboratory impactor (Aries Inc., Mercer et al., 1970) was about 1.6 μm.

Tracer particles were aerosolized, dried, brought to charge equilibrium, and passed into a nose-only exposure chamber similar to that described by Phalen et al. (1975) and Raabe et al. (1973). The individual tubes for holding rats in the device were made of perforated metal and were thin-walled to reduce thermal stress due to body heat. The apparatus and procedures for use have been described (Phalen et al., 1975).

The average amount of tracer material deposited per rat was less than 0.1 μCi, which is contained in less than 1 μg of particles. After the deposition of tracer particles was completed, the rats' noses were washed with water to remove radioactive particles. The animals were then placed in individual plastic counting tubes and inserted in a collimated counting apparatus such that radiation emitted from the respiratory tract was favored for detection over that from the stomach and intestines. Upper- and lower-level pulse height discrimination was used to detect the ^{51}Cr gamma rays (320 keV). All rats that underwent deposition of PSL particles were counted twice for 100 s in this apparatus before they were placed in the pollutant exposure chambers. When the 4-h clean air or pollutant exposure was completed, the animals were periodically put into individual plastic counting tubes and the amount of radioactivity in the respiratory tract was determined at five additional predetermined times for up to 17 d. Fecal samples were collected from each rat 11 times during the first 48 h after tracer particle deposition. Coprophagy was minimized by the use of $\frac{1}{2}$-in mesh wire cage bottoms and by the frequent fecal collections.

Clearance curves were determined for each animal and half-times obtained from least-squares fits for short- and long-term clearance data. Group mean values for pollutant-exposed and sham-exposed (control) groups were calculated. Half-times for experimental groups were subtracted
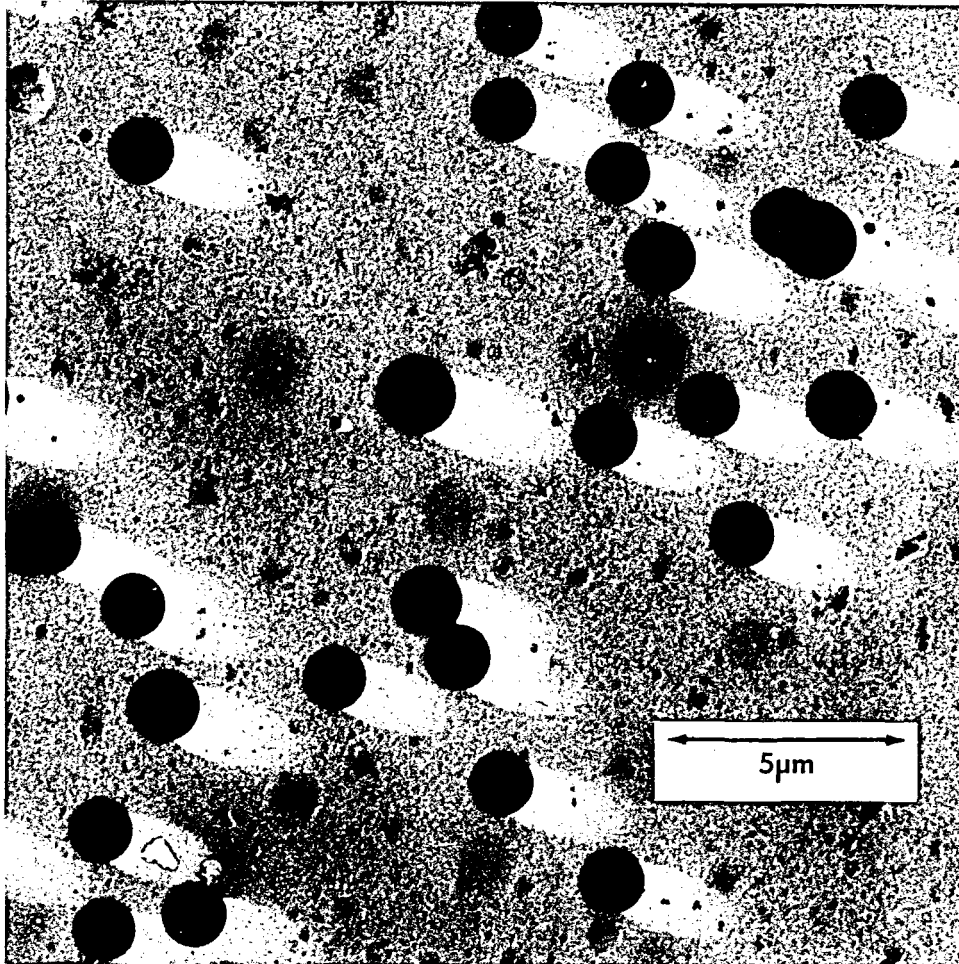
FIGURE 1. Electron micrograph of radiolabeled monodisperse PSL particles. Reaction chemistry debris and surfactant in the aerosolized suspension formed the smaller particles. More than 99% of the radioactivity is bound to the monodisperse particles.

from those for control groups and the differences tested for significance at the 90% level, using a two-tailed t-test.

Purified air supplied to the exposure chambers had been passed successively through a coarse particulate filter, a humidifier, a heater, and a high-efficiency particulate (HEPA) filter. Both temperature and humidity were controlled in this system. Air was supplied to the chambers at about 0.1–0.3 m^3/min. Ammonia levels, due to the presence of rats, were measured as about 0.25 ppm or less under these conditions of exposure.

Stable, controllable salt aerosols with MMAD between 0.4 and 0.6 μm and sulfuric acid aerosols with MMAD of 1.0 μm, at mass concentrations

up to 3 or 4 mg/m^3 in air, were generated with compressed-air nebulizers loaded with aqueous sulfate solutions. A Collison-type three-jet nebulizer (May, 1973), followed by a ^{85}Kr charge neutralizer and air-dilution drier, was used for ammonium sulfate and ferric sulfate particle generation (Sierra Instruments Inc., St. Paul, Minn., model 7330). Under low-humidity (30–40%) chamber conditions these aerosols were dry and were sized by electron microscopy. Ferric sulfate an ammonium sulfate aerosols were collected on electron microscope grids, using an electrostatic precipitator (Morrow and Mercer, 1964) (Aries, Inc.). The size distribution, count median diameter, mass median diameter, and geometric standard deviation were then determined by analysis of photographs with a Zeiss TGZ-3 particle size analyzer. At high humidity (greater than 80%) the aerosols were wet and the multistage laboratory impactor (Mercer et al., 1970) was used. Our techniques for generation and characterization of these aerosols have been published (Ho et al., 1980).

Sulfuric acid aerosols were generated from solution by an all-glass compressed-air nebulizer nearly identical to that described by Cavender et al. (1977b). Sizing was performed by determining the titratable acidity on the multistage impactor collection plates and backup filter and fitting the data to a lognormal distribution function.

Airborne mass concentrations were determined by putting two fiberglass filters in series inside the chamber and sampling at constant flow rates for up to 1 h. The first filter captured the aerosol and the second filter gave the change of filter weight due to humidity and allowed the efficiency of the primary sample filter to be verified. In the case of sulfuric acid, the fiberglass filters were pretreated to remove any contaminant alkali, and titration of the deposit was used to determine the airborne mass concentrations.

Ozone, produced by passing medical grade oxygen through an electrical ozone generator (Sander Ozonizer, type III, Osterberg, West Germany), was introduced into a chamber run at constant flow rate and slight negative pressure. A Dasibi (Dasibi Environmental Corp., Glendale, Calif.) ultraviolet monitor was used to determine the ozone levels. The instrument was calibrated before and after each exposure with a factory calibrator.

All samples for aerosol and gas characterization were acquired from the center of the breathing zone of the animals. Sampling lines were large-bore stainless steel for aerosol with Teflon for ozone.

RESULTS

Atmospheres

Concentrations and other characteristics of the atmospheres were remarkably stable from one exposure to another. Average data for all runs

with standard deviations were: ozone, 0.79 ± 0.02 ppm; aerosol concentrations, 3.6 ± 0.4 mg/m^3; low relative humidity, $39 \pm 3\%$; high humidity, $85 \pm 4\%$; MMAD of salt aerosols, 0.4 ± 0.1 μm; and MMAD of sulfuric acid aerosols, 1.0 ± 0.2 μm. The aerosols had average estimated geometric standard deviations of 1.9–2.3 from impactor data. Electron microscopy indicated geometric standard deviations of 1.6–1.7.

Clearance Measurements

The low relative humidity sham-exposed animals were selected as primary controls to examine the effect of high relative humidity as a potential cotoxin. A total of 12 groups of 10–15 rats each were exposed to low-humidity clean air. The clearance data for these animals are given in Table 1. Some animals were excluded from the data analysis because they did not consume food or water at a sufficient rate to remove tracer particles from the gastrointestinal tract. When this occurred, in about 5% of the rats, clearance half-time values could not be obtained.

As seen in Table 1, the effect of high humidity on clearance is interesting. The short-term clearance half-time was longer in the high-humidity group by 0.9 h; the long-term clearance half-time was diminished by high relative humidity by about 90 h (significant at $p = 0.1$).

As shown in Table 2, ozone alone at 0.8 ppm and low humidity statistically significantly slowed early clearance and accelerated late clearance. These effects were even greater at high humidity, the effects of humidity being roughly additive to those of ozone.

Ammonium sulfate at high or low humidity did not have any significant effects on early or late clearance compared to that in low-humidity clean-air controls.

Ferric sulfate gave one statistically significant positive result: at low relative humidity it appeared to inhibit late clearance. This result will be discussed in the following section.

Sulfuric acid alone at low humidity was associated with a significant deceleration of late clearance.

The clearance data for aerosols combined with ozone are very similar to those for ozone alone. In no case is there a statistically significant

TABLE 1. Clearance Data for Rats Exposed to Low- and High-Humidity Clean Air

Humidity	Number of animals		Clearance half-time (h)[a]	
	Short-term	Long-term	Short-term	Long-term
Low	124	123	11.17 ± 2.29	465 ± 328
High	98	96	12.06 ± 2.31	376 ± 250
Difference $\pm$ SE			0.89 ± 0.33	-89 ± 42

[a]Mean $\pm$ SD.

TABLE 2. Particle Clearance Responses of Rats Exposed for 4 h to Ozone and Sulfate Aerosols (3.5 mg/m³): Group Mean Differences from Low-Humidity Sham-exposed Animals

Treatment	Change in clearance half-time (h)[a]	
	Short-term	Long-term
Low relative humidity		
Sham	0 ± 0.3 (124)[b]	0 ± 42 (123)
0.8 ppm O_3[d]	0.9 ± 0.5^c (39)	-152 ± 66^c (40)
$(NH_4)_2SO_4$[d]	0.1 ± 0.6 (29)	-14 ± 38 (29)
$Fe_2(SO_4)_3$[d]	-0.3 ± 0.7 (28)	113 ± 53^c (30)
H_2SO_4[d]	0.5 ± 0.7 (30)	79 ± 37^c (30)
$(NH_4)_2SO_4$ + 0.8 ppm O_3	1.5 ± 0.6^c (33)	-104 ± 53^c (33)
$Fe_2(SO_4)_3$ + 0.8 ppm O_3	1.0 ± 0.6 (30)	-107 ± 95 (30)
$H_2SO_4 \pm 0.8$ ppm O_3	0.7 ± 0.5 (40)	-208 ± 79^c (40)
High relative humidity		
Sham	0.9 ± 0.3^c (98)	-89 ± 42^c (96)
0.8 ppm O_3	1.7 ± 0.8^c (38)	-174 ± 85^c (40)
$(NH_4)_2SO_4$	0.6 ± 0.8 (29)	38 ± 80 (30)
$Fe_2(SO_4)_3$	0.4 ± 0.8 (29)	-78 ± 79 (30)
H_2SO_4	1.3 ± 0.8 (29)	-69 ± 79 (30)
$(NH_4)_2SO_4$ + 0.8 ppm O_3	0.9 ± 0.7 (39)	-153 ± 107 (30)
$Fe_2(SO_4)_3$ + 0.8 ppm O_3	0.8 ± 0.9 (29)	-133 ± 92 (29)
H_2SO_4 + 0.8 ppm O_3	2.6 ± 0.8^c (40)	-158 ± 99 (27)

[a]Mean ± SE. Positive values represent slowing and negative values acceleration of clearance.
[b]Number of rats in each group is shown in parentheses.
[c]Significantly different from low-humidity sham-exposed animals ($p < 0.1$, two-tailed t-test).
[d]Form inhaled is not usually the pure undissociated sulfate.

difference between ozone alone and ozone with an aerosol at the same humidity. Further, in the majority of cases clearance patterns with sulfate particles and ozone both present lie between those for sham-expoed groups and groups exposed to ozone only. The atmosphere with the greatest effect on short-term clearance was sulfuric acid mist with ozone at high humidity.

DISCUSSION

Ozone alone retarded early clearance and stimulated late clearance. These effects were not entirely unexpected. The gas is known to cause extensive epithelial damage and destruction of cilia even after brief exposures to 1 ppm or less (National Academy of Sciences, 1977), which should tend to reduce the rate of early clearance. Our data on con-

sumption of water and volume of fecal output after exposure to ozone show that both drinking and defecation volumes can be decreased during the first few hours after exposure. This effect, when present, could add to and amplify the direct effect of ozone on early clearance by causing a holdup of tracer particles in the oral pharynx and stomach. Although our data alone do not prove that the clearance delay occurs in the respiratory system, interference with mucociliary transport is a probable major mechanism.

The stimulation of late clearance of particles from the lung correlates with the increased number of free cells we observed in histological sections in alveoli after exposure. It appears that ozone produces both a sloughing of parenchymal cells and increased numbers of free phagocytic macrophages.

The delay of early clearance and stimulation of late clearance by high humidity is more difficult to explain. However, in this case, as with ozone exposure, water consumption immediately after exposure was depressed and histological examination showed greater than usual numbers of free cells in alveoli.

The effects of ozone and humidity on clearance appear to be roughly additive. Since high-humidity studies were done only with 0.8 ppm ozone, we cannot say whether this apparent additive effect would occur at other dose levels. The finding does, however, show the need to control humidity in inhalation studies that rely on physiological end points.

The two sulfate-only atmospheres that had statistically significant effects on clearance—ferric sulfate and sulfuric acid mist, both at low humidity—are not present in submicrometer particles in the environment at the high concentrations studied here. Histological examinations of rats exposed to these two atmospheres did not indicate that they affect the lung in any unique way in comparison to the other sulfate atmospheres. However, if ferric sulfate does genuinely inhibit deep lung clearance, and the statistically significant result is not spurious, it should be considered a potentially serious lung toxin. In chambers, sulfuric acid is partly neutralized by ammonia. In this study the airborne concentration was maintained to yield a titratable acidity of 3-4 mg/m^3 in air. When the neutralized fraction is taken into account, the total aerosol exposure level was on the order of 5 mg/m^3, which is very high compared to probable environmental levels.

Data for groups exposed to ozone alone can be compared to data for groups exposed, at corresponding humidity, to ozone in combination with sulfate aerosols to determine whether the effect of combined exposure is best described as antagonism (less than additive), independent (additive) action, or synergism (greater than additive). We found that the effects of aerosols with ozone are qualitatively similar to those of ozone alone, and in most cases the effects of the combination are less than those of ozone alone at a given humidity. The data do not support a synergism

model for the parameters measured. The only exception may be sulfuric acid mist at low humidity with late clearance as the end point; but even here the comparison in a one-tailed t-test is not significant at $p = 0.1$.

In short, a less than additive effect was seen more often than a greater than additive effect. Since environmental levels of these aerosols are far below those used in our studies, we would not expect synergism between sulfates and ozone in the environment when clearance is used as the end point. This conclusion should not be extended to include physiological events that were not studied. For example, work by others (Last and Cross, 1978) on tracheal explants implies that sulfuric acid mist may indeed have a greater than additive effect with ozone when mucus production is used as the end point. A combination that has irritant properties greater than those of either component alone and is synergistic with respect to upper airway mucus secretion could, for example, induce a rapid shallow-breathing response that would be protective by reducing exposure to the deeper lung. Such a protective response could diminish the effects on particle clearance.

What are the health implications of our results? Delays in short-term clearance on the order of 1 h could be significant in terms of susceptibility to upper respiratory tract infections. The doubling time for microorganisms at body temperature can be on the order of hours, so increased susceptibility to infection could be one consequence of impaired early clearance. The effect of ozone in accelerating long-term clearance is not in itself harmful. However, the results of others imply that stimulation of clearance from the deep lung is associated with loss of ability to inactivate microorganisms (Goldstein et al., 1971). This could, of course, be a possible health hazard. Finally, the net effect of ozone on overall clearance was to diminish the amount of label in the lung at the time of termination of the experiment. Therefore one would not expect a single acute ozone exposure to contribute to the overall accumulation of inhaled insoluble dusts in the respiratory tract.

REFERENCES

Albert, R. E., Lippmann, M., and Briscoe, W. 1969. The characteristics of bronchial clearance in humans and the effects of cigarette smoking. *Arch. Environ. Health* 18:738-755.

Albert, R. E., Lippmann, M., and Peterson, H. T., Jr. 1970. The effects of cigarette smoking on the kinetics of bronchial clearance in humans and donkeys. In *Inhaled Particles III*, ed. E. H. Walton, vol. 1, pp. 165-180. Surrey, England: Unwin.

Albert, R. E., Berger, J., Sanborn, K., and Lippmann, M. 1974. Effects of cigarette smoke components on bronchial clearance in the donkey. *Arch. Environ. Health* 29:96-101.

Amdur, M. O., Bayles, J., Ugro, V., and Underhill, D. W. 1978. Comparative irritant potency of sulfate salts. *Environ. Res.* 16:1-8.

Appel, B. R., Kothny, E. L., Hoffer, E. M., Hidy, G. M., and Wesolowski, J. L. 1978. Sulfate and nitrate data from the California aerosol characterization experiment. *Environ. Sci. Technol.* 12:418-425.

Camner, P. 1973. Tracheobronchial clearance in patients with influenza. *Am. Rev. Respir. Dis.* 108:131–135.

Cavender, F. L., Steinhagen, W. H., Ulrich, C. E., Busey, W. M., Cockrell, B. Y., Haseman, J. K., Hogan, M. D., and Drew. R. T. 1977a. Effects in rats and guinea pigs of short term exposures to sulfuric acid mist, ozone and their combination. *J. Toxicol. Environ. Health* 3:521–533.

Cavender, F. L., Williams, J. L., Steinhagen, W. H., and Woods, D. 1977b. Thermodynamics and toxicity of sulfuric acid mists. *J. Toxicol. Environ. Health* 2:1147–1159.

Creasia, D. A., Nettesheim, P., and Hammons, A. S. 1973. Impairment of deep lung clearance by influenza virus infection. *Arch. Environ. Health* 26:197–201.

Fairchild, G. A., Stultz, S., and Coffin, D. L. 1975. Sulfuric acid effect on the deposition of radioactive aerosol in the respiratory tract of guinea pigs. *Am. Ind. Hyg. Assoc. J.* 36:584–594.

Ferin, J. and Leach, L. J. 1973. The effect of SO$_2$ on lung clearance of TiO$_2$ particles in rats. *Am. Ind. Hyg. Assoc. J.* 34:260–263.

Gardner, D. E., Miller, F. J., Illing, J. W., and Kirtz, J. M. 1977. Increased infectivity with exposure to ozone and sulfuric acid. *Toxicol. Lett.* 1:59–64.

Gloria, H. R., Bradburn, G., Reinisch, R. F., Pitts, J. N., Jr., Behar, J. V., and Zatonte, L. 1974. Airborne survey of major air basins in California. *J. Air. Pollut. Control Assoc.* 24:645–652.

Goldstein, E., Tyler, W. S., Hoeprich, P. D., and Eagle, C. 1971. Ozone and the antibacterial defense mechanisms of the murine lung. *Arch. Intern. Med.* 127:1099–1102.

Green, G. M. 1965. Patterns of bacterial clearance in murine influenza. In *Antimicrobial Agents and Chemotherapy,* pp. 26–29. Washington, D.C.: American Society for Microbiology.

Hackney, J. D., Linn, W. S., and Bell, K. A. Experimental studies of the human health effects of sulfur oxides. *Bull. N.Y. Acad. Med.* 54:1177–1185.

Henry, M. C., Spangler, J., Findlay, J., and Ehrlich, R. 1970. Effects of nitrogen dioxide and tobacco smoke on retention of inhaled bacteria. In *Inhaled Particles III,* vol. 1, pp. 527–533. Surrey, England: Unwin.

Hinrichs, R. J., Kenoyer, J. L., Phalen, R. F., and Crocker, T. T. 1978. Labeling of monodisperse polystyrene Microspheres with tightly bound ^{51}Cr. *Am. Ind. Hyg. Assoc. J.* 39:570–575.

Ho, A. T., Phalen, R. F., and Crocker, T. T. 1980. Laboratory production of ammonium and ferric sulfate aerosols. *Am. Ind. Hyg. Assoc. J.* 41:346–351.

Juhos, L. T., Evans, M. J., Mussenden-Harvey, R., Furiosi, N. J., Lapple, C. E., and Freeman, G. 1978. Limited exposure of rats to H$_2$SO$_4$ with and without ozone. *J. Environ. Sci. Health* C13:33–47.

Kinosian, J. R. and Duckworth, S. 1973. *Oxidant Trends in the South Coast Air Basin 1963–1972.* Sacramento: California Air Resources Board.

Last, J. A. and Cross, C. E. 1978. A new model for health effects of air pollutants: Evidence for synergistic effects of mixtures of ozone and sulfuric acid on rat lungs. *J. Lab. Clin. Med.* 91:328–339.

May, K. R. 1973. The Collison nebulizer: Description, performance and application. *Aerosol Sci.* 4:235–243.

McJilton, C. E., Frank, R., and Charlson, R. J. 1976. Influence of relative humidity on functional effects of an inhaled SO$_2$ aerosol mixture. *Am. Rev. Respir. Dis.* 113:163–169.

Mercer, T. T., Tillery, M. I., and Newton, G. J. 1970. A multistage low flow rate cascade impactor. *J. Aerosol Sci.* 1:9–15.

Morrow, P. E. 1977. Clearance kinetics of inhaled particles. In *Respiratory Defense Mechanisms,* eds. J. D. Brain, D. F. Proctor, and L. M. Reid, pt. 2, pp. 491–543. New York: Dekker.

Morrow, P. and Mercer, T. T. 1964. A point-to-plane electrostatic precipitator for particle size sampling. *Am. Ind. Hyg. Assoc. J.* 25:8–14.

National Academy of Sciences, Committee on Medical and Biological Effects of Environmental Pollutants. 1977. *Ozone and Other Photochemical Oxidants.* Washington, D.C.: National Academy of Sciences.

Phalen, R. F., Hallford, J. D., and Kenoyer, J. L. 1975. Particle disposition and clearance as a test of toxic effect. *Proc. Annu. Conf. Environ. Toxicol.* 6:83–96.

Phalen, R. F., Kenoyer, J. L., and Davis, J. 1977. Deposition and clearance of inhaled particles: Comparison of mammalian species. *Proc. Annu. Conf. Environ. Toxicol.* 7:159–170.

Raabe, O. G., Bennick, J. E., Light, M. E., Hobbs, C. H., Thomas, R. L., and Tillery, M. I. 1973. An improved apparatus for acute inhalation exposure of rodents to radioactive aerosols. *Toxicol. Appl. Pharmacol.* 26:264–273.

Sackner, M. A., Ford, D., Fernandez, R., Cipley, J., Perez, D., Kwoka, M., Reinhart, M., Michaelson, E., Schreck, R., and Wanner, A. 1978. Effects of sulfuric acid aerosol on cardiopulmonary function in dogs, sheep and humans. *Am. Rev. Respir. Dis.* 118:497–510.

Schlesinger, R. B., Lippmann, M., Albert, R. E. 1978. Effects of short-term exposures to sulfuric acid and ammonium sulfate aerosols upon bronchial airway function in the donkey. *Am. Ind. Hyg. Assoc. J.* 39:275–286.

Schlesinger, R. B., Halpern, M., Albert, R. E., and Lippmann, M. 1979. Effect of chronic inhalation of sulfuric acid mist upon mucociliary clearance from the lungs of donkeys. *J. Environ. Pathol. Toxicol.* 2:1351–1367.

Spiegelman, J. R., Hanson, G. D., Lazarus, A., Bennett, R. J., Lippmann, M., and Albert, R. E. 1968. Effects of acute sulfur dioxide exposure on bronchial clearance in the donkey. *Arch. Environ. Health* 17:321–326.

U.S. Environmental Protection Agency. 1976. *Monitoring and Air Quality Trends Report, 1974,* EPA-450/1-76/001. Research Triangle Park, N.C.: U.S. Environmental Protection Agency.