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The effect of a 4 hour exposure to a model sulfur pollutant atmosphere on the clearance of inhaled insoluble tracer particles from the lungs of rats has been studied. The pollutant combination consisted of 5 ppm of sulfur dioxide gas and 1.5 mg/m³ of sulfate aerosol at 80-85% relative humidity. The exposure atmosphere was aged for 30 minutes upstream of the exposure chamber in an aging line in order to provide for gas/particle interactions such as those occurring in industrial and environmental atmospheres. Results indicate that the sulfur pollutant atmosphere did not produce a statistically significant alteration in early (nasopharyngeal and tracheobronchial) or late (parenchymal) clearance rates such as those which have been identified in this laboratory following exposure to ozone-containing atmospheres.

Effect of sulfur dioxide-sulfate exposure on rat respiratory tract clearance

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introduction

Gaseous sulfur dioxide (SO₂) is an upper respiratory tract irritant which has been identified as an atmospheric contaminant in a variety of environments. SO₂ can be oxidized to sulfate particles via a variety of reactions, some of which may be catalyzed by transition metals such as iron and manganese. Thus, SO₂ inhalation often occurs in the presence of sulfate particles. The water solubility of SO₂ accounts for the uptake of the gas in the upper airways, while the sorption of SO₂ to aerosols leads to enhanced transport of the gas to the deep lung.

Several investigators have undertaken studies in order to determine the effects of either SO₂ or sulfate aerosols upon respiratory tract clearance phenomena. Ferin and Leach⁽¹⁾ described an experiment in which inert TiO₂ particles were deposited in the lungs of rats following a series of exposures to SO₂ (1 ppm, 7 hours/day, 5 days/week, 10-25 days). Lung clearance was either accelerated or at control levels in rats exposed for 10 to 20 days. However, rats exposed for 25 days exhibited a marked depression in clearance as measured at sacrifice 25 days after deposition of the particles. A delay in the bronchial clearance of monodisperse Fe₂O₃ particles was observed in donkeys following a brief exposure (300 ppm SO₂, 30 minutes) by Spiegelman *et al.*⁽²⁾ The effects of SO₂ (5 ppm, 3 hours) on tracheobronchial clearance in healthy, nonsmoking adults were studied by Wolff *et al.*⁽³⁾ No significant alteration of the rate of mucociliary clearance occurred in resting subjects, except for a small transient increase seen one hour after exposure. Newhouse *et al.*⁽⁴⁾ reported an increase in bronchial clearance in healthy, nonsmoking adults following an exposure to SO₂ (5 ppm, 2 hours) during intermittent exercise.

Tests in the laboratory to evaluate the effect of sulfate particles on respiratory tract clearance rates have also been performed. Schlesinger *et al.*⁽⁵⁾ observed a slowing of tra-

cheobronchial clearance of tracer particles in donkeys following an exposure to sulfuric acid mist (0.5 µm mass median aerodynamic diameter (MMAD), 0.194-1.364 mg/m³, 1 hour). Schlesinger *et al.*⁽⁶⁾ also studied the effect of chronic inhalation exposures of donkeys to sulfuric acid mist (0.5 µm MMAD, 0.1 mg/m³, 1 hour/day, 5 days/week for 6 months) on mucociliary clearance. Bronchial clearance became erratic within the first week of exposure and the clearance rates of the animals after exposure were, in general, significantly slower than the clearance rates observed prior to exposure. Phalen *et al.*⁽⁷⁾ found that both ferric sulfate (0.4 µm MMAD, 3.6 mg/m³, 4 hours) and sulfuric acid mist (1.0 µm MMAD, 3.6 mg/m³, 4 hours) at low relative humidity (30-40%) slowed deep lung clearance. But the same atmospheres at high relative humidity (>80%) did not significantly affect the clearance rates. Leikauf *et al.*⁽⁸⁾ reported a study in which healthy, nonsmoking adults were exposed to various levels of sulfuric acid mist (0.5 µm MMAD) via a nasal mask for 1 hour following the inhalation of a radiolabeled Fe₂O₃ aerosol. At a level selected by the investigators to represent "peak" episodic environmental levels (0.11 mg/m³) the acid accelerated bronchial clearance, while higher levels (0.98 mg/m³ and above) significantly delayed the clearance of the tracer. Wolff *et al.*⁽⁹⁾ measured changes in tracheal mucous clearance in beagle dogs after 1 hour exposures to sulfuric acid mist. For 1.0 mg/m³, 0.9 µm MMAD exposures, tracheal mucous velocities were significantly depressed after 30 minutes, 1 day, and 1 week — but had returned to normal 5 weeks after exposure. For 0.5 mg/m³, 0.9 µm MMAD exposures, nonsignificant increases in clearance were seen after 30 minutes and 1 day, with a significant depression in clearance occurring at 1 week. No significant alterations in clearance appeared following exposures to 0.3 µm MMAD particles at concentrations up to 5.0 mg/m³.

Figure 1 — Nose-only exposure system used to deposit tracer aerosols into the respiratory systems of rats.

While a considerable amount of data has accumulated concerning the effects upon clearance of SO_2 and sulfates studied individually, no information is currently available which describes the effects of SO_2 -sulfate mixtures on clearance rates. SO_2 , due to its high solubility in water, deposits primarily in the upper respiratory tract.⁽¹⁰⁾ For this reason it would generally be expected to have little, if any, effect on deep lung clearance. However, the reactions that occur between SO_2 and environmental aerosols, and the gas-to-particle conversions which may occur, can greatly influence the regional deposition of biologically active chemical species. Since SO_2 is, again, highly soluble in water, droplet aerosols, including those formed by deliquescent particles, will collect dissolved SO_2 and can carry the resulting acids of sulfur, including sulfuric and sulfurous acids, deep into the lung. As a result of this the clearance pattern of the lung parenchyma (respiratory bronchioles and more distal structures) may be affected.

The objective of this study was to determine the effect on particle clearance of a mixture of SO_2 and sulfates which is often present in both industrial and environmental atmospheres. The exposure concentration of SO_2 , 5 ppm, is the Federal time-weighted average permissible exposure limit (PEL). The sulfate aerosol employed consisted of a droplet aerosol ($0.5 \mu\text{m}$ MMAD) generated from a single solution of ammonium sulfate and ferric sulfate (0.85 M NH_4^+ ; 0.25 M Fe^{+3} ; $0.80 \text{ M total SO}_4^{2-}$). The airborne mass concentration, excluding droplet solution water, was 1.5 mg/m^3 . (Although sulfates generally are not regulated in the workplace, levels above 5 mg/m^3 would exceed the Federal PEL for the respirable fraction of nuisance dust.) Ammonia gas is ubiquitous to human environments and is often present in quantities sufficient to account for the majority of sulfate present being ammonium sulfate. Amdur and Underhill⁽¹¹⁾ have postulated that soluble metallic salts, when present in droplet

aerosols that are mixed with SO_2 , catalyze the conversion of SO_2 to sulfate. Thus, the inclusion of ferric sulfate theoretically allows for this conversion during aging prior to exposure. In addition, it is presumed that any intermediate products in such reactions, although not directly characterized, should also be present in the exposure atmosphere. For this reason the SO_2 -sulfate mixture was allowed to react for 30 minutes in an aging line system prior to inhalation by the rats.

An exposure temperature of 70°F was selected for this study in order to ensure the thermal comfort of the test animals. The relative humidity was controlled between 80-85% so that the sulfate aerosol present would be in a droplet,

Figure 2 — Thoracic counting system used to determine the amount of radioactivity in the respiratory tract of a rat.

TABLE I
Effect of Aged Sulfur Pollutant Exposure on Early and Late Clearance
of Radiolabeled Tracer Microspheres

Early Clearance ^A					
Atmosphere	Number	$\bar{T}_{50\%} \pm \text{SD (hr)}$		$\Delta T_{50\%} \pm \text{SE (hr)}$	p^C
Clean Air	30	10.0 $\pm$	2.7	—	—
Sulfur Pollutants	26	9.3 $\pm$	2.4	-0.7 $\pm$ 0.7 ^B	0.29
Late Clearance					
Atmosphere	Number	$\bar{T}_L \pm \text{SD (hr)}$		$\Delta T_L \pm \text{SE (hr)}$	p^C
Clean Air	32	885 $\pm$	262	—	—
Sulfur Pollutants	31	866 $\pm$	321	-19 $\pm$ 74 ^B	0.80
A_{30} (Index of Late Clearance)					
Atmosphere	Number	$\bar{A}_{30} \pm \text{SD}$		$\Delta A_{30} \pm \text{SE}$	p^C
Clean Air	32	23.8 $\pm$	2.5	—	—
Sulfur Pollutants	31	24.3 $\pm$	3.5	0.5 $\pm$ 0.8	0.52

Exposures were 4 hours in length. $T_{50\%}$ = Time required to excrete 50% of total activity excreted through 50 hours postdeposition. T_L = Long-term biological half-time.

^ASeven rats were excluded from the early clearance data analysis either for failure to drink water, or for failure to defecate during three successive fecal sampling intervals.

^BNegative sign (-) implies an acceleration in clearance.

^CTwo-tailed t-test.

rather than a dry, form. This was presumed to permit greater reactivity between the SO₂ and aerosol in the aging process.

materials and methods

The animals used in this experiment were barrier-reared Sprague Dawley rats (Hilltop Lab Animals, Inc., Chatsworth, CA). Male rats weighing approximately 200 g were delivered to the laboratory in filtered shipping containers in order to minimize prior exposure of the animals to pollutants. The rats were housed in a laminar air barrier caging system in wire-bottom stainless steel cages over a nonstandard, relatively dust-free, sodium chloride litter for about 1 week before being randomly placed in the experimental exposure protocol. Histopathologically, the lungs were relatively free of infection sites, and microbiological assays (supplied by Hilltop Lab Animals) indicated freedom from mycoplasmosis and other common respiratory infections.

The tracer microspheres were labeled at this laboratory with tightly bound ⁵¹Cr.⁽¹²⁾ The particles labeled for this study were produced from commercial monodisperse, polystyrene latex microspheres (Dow Chemical Company, Midland, MI). After aerosolization with a Lovelace-type laboratory compressed air nebulizer⁽¹³⁾ (ARIES, Inc., Davis, CA) of a 0.1% (by volume) aqueous suspension of particles, the activity median aerodynamic diameter (AMAD) of dried particles was measured using a calibrated seven-stage impactor⁽¹⁴⁾ (ARIES, Inc.). The aerosol, which was measured in the breathing zone of the rats, had an AMAD of 1.9 μm with a geometric standard deviation (GSD) of 1.3. Less than 1% of the radioactivity was in the fine fraction collected on the fiberglass backup filter in the impactor. Electron microscopy samples collected from the exposure unit using a point-

to-plane electrostatic precipitator⁽¹⁵⁾ (ARIES, Inc.) indicated that approximately 80% of the particles deposited on the collection surfaces were singlets. The aerosol size distributions of the tracer microspheres closely approximated log-normal functions. *In vitro* leaching studies indicated a leaching rate of ⁵¹Cr from the particles of less than 0.1% per day.⁽¹²⁾ The specific activity of the labeled microspheres was calculated to be 500 Ci/gm. The estimated dose to a rat over the length of the experiment from the ⁵¹Cr, which emits a 0.32 MeV gamma ray, was on the order of several millirem. Counting times in the thoracic activity counting system ranged from 100 seconds (at the initial activity measurement) to 300 seconds for the final count of low-activity animals. A minimum of 1000 gross counts were obtained for each thoracic activity determination.

The aerosolized particles were dried by heating and dilution with clean air and passed through a ⁸⁵Kr discharger (TSI Inc., St. Paul, MN) before entering the nose-only exposure chamber at an airborne mass concentration of about 0.2 $\mu\text{g}/\text{m}^3$. This exposure chamber has been described previously.⁽¹⁶⁾ Sixteen rats were exposed simultaneously to the radioactive aerosol in this system (Figure 1) for 20 minutes. The nose-only tubes that held the unanesthetized animals were constructed of perforated metal to minimize thermal stress to the animals due to a buildup of body heat.

After the deposition was completed the rats were removed from the system and their noses washed with wet paper towels in order to reduce the quantity of externally deposited radioactivity. The animals were then placed in plastic counting tubes and positioned beneath a collimated 3-in NaI (Tl) gamma ray detection system (Figure 2) shielded from background radiation with lead. If an animal moved forward or backward or twisted sideways in the tube while being

counted, then it was recounted. The initial total amount of deposited activity (defined as 100% for each rat) was determined before the rats went into either sulfur pollutant or control (clean air) atmospheres. The amount of time between the termination of the tracer particle deposition and the initial count of the first and last animals ranged from 3-40 minutes, respectively. While there is some particle clearance from the respiratory system during this time (using the early clearance rate data an estimated 5% clears in the first 40 minutes), the effect on the subsequent data analysis is minimized by using the midpoint of the group counting interval in the calculations. (Even after the nose wiping about 30% of the total body burden is initially in and on the head. For this reason early thoracic counts were considered to be only crude estimators of lung activity and the short term clearance curves were determined using the serial fecal collections described later.)

One hour after the exposure to the radiolabeled particles, the rats were randomly divided into two groups and placed in individual compartments of open-mesh stainless steel exposure cages. The cages were then positioned on a single level in rectangular cross section 1 m³ volume stainless steel chambers for a 4 hour exposure to either clean air or an aged sulfur pollutant atmosphere consisting of 5 ppm of SO₂ and 1.5 mg/m³ of sulfate aerosol. For each sulfur pollutant exposure, clean air exposed animals from the same supply batch were used as controls.

The aging line used in this study has been described.⁽¹⁷⁾ Briefly, the aging line is a nearly-square (89 cm high by 99 cm wide) stainless steel duct 9.3 m in length which terminates at

a rectangular stainless steel exposure chamber. Pollutants are continuously injected into purified air in a turbulent mixing pipe connected through a diffuser to the aging line. The exposure atmosphere was allowed to age for 30 minutes prior to inhalation by the rats in order to simulate the aging times of industrial and environmental atmospheres. During passage through the aging line SO₂ losses were less than 3% and particle losses in the size range used in the study were less than 10% (by mass). These modest SO₂ losses were consistent with the calculated depletion of the gas due to dissolution in the droplet particles.

Both of the exposure chambers were supplied with clean air from a high pressure air purification system. Outside air was first filtered and then compressed to 100 psig using a liquid ring compressor (Nash Engineering Company, Norwalk, CT). The high pressure air then passed through a fixed Purafil (KMnO₄ on Alumina; HE Burroughs, Inc.) bed to remove such contaminants as O₃, SO₂, NH₃, NO_x, and some hydrocarbons. Carbon monoxide was removed by passage through Hopcalite catalyst; a heatless dryer located just upstream of the catalyst bed prevented catalyst inactivation by moisture and provided further air purification. After filtration and thermal equilibration to laboratory temperature, the high pressure purified air was throttled down to ambient pressure and humidified to 80-85% RH. Delivery of clean air to the control chamber followed a final HEPA filtration. In the case of the experimental (exposure) chamber, the air was filtered, pollutants were injected, and the air stream then entered the aging line prior to delivery to the chamber.

Figure 3 — Difference in activity excretion curves (clean air minus sulfur pollutant exposed) as a function of hours postdeposition. Error bars represent ± 1 standard error.

SO₂, obtained from a cylinder of research quality gas (1% SO₂ in zero air), was monitored continuously at the breathing zone of the animals through teflon sampling lines using a calibrated pulsed fluorescent SO₂ analyzer (Thermo Electron Corp., Hopkinton, MA). A single Collison-type nebulizer,⁽¹⁸⁾ followed by a ⁸⁵Kr discharger, was used for sulfate particle generation (Sierra Instruments, Inc., St. Paul, MN, Model 7330). The average airborne mass concentration of sulfate aerosol (1.5 mg/m³) in the breathing zone was determined gravimetrically from six spectrograde glass fiber filter samples collected during the actual 4 hour exposure with rats in the chamber. (The stated mass concentration includes ammonium sulfate and ferric sulfate residues, and the water of hydration, but excludes droplet solution water.) The calculated airborne concentration of sulfate ion (1.4 mg/m³) was obtained by chemical analysis from the breathing zone filter samples by manual barium perchlorate titration using Thorin indicator.⁽¹⁹⁾ The quantity of ferric ion (0.14 mg/m³) was determined from the filter samples using atomic absorption spectroscopy. To ensure accurate determination of the aerodynamic size distribution of the droplet aerosol, a calibrated seven-stage cascade impactor⁽¹⁴⁾ (ARIES) was used. An ammonium ion analysis of the impactor stages yielded a MMAD of 0.5 μm and a GSD of 1.6. Because a solution of ammonium sulfate and ferric sulfate had been nebulized, it is assumed that the ratios of ammonium, sulfate, and ferric ions are independent of particle size.

After the rats were removed from the chambers following the 4 hour exposure, the feces were collected at fixed intervals selected in order to allow for the determination of the fecal activity curve. Early clearance was determined from these excretion curves due to the interference of early gastrointestinal tract activity with the thoracic counts. In total, eleven fecal collections from each rat were made during the first 50 hours after the deposition of the tracer microspheres. The feces were not collected after this time because levels of excreted radioactivity were negligible. Fecal excretion curves were fitted to a log-normal function and the time at which 50% of the total activity was excreted was determined for each rat. This time is referred to as the 50% clearance time (T_{50%}) rather than a half-time because this phase of clearance was not strictly exponential. If the T_{50%} for the sulfur pollutant exposed animals was less than that for the control (clean air exposed) animals, then the difference (negative) between them would indicate an acceleration of early clearance. During the time interval of 50-400 hours postdeposition of the radiolabeled particles, five thoracic counts were performed on each animal using the plastic counting tubes. The percentages of these counts of the initial respiratory tract activity were calculated, plotted versus time, and fit to a single exponential function. From this function a biological half-time for long-term clearance (T_L) of the radioactive microspheres, decay corrected to the time of particle deposition, was obtained for each rat. This long-term clearance phase was sufficiently exponential to warrant the use of the half-time concept. Means and standard deviations were calculated for both the exposed and control groups of rats and the

early 50% clearance times and the late clearance biological half-times were compared using two-tailed t-tests.

As an independent measure of lung clearance, the rats were sacrificed at 30 days postdeposition and their excised lungs were counted (left and right lung individually) in a well counter in order to determine the residual lung radioactivity. This was done because by this time the rats had outgrown the thoracic counting system. The total lung activity for each rat was first corrected for the decay of the ⁵¹Cr back to 50 hours postdeposition, and then normalized by dividing by the thoracic count obtained for the same rat at 50 hours postdeposition. (This normalization using two separate detection systems is necessary in order to account for the differences in the quantity of radioactivity deposited in the slow-clearing lung compartment of the individual rats.) The resultant values constitute an index of late clearance, termed the A₃₀. Statistical analysis of the grouped A₃₀ values was then performed. Should the mean A₃₀ value for the sulfur pollutant exposed group be larger than the corresponding value for the control group, this would indicate that a greater fraction of radioactivity had been retained in the lungs of the exposed group. This would be identified as an inhibition of late clearance.

Figure 4 — Clearance curves of ⁵¹Cr-labeled tracer particles as measured by thoracic counting. Error bars represent ± 1 standard error of the mean for the clean air exposed group.

Some animals were not included in the data analysis. Animals that did not drink water, and animals that did not defecate during three successive fecal sampling intervals, were excluded from the short-term clearance study.

results

The effect of the 4 hour exposure to the sulfur pollutant atmosphere on the clearance of the radiolabeled tracer particles was measured (Table I). No significant alteration of either early (0-50 hours postdeposition) clearance or long-term (50-400 hours postdeposition) clearance was detected using a pre-selected criterion of $p < 0.1$. (All of the p values reported in this paper were obtained using two-tailed t -tests.)

The effect of exposure to the pollutant atmosphere on the early clearance of the radiolabeled particles is shown in Figure 3, where the difference between the group activity excretion curves (clean air minus sulfur pollutant exposed) is plotted versus time postdeposition. Calculations indicate that the time required for the exposed animals to excrete 50% of the total amount of radioactivity excreted during the first 50 hours postdeposition was 0.7 ± 0.7 (SE) hours less than the time required by the control animals. However, this acceleration of the clearance rate in the exposed group was not statistically significant.

The effect of exposure to the sulfur pollutants on the retention of thoracic radioactivity after 50 hours postdeposition is shown in Figure 4. Here, the radioactivity in the respiratory tract (as a percentage of the initial activity) is plotted versus time for both the exposed and clean air exposed groups. These clearance curves have nearly identical slopes and the calculated difference in long-term biological half-times (19 ± 74 (SE) hours acceleration in the exposed group) was not significant.

The index of late clearance A_{30} , while proving to be a precise measure of long-term clearance ($\%SD < 15\%$), indicated a nonsignificant delay in late clearance.

discussion

The purpose of this study was to determine the effect of a mixture of sulfur pollutants commonly found in industrial and environmental atmospheres on the ability of the lung to mechanically remove inhaled particles. Many industrial workers, including brewery workers, furnace operators, ore smelter workers, paper makers and chemical workers are potentially exposed to levels of SO_2 up to, but hopefully not exceeding, the Federal PEL of 5 ppm. For reasons mentioned previously sulfate particles are often present in atmospheres containing SO_2 . Sulfate particles and SO_2 are generally present in both occupational settings and in urban atmospheres as a result of the burning of sulfur-laden fossil fuels. Therefore these sulfur pollutants were studied together in order to determine their combined effect upon lung clearance kinetics.

The particle clearance methods described here have, in several previous studies, proven to be a useful test of the toxicity of various inhaled pollutants. Employing an almost identical protocol and similar ^{51}Cr -labeled microspheres

Kenoyer *et al.*⁽²⁰⁾ showed that ozone studied at levels of 0.8 ppm and above caused a significant ($p < 0.1$) delay in early clearance and acceleration in late clearance in rats. At an ozone concentration of 1.0 ppm their data revealed a 4.8 hour delay in the early 50% clearance time ($T_{50\%}$) of the exposed group ($p < 0.05$). In addition, the long-term biological half-time for the ozone-exposed rats was 326 hours less than that of the control animals ($p < 0.05$). Phalen *et al.*,⁽⁷⁾ in an experiment mentioned earlier, utilized similar methodology in determining that sulfuric acid mist and ferric sulfate, at low relative humidity, each accelerate long-term clearance in rats ($p < 0.1$). Therefore, the methods described here are capable of detecting alterations in both long and short term clearance rates following the inhalation of potentially toxic airborne pollutants.

In the present study neither the early nor late clearance patterns were significantly affected by exposure to the mixed sulfur pollutant atmosphere. However, while the 50% clearance times ($T_{50\%}$) for the two groups were not resolvable using the statistical tests employed, there did appear to be a slight, if statistically insignificant, acceleration in early clearance. Both of the endpoints used in characterizing late clearance are in agreement that the sulfur pollutant atmosphere studied did not significantly affect long-term clearance. Therefore, the conclusion here is that the 4 hour exposure to the mixed sulfur pollutants at high relative humidity did not alter either the early or late clearance of the radioactive tracer particles.

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