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EFFECTS OF OXIDANT AND SULFATE INTERACTION ON PRODUCTION OF LUNG LESIONS

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EFFECTS OF OXIDANT AND SULFATE INTERACTION ON PRODUCTION OF LUNG LESIONS

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

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16. ABSTRACT

This line of investigation is designed primarily to determine the sub-acute and chronic effects of sulfuroxide inhalation alone and in combination with oxidant exposure on the respiratory system of laboratory animals. Preliminary experiments are being conducted to determine the optimum concentration of small-particle H_2SO_4 exposure to use in subsequent H_2SO_4-oxidant experiments. The comparative response of rats, guinea pigs and monkeys is being determined. The effects of SO_2-O_3 mixtures will be determined also.

The principal biologic responses being determined are the histopathologic response, including ultrastructural studies and autoradiographic assessment of cell turnover rates, biochemical studies and physiologic measurements.

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EFFECTS OF OXIDANT AND SULFATE INTERACTION ON PRODUCTION OF LUNG LESIONS

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INTRODUCTION

The objectives of the research during the final quarter (June 16 to October 31, 1975) of Contract No. 68-02-1944 were to:

- Evaluate in the lungs of monkeys the effects of nine years of exposure to 2 and 9 ppm nitrogen dioxide (NO_2).
- Continue exposure of monkeys to mixtures of the interdependent atmospheric oxidants NO_2 and ozone (O_3) at realistic concentrations.
- Continue investigations of the interaction of atmospheric oxidants and particulate (aerosolized) sulfate in rodents.

PROGRESS DURING THE QUARTER

Measurements of Body Weights and Respiratory Rates in Monkeys

Monitoring of the clinical conditions of monkeys in the various exposure environments continued by measurement of body weights and respiratory rates. Figure 1 shows the means of body weights and respiratory rates of adult monkeys, and Figure 2 shows the weights of juvenile monkeys. No alterations from previous norms are evident; also, the body weights of juveniles that appear to have reached adult size have become stable.

Exposure of Monkeys to Mixtures of NO_2 and O_3

Exposure of three young-adult female monkeys to mixtures of 2 ppm NO_2 and 0.9 ppm O_3 continued. This exposure had been initiated in August 1975. We are continuing to observe these monkeys clinically.

Monitoring of Exposure Chambers

The concentrations of NO_2 and O_3 alone and as mixtures in exposure chambers were monitored as described in Quarterly Report No. 1.

Quarterly Report No. 3 contains a description of the all-glass nebulizer used for the generation of sulfuric acid mist and the airsteam-heater installed for the production of smaller mean particle size. The generated particle size and the mass distribution, which was produced with and without the use of the airstream-heater, were analyzed in detail during the fourth quarter. We used a Royco Model 225 particle-size analyzer of sheath-flow and forward-light-scatter design, equipped with a Model 241 five-channel simultaneous digital analyzer with printout; the Chemical Engineering Department of SRI let us use this apparatus at no cost to the contract. Table 1 shows the typical particle size distribution of sulfuric acid mist generated with and without the use of the inlet airstream-heater.

We used the information on particle size, presented in Table 1 and Figure 3, to calculate the mass distribution as a function of particle size, assuming unit density and spherical configuration of sulfuric acid particles. Figure 4 presents these data. The data in Figure 3 suggest that the diameters of 50% of the particles are smaller than either 0.73 or 0.46 μ for the unheated and heated modes of generation, respectively. As is evident from Figure 4, these particles represent only 0.7 or 2% of the mass of acid in the chamber. Figure 4 also shows that 50% of

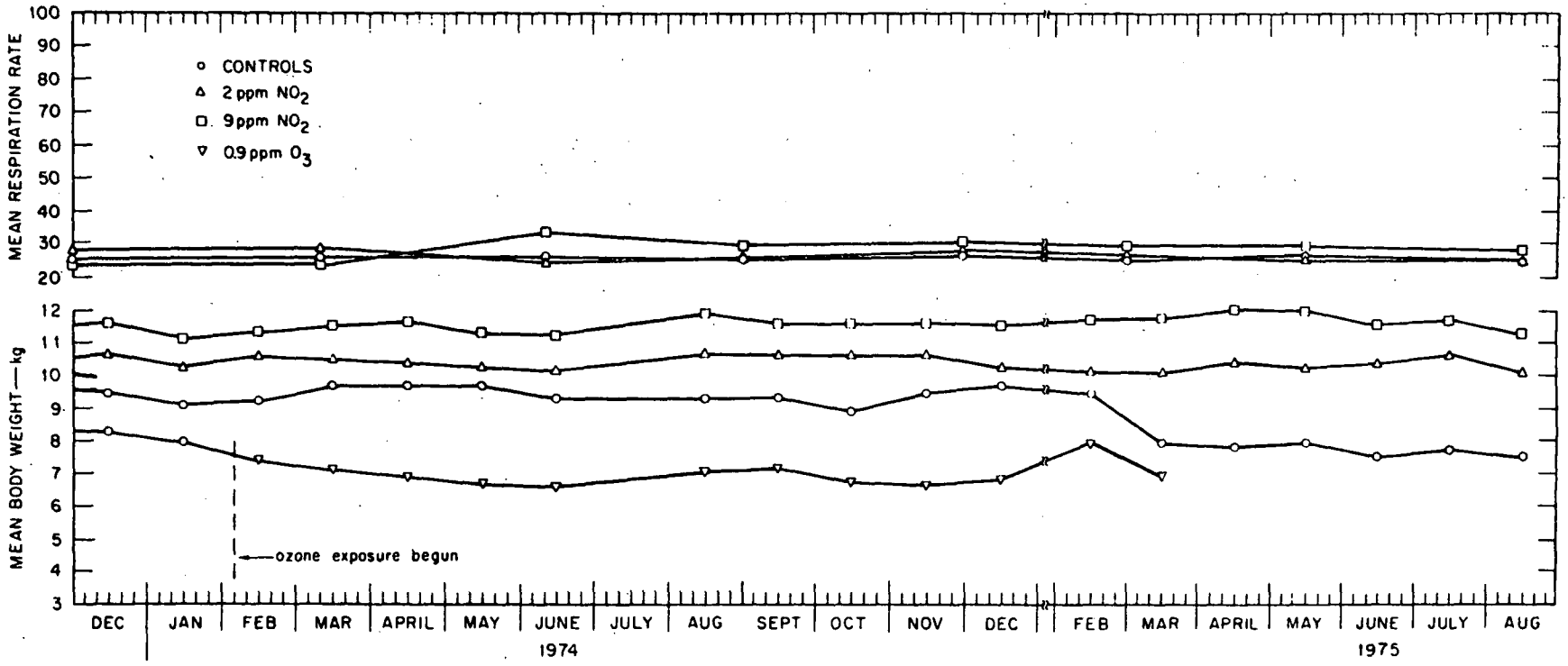


FIGURE 1 MEAN BODY WEIGHTS AND MEAN RESPIRATION RATES OF MONKEYS ON 0, 2, AND 9 ppm NO_2

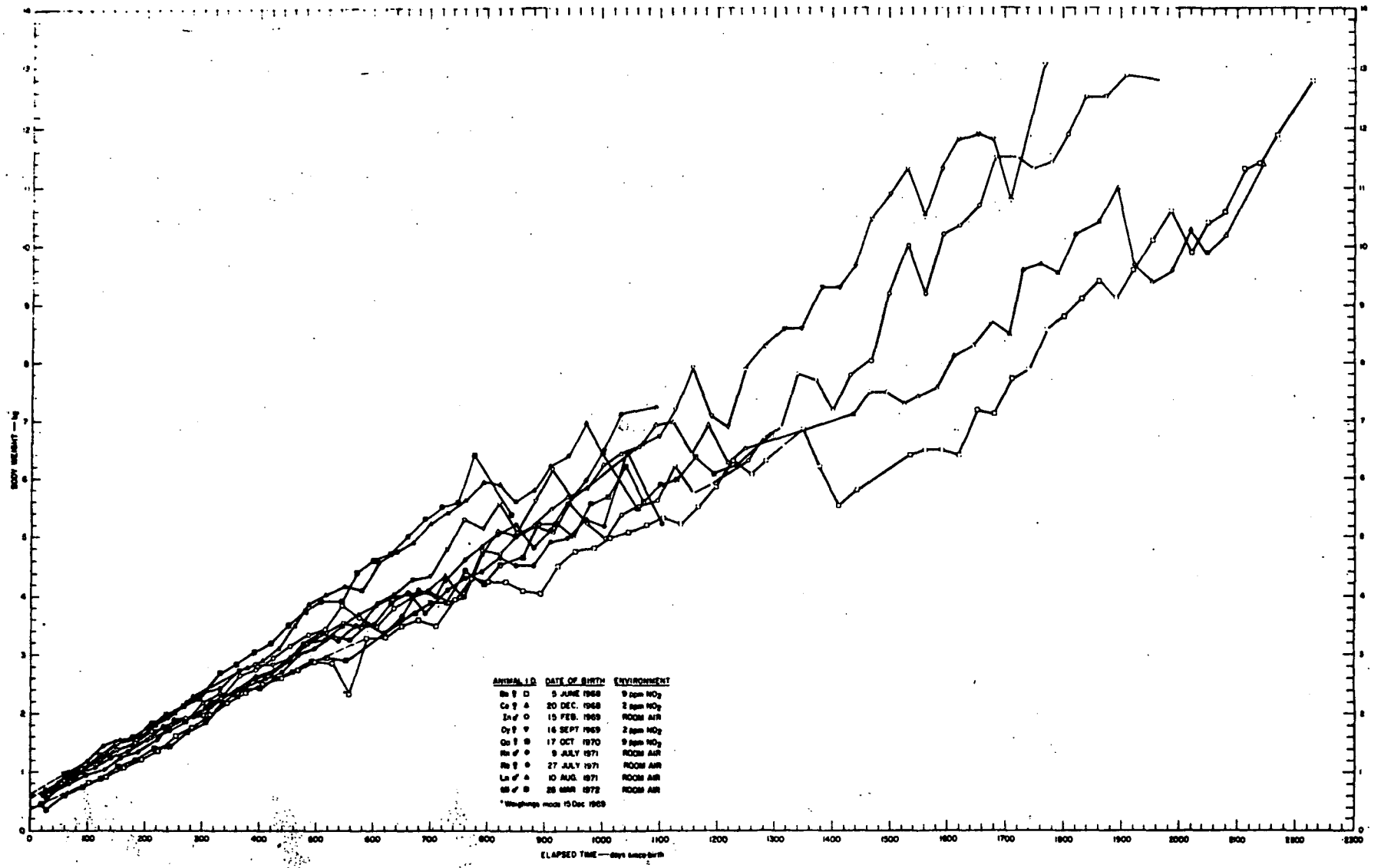


FIGURE 2 WEIGHT CURVES OF GROWING STUMP-TAILED MACAQUES ON 0, 2 AND 9 ppm NO_2

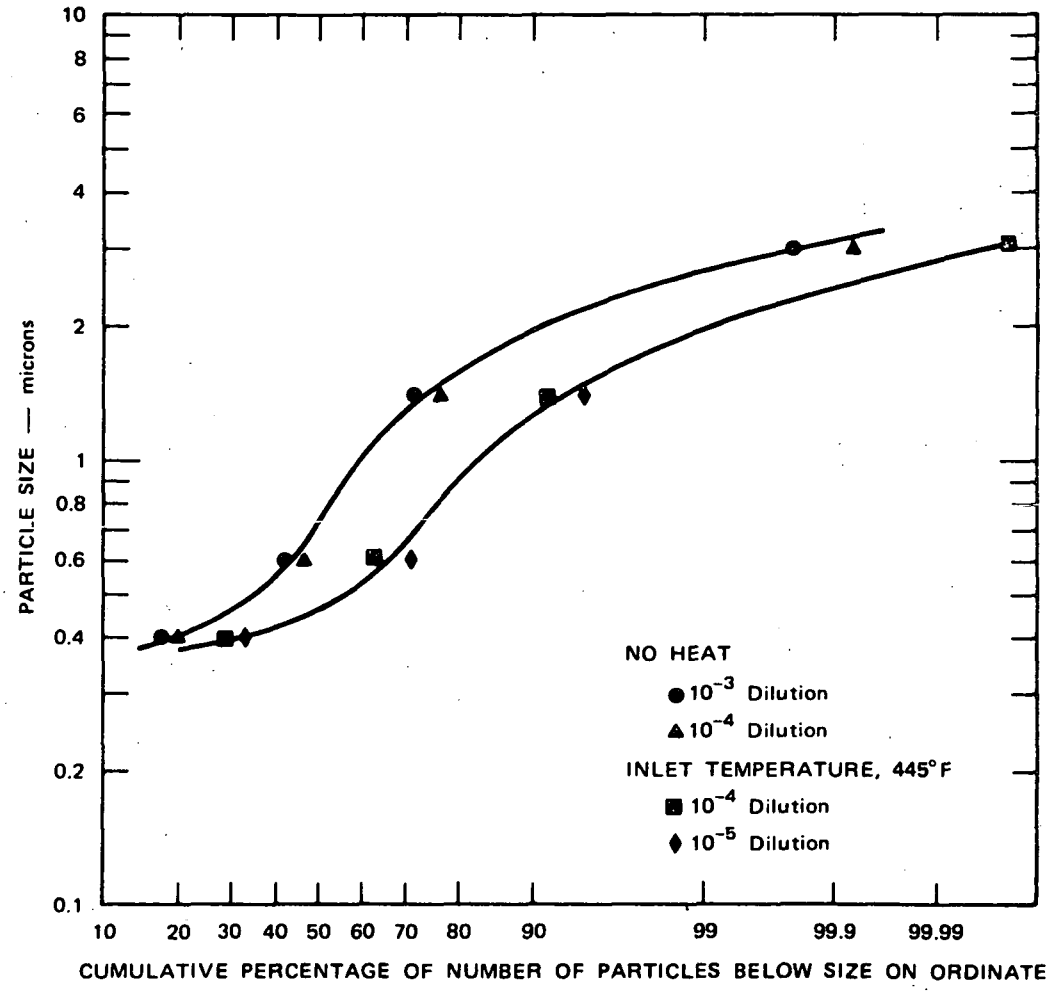


FIGURE 3 PARTICLE SIZE DISTRIBUTION FOR SULFURIC ACID MIST

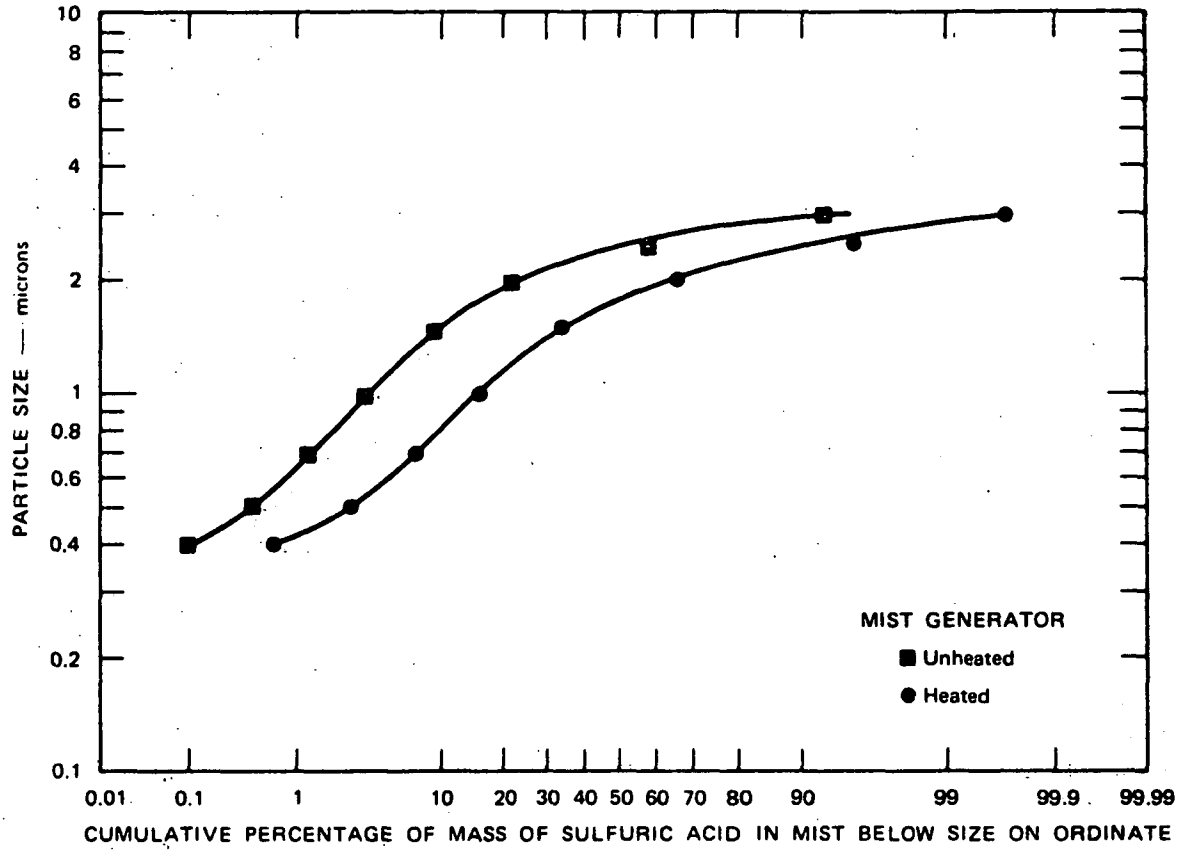


FIGURE 4 MASS DISTRIBUTION FOR SULFURIC ACID IN THE MIST

Table 1

PARTICLE-SIZE DISTRIBUTION OF SULFURIC ACID MIST

Condition of Generation	Nominal Royco Dilution	Channel Number	Average Count, Corrected*	Particle Size (μ)	Number of Particles Greater than Particle Size Shown	Number of Particles Greater than Particle Size (Cumulative %)
84 to 90 psig on pneumatic atomizer; no heat	10^{-3}	5	509.5	3	509.5	0.239
		4	60,003	1.4	60,513	28.34
		3	63,145	0.6	123,658	57.90
		2	52,927	0.4	176,585	82.68
		1	36,986	0.3	213,571	100
	10^{-4}	5	18	3	18	0.0631
		4	6,675	1.4	6,693	23.49
		3	8,535	0.6	15,228	53.44
		2	7,647	0.4	22,875	80.27
		1	5,621	0.3	28,496	100
84 to 90 psig on pneumatic atomizer; inlet to chamber (435 to 445°F) 85 psig on diluting air	10^{-4}	5	3.67	3	3.67	0.000779
		4	11,088	1.4	11,092	8.64
		3	35,783	0.6	46,875	36.52
		2	45,312	0.4	92,187	71.83
		1	36,156	0.3	128,343	100
	10^{-5}	5	0	3	0	0
		4	397	1.4	397	5.63
		3	1,631	0.6	2,028	28.74
		2	2,659	0.4	4,687	66.42
		1	2,370	0.3	7,057	100

*Background count without the atomizer in operation was determined and subtracted.

the aerosolized sulfuric acid particles have diameters of 2.5 or 1.78 μ, respectively, when generated with and without the inlet airstream-heater. The above measurements were made by nebulizing a 35% (w/w) solution of sulfuric acid. The heated aerosol generator maintained the internal chamber temperature below 75°F during exposure of animals.

Determination of Gross Toxicity of Sulfuric Acid Mist

From the physicochemical constants for the vapor pressure of sulfuric acid, we computed the concentration of acid in the aerosol droplets as a function of ambient humidity. Figure 5 shows that, at 50% ambient humidity and at a temperature of 20°C, the equilibrium concentration of the sulfuric acid droplet is about 43% (w/w). If the emitted aqueous aerosol has less than a 43% sulfuric acid content (w/w), it will lose water content and, therefore, particle size. If the concentration of the sulfuric acid is greater than 43% (at 20°C and 50% humidity), the aerosol will pick up moisture from the environment, and particle size will increase until an equilibrium concentration of 43% (w/w) is attained. An extension of the latter circumstance is likely to pertain once the sulfuric acid aerosol enters the physiological environment of the mammalian body through respiration. The humidity gradient from the ambient air (about 50% humidity) outside the nasal or oral cavity to the saturated intrarespiratory environment (100% humidity) effects a rapid dilution of acid concentration with attendant increase in particle size.

In presenting theoretical growth rates of particle size in Quarterly Report No. 3, we did not consider the effects of heat transfer and slip flow. Additional theoretical computations suggest that the time factor "t" (Equations 4 and 5 in Quarterly Report No. 3) was underestimated by a factor of 6.4 when effects of heat transfer were considered. This was calculated from

$$t = 1 + \frac{D_v C_{*_o} \Delta H_v^2}{kMRT_o^2}$$

where t = time of particle size growth (sec)

D_v = vapor diffusion coefficient, 0.26 cm^2/sec

ΔH_v = molar heat of vaporization (4.2×10^{11} erg/g mole)

C_{*_o} = moisture content of ambient air at saturation (4.4×10^{-5} g/cm^3)

k = thermal conductivity of air (2,600 erg)(sec)(cm)(K^o)

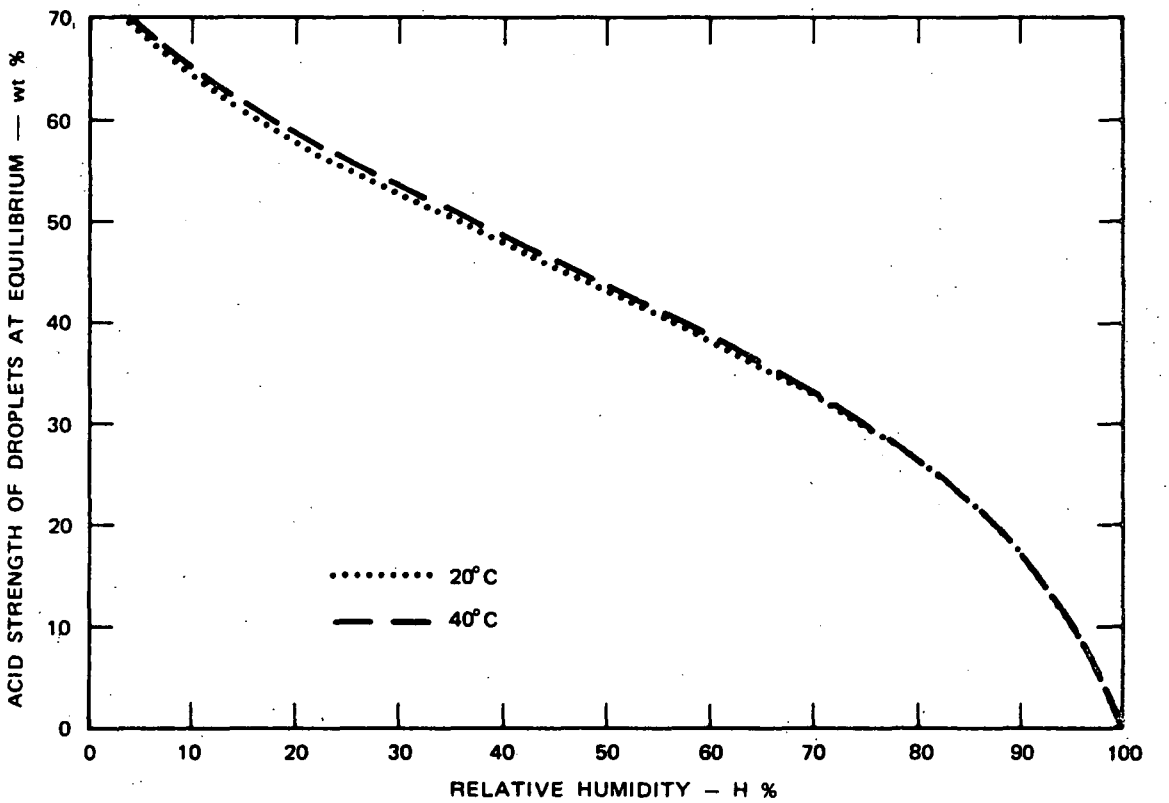


FIGURE 5 EQUILIBRIUM CONCENTRATION FOR SULFURIC ACID AEROSOL AS A FUNCTION OF HUMIDITY

M = molecular weight of water (18 g/mole)

R = gas constant, 8.31×10^7 erg/($^\circ$K)(g mole)

T_o = absolute temperature of ambient air ($^\circ$K)

We conducted four experiments to study the toxicity of sulfuric acid mist to guinea pigs. Two concentrations of stock sulfuric acid were nebulized, each with and without heating the diluting air. Table 2 summarizes the results of these experiments, in each of which six guinea pigs were used.

Table 2

TOXICITY OF SULFURIC ACID MIST TO GUINEA PIGS

Experiment Number	Concentration of Nebulized H_2SO_4 (w/w) (%)	Inlet Airstream-Heater (450°F)	Chamber Concentration of H_2SO_4 (mg/m^3)	Death of Guinea Pigs (LD_{50})
1	50	No	84	24 hr
2	50	Yes	60	24 hr
3	35	No	58	1 week
4	35	Yes	52	1 week

The apparent difference in the concentration of aerosolized sulfuric acid in the chamber with and without the use of the inlet airstream-heater is puzzling. Since neither the rate of acid nebulized into the chamber nor the airflow rate through the chamber differed, the total acid concentration should have remained the same both with and without the use of the inlet airstream-heater. We are considering possible explanations for the difference in acid concentration; among them are the following:

- Because of the reduced median particle size (from 0.73 to 0.46 μ) and median mass (from 2.5 to 1.78 μ)--see Figures 3 and 4--as a function of use of the inlet airstream-heater, the sample collection efficiency may have been reduced for the chemical determination of acid content from the sampled volume of air containing the relatively smaller aerosol particles generated by the inlet airstream-heater.

- The reduced particle size generated by the inlet airstream-heater probably represents greater reactivity of the acid particles with the hardware components (corrosion and other factors), which would be an actual loss of acid content in the chamber.

The relative toxicity of the mist generated by the inlet airstream-heater appears to be greater, which may well be related to the reduced mean particle size. However, on theoretical grounds, as suggested from the information in Figure 5, the concentration of the individual sulfuric acid droplet and its size in the chamber may be different from that reaching the respiratory tissue because of dilution and particle size growth during transit from the environment of 50% chamber humidity to the close-to-saturation humidity of the physiological environment. This change in the concentration of the acid content of the individual acid droplet and the attendant particle size growth would not influence the total dose of acid that enters the nasooropharynx of the individual guinea pig to have a systemic effect.

Charles Lapple and Clyde Witham aided in preparing and evaluating the nature of the H_2SO_4 aerosol.

Examination of Monkeys Exposed to 2 or 9 ppm NO_2 for Nine Years or to 0.9 ppm O_3 for Thirteen Months

Quarterly Report No. 3 presents results of initial studies of this first group of monkeys. We had found that the mature male monkey Cy, manifesting clinical respiratory symptoms and signs, developed elevated erythrocyte and hematocrit counts during the exposure period (Table 6 in QR #3). Cy's CO diffusion rate was found to be reduced relatively early during the nine years of exposure (Table 12 in QR #3). Functional studies conducted before sacrifice of Cy revealed that his transpulmonary resistance to airflow was increased, and the ratio of duration between inspiration and expiration favored prolongation of the latter compared with ratios in control monkeys (Table 12 in QR #3).

A second male monkey, Gr, that had been treated identically but had less evidence of disease clinically was sacrificed subsequently. Although Gr exhibited increased erythrocyte levels during life and reduced rates of CO diffusion, his lungs postmortem were neither heavier nor more voluminous than control lungs, but they did have large depressed areas of atelectasis. These areas were easily reinflated through the trachea and became homogeneous with the rest of the pleural surfaces. Microscopic examination of the lungs revealed disease quite similar to that seen in Cy, except for a lack of disruption of alveolar tissue in the peripheral portions of the parenchyma.

Two mature monkeys, Ls and Gy, that also had been exposed to 2 ppm NO_2 for nine years were examined. Neither of these animals had developed clinical evidence of disease except for elevations in erythrocyte counts early during the exposure period. Their lung-to-body weights were not

different from the average for control animals. In Ls, the lung capacity, when equilibrated at 1 atmosphere of pressure, was 10% greater than the mean value for control animals. Neither the end-expiratory lung capacity nor the capacity at atmospheric pressure was increased in Gy. In neither case could we conclude that the pulmonary capacity of the lungs intrathoracically was not reduced by leakage either through the trachea or pleurae resulting from postmortem manipulation.

Pre- and postmortem physiological studies were conducted principally by Laszlo T. Juhos and Rowena Mussenden-Harvey.

Cy ♂--Nine Years of Exposure to 9 ppm NO_2

Gross Pathology

After the trachea was clamped off following exposure of the thoracic cavity, extremely pale-pink lungs without discoloration bulged from the cavity. They were overdistended and easily removed, the pleurae being free of adhesions. The sharp anterior and diaphragmatic angles of the light and fluffy lungs were pale, blending in with the lighter colored upper lobes. When we touched the surfaces lightly, the pulmonary tissue was indented, and the finger depressions were retained.

The lung capacity at the end-expiratory state (equivalent to functional residual capacity) was increased 176% over that of controls. When the intrathoracic pressure was allowed to equilibrate with the atmosphere, a 37% loss in capacity occurred, and 288 ml of air was retained--an amount 248% greater than that retained by unexposed control lungs. Based on body weight, the lungs of Cy were 42% heavier than control lungs.

The lungs weighed 88.9 g and had an intrathoracic volume of 544.7 ml, which was reduced to 376.6 ml at atmospheric pressure. Thus, the moderate increase in lung weight relative to body weight was overshadowed by the extraordinary volume under the described conditions of pressure. The thoracic cage flared out abnormally and progressively from the apex to the diaphragmatic level, reflecting a large sustained volume during life.

Grossly, the other organs appeared not to have been affected.

Microscopic Pathology

The tissue elements proximal and distal to the alveolar ducts contrasted in response. Parenchymal changes extended peripherally for about three to four alveoli in depth beyond the smallest airways. Bronchiolar and ductal walls and their basement membranes were thicker than normal. Ducts and alveoli contained excessive free cells comprising macrophages, other less phagocytic mononuclear cells, occasional lymphocytes, and dead or dying cells that probably were epithelial and phagocytic

in type. Polymorphonuclear leukocytes also were seen occasionally in the interstitium and among the free cells. In contrast, the more peripheral alveolar walls were strikingly attenuated. Whereas epithelium in the proximal alveoli was readily visible with prominent cells, equivalent but attenuated alveolar epithelium from the distended areas of the parenchyma was identifiable only with considerable uncertainty. Bronchiolar epithelium was hypertrophic, often hyperplastic, and sporadically metaplastic. Capillaries in proximal alveoli were less prominent than normal, and their endothelial cells could not be distinguished with confidence from fibroblasts or muscle cells.

Areas characterized by extremely large air spaces were enclosed by very thin walls that were frequently discontinous from loss of parietal tissue, as shown in Figure 6. Such areas were characteristic of developing emphysema and suggestive of microscopic bullae. Interstitially, collagen was deposited liberally wherever the alveolar, ductal, or bronchiolar epithelium was hyperplastic. This was seen most often in ducts and in affected alveoli where smooth muscle also was abundant. In contrast, collagen was rather sparse in areas of attenuated alveolar walls. Elastic fibers were uniformly distributed, although in attenuated areas they often appeared frayed and fractured.

The other lung of the pair was "respired" mechanically while functional studies were being conducted. Microscopy revealed striking disruption of the parenchyma in areas of distended and attenuated alveoli. Alveolar tissue was fragmented, suggesting that the tissue was more fragile under such stress than normal parenchyma would be.

Microscopic examination of other organs revealed some interesting changes. Randomly situated parenchymal cells, in an otherwise intact liver, were filled with aggregations of particulate pigment, the nature of which is not now known. The pulp of the spleen was "loosely" arranged, and the germinal centers were indistinct. Basement membranes of renal glomeruli were sometimes prominent but not remarkably so. The weight of the intact heart was relatively light for the body, possibly reflecting sedentary behavior imposed by a respiratory limitation to physical activity. The ventricles did not appear disproportionately thick, but they were not weighed separately.

Ls ♀--Nine Years of Exposure to 2 ppm NO_2

Gross Pathology

The lungs, exposed while in the chest, were pink and full and had a ridged surface conforming to the pattern of the rib cage. The more inflated portions were very light pink and contrasted with a few smaller, scattered but circumscribed, depressed red blotches of varying sizes. The lungs on the surface were otherwise light and fluffy and appeared somewhat larger than normal. They weighed 50 g and occupied 213.4 ml of space while under intrathoracic pressure. At ambient pressure,

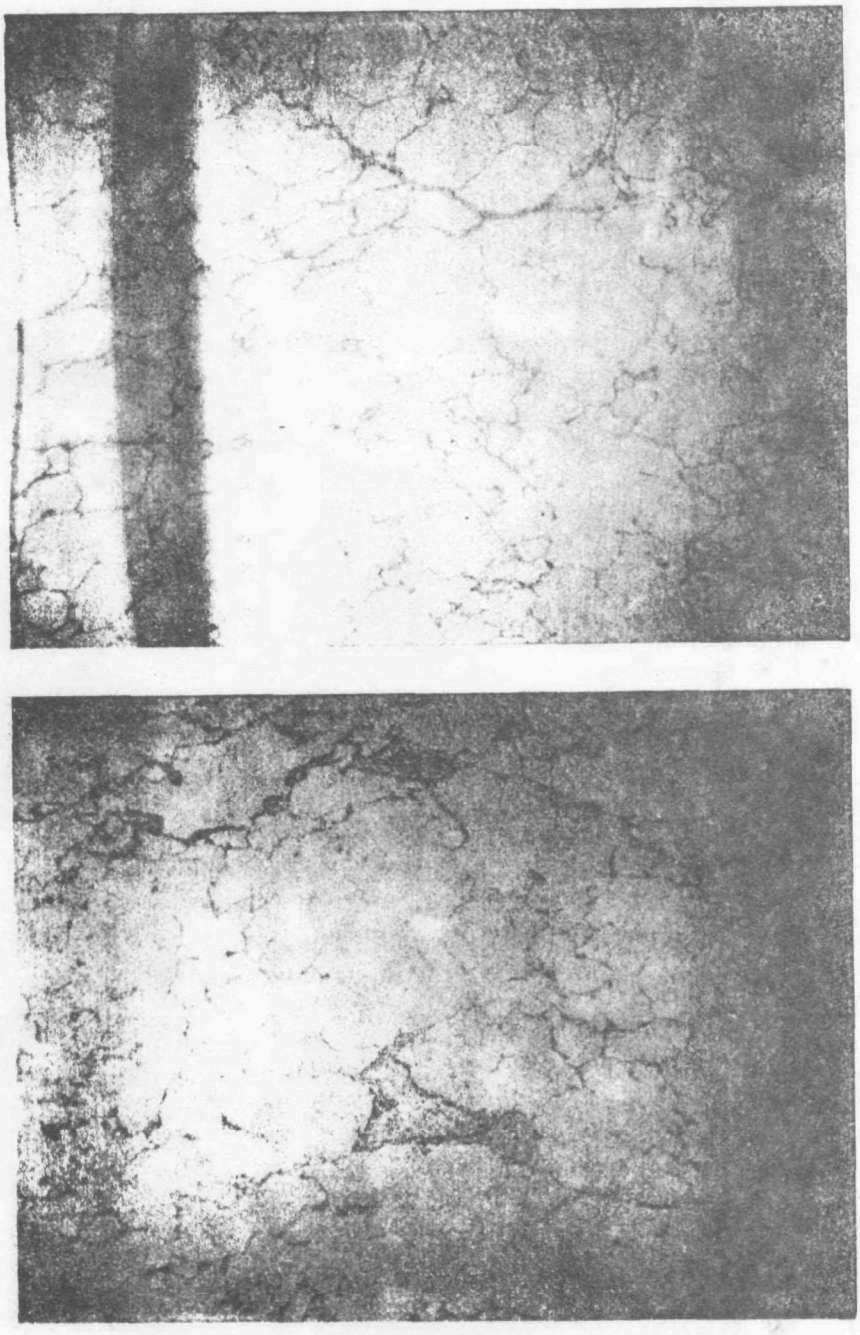


FIGURE 6 EMPHYSEMA FOLLOWING NINE YEARS OF EXPOSURE TO ABOUT 9 ppm NO_2 IN MONKEY Cy

H&E stain of 4-μ paraffin section
20x magnification

the volume fell to 141.4 ml. The volume-to-weight relationship of the lungs was slightly but significantly increased over normal.

The remaining viscera were not unusual in gross appearance.

Microscopic Pathology

A modest degree of distension of the alveoli was evident in some portions of the lungs. The long respiratory bronchioles of Ls were distinguished by thickening with subepithelial connective tissue, smooth muscle, and tall, hypertrophic epithelium. The epithelium was variously columnar or cuboidal or was composed of enlarged, rounded pseudosquamous or hyperplastic cells. The prominence of the peripheral bronchiolar epithelium contrasted with the relatively flat, squamous type normally seen in the monkey. Figure 7, prepared by Dr. Robert J. Stephens, demonstrates hyperplasia. Collagen was more plentiful than usual both in the respiratory bronchioles and in the walls of the alveolar ducts. The alveoli, however, were relatively unaffected. Histologically, elastic fibers intermingled with collagen and appeared to be "teased apart" at affected sites such as those underlying hypertrophic epithelium.

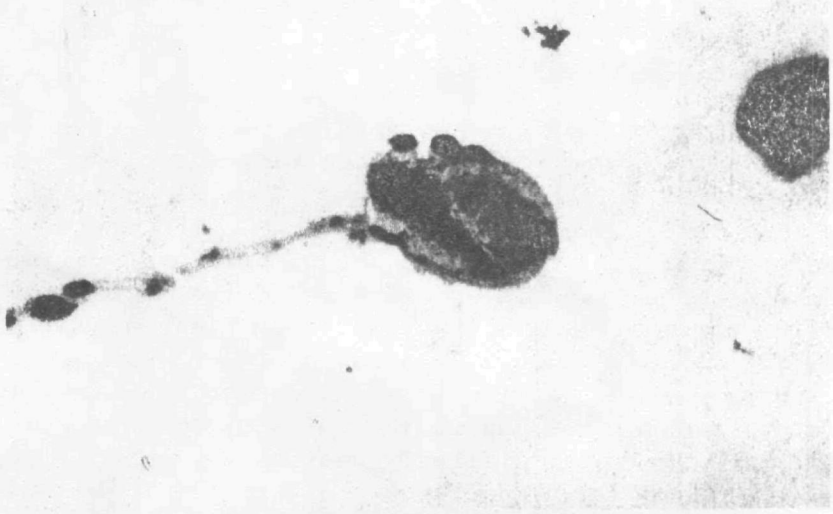
Other organs had no remarkable changes.

Rh ♀--Thirteen Months of Exposure to 0.9 ppm O_3

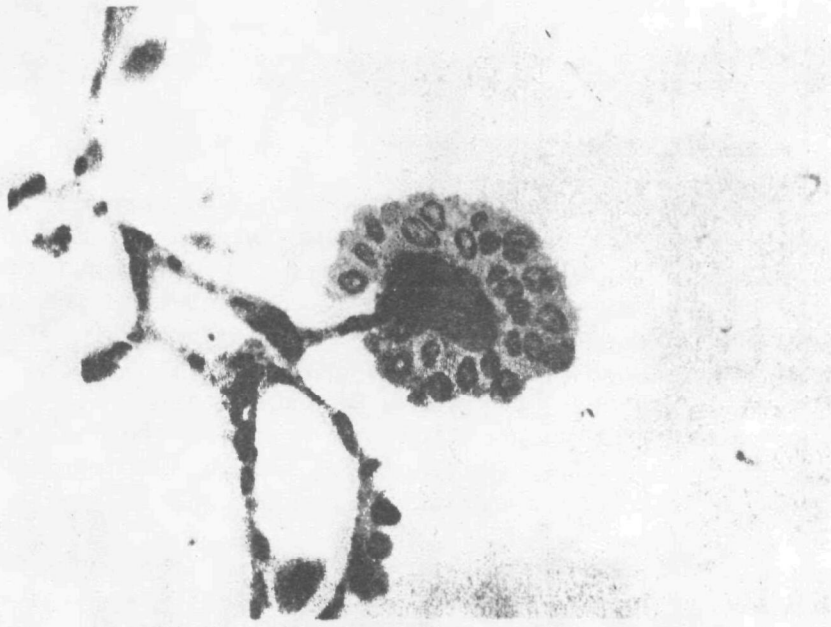
Gross Pathology

In addition to the group of monkeys exposed to NO_2, one animal (Rh) was observed for 13 months during continuous exposure to 0.9 ppm O_3. We sacrificed Rh to compare pathological effects with those in the NO_2 group. Although Rh did not manifest clinical evidence of pulmonary disease (functional pulmonary tests were not made *in vivo*), postmortem her lungs were found to be 34% heavier per unit of body weight than normal control lungs. Rh's lungs appeared to be inflated at the intrathoracic end-expiratory position and proved to be 8.4% greater in volume than normal lungs. They continued to retain 30% more air than lungs of control animals under atmospheric conditions.

In the fixed end-expiratory state, the lungs appeared overinflated in the opened thoracic cavity. They were uniformly light pink except for the angular lobar margins anteriorly, and, at the costophrenic angle, where the pleurae overlaid white, translucent alveolar tissue extending as a marginal border 1- to 2-mm in breadth. The lungs were dry and fluffy. Removal of the tracheal plug initiated release of air under atmospheric pressure, but the lungs maintained an inflated appearance. The light-pink surfaces were marked homogeneously with small, spider-like vessels radiating in several directions from their centers for about 0.5 mm, suggesting an anatomically defined distribution. These vascular units appeared a few millimeters apart over the pleural surfaces. The



(a) NORMAL ALVEOLAR DUCTAL EPITHELIUM COVERING MUSCULAR RING



(b) HYPERPLASIA OF ALVEOLAR DUCTAL EPITHELIUM

FIGURE 7 RESPONSE TO EXPOSURE TO 2 ppm NO_2 FOR NINE YEARS IN MONKEY Ls
Toluidine Blue stain of 1-μ plastic section
40x magnification

posterior surfaces of the right lower and left upper lobes revealed retracted (atalectatic), beefy-red areas occupying much of the pleural surface. The lungs had at least twice the expected normal volumes at intrathoracic and atmospheric pressures--237 and 165 ml, respectively.

Microscopic Pathology

The principal changes occurred in the respiratory bronchioles and alveolar ducts in which the subepithelial interstitial tissue was considerably thicker and less compact than normal. This area appeared to be the site of fibroblastic activity. The epithelium at this level was hypertrophic, having rounded, cuboidal cells in place of the usual flatter or squamous type. The thickened ductal septa extending into the proximal alveolar walls also contained fibroblast-like cells. A characteristic feature of these lungs was the abundance of pigment-loaded macrophages that aggregated in the affected proximal alveoli and in the interstitial tissue. In addition to these, other mononuclear cells were evident within the broad septa. The more proximal, uninterrupted bronchioles were well ciliated, whereas the respiratory portions were not. Deposition of collagen appeared excessive in the thick walls where "active" fibroblasts could be seen. The remaining viscera did not appear to have been affected.

Rh had developed evidence of polycythemia during her lifetime, similar to that seen in the monkeys exposed to NO_2. The only basis for comparison of Rh with the monkeys exposed to NO_2 was that pulmonary disease was induced by exposure to atmospheric "oxidants."

Pulmonary Cell Renewal Rates

In determing the replication rates of pulmonary cells in monkeys, Dr. Michael J. Evans found the rates for normal unexposed monkeys to be similar to those for normal, 11- and 19-month-old rats, as shown in Table 3.

The turnover rates for terminal bronchiolar epithelial cells and for Type 2 alveolar epithelial cells at the time of sacrifice of the more affected monkeys exposed to either 9 ppm NO_2 or 0.9 ppm O_3 were very significantly elevated. In the monkey exposed to 2 ppm NO_2 and in the one exposed to 9 ppm (whose disease was less advanced), evidence of increased labeling of DNA with tritiated thymidine was uncertain. The "other" category of cells in Table 3 comprises all other cell types in the bronchiolar and alveolar areas such as endothelial, migratory, and fibroblastic cells.

Hematological Changes in Monkeys

The principal hematological effect caused by exposure of monkeys to NO_2 and to O_3 was an average elevation of the red cell counts. In

Table 3

PULMONARY CELL RENEWAL RATES

	Cell Type (Labeled Cells/1000 Cells)*			
	Terminal Bronchiolar	Type 2 Pneumonocyte	Alveolar Macrophage	Other
Rats (11 mo), controls†	-	0.2 ± 0.2	0.1 ± 0.1	8.0 ± 3.3
Rats (19 mo), controls†	-	0.5 ± 0.4	0.2 ± 0.2	3.8 ± 3.0
Monkey An, control	0.1	0.5	0.1	5.0
Monkey Br, control	0.1	0.5	0.1	6.5
Monkey In, control	0.1	<0.1	0.1	2.0
Monkey Ls, 2 ppm NO_2‡	0.6	0.1	1.0	6.0
Monkey Cy, 9 ppm NO_2‡	4.0	5.0	<0.1	19.0
Monkey Rh, 0.9 ppm O_3**	2.3	8.0	0.3	5.6
Monkey Gr, 9 ppm NO_2‡	-	0.5	0.0	3.5
Monkey Gy, 2 ppm NO_2‡	-	0.5	0.0	2.5

*3000 cells counted.

†Six rats in each group.

‡Exposed for about 9 years.

**Exposed for about 13 months.

those animals exposed to 9 ppm NO_2, the rise was more than 1 million/mm^3 over controls during the first year and about 3 million greater during the second year of continuous exposure. During the following four years, the red cell concentration tended to fall toward normal, and normal levels were attained by the ninth year of exposure. Monkeys exposed to 2 ppm NO_2 exhibited a similar pattern, but it was characterized by a slower rate of increase, a peak of somewhat less than 2 million over normal during the second year, and a slow return to normal by the sixth year; normal levels were maintained into the ninth year.

The hematocrit counts rose with the elevation of red cell counts in animals exposed to 9 ppm NO_2 but to a lesser extent proportionally. These counts did not change in monkeys exposed to 2 ppm NO_2. The hemoglobin levels rose, as did the red cell counts, only during growth and maturation of the monkeys but not in relation to elevations in cell numbers based on exposure to NO_2. Simultaneously, the mean corpuscular volumes (MCVs) fell during maturation of both normal and exposed animals, while their red cell counts were rising as part of their natural growth. Then, as polycythemia developed with exposure to NO_2 (but not in controls), the MCVs were reduced in proportion to the elevation of cell counts and elevated to normal as the polycythemia waned during the latter years of exposure to NO_2. Accordingly, the microcytosis was exaggerated in the group of monkeys exposed to 9 ppm NO_2. The mean corpuscular hemoglobin concentration remained steady, indicating an increase in overall red cell surface area during phases of polycythemia based on increased numbers of smaller cellular units.

The leukocyte counts varied widely but manifested a tendency for the ratio of polymorphonuclear cells to lymphocytes to rise with elevations of total white cell counts. This was more pronounced in animals exposed to 9 ppm NO_2.

Hematological studies were conducted by Nazzareno J. Furiosi.

SUMMARY OF LONG-RANGE STUDIES OF MONKEYS

For approximately nine years, we have been observing monkeys (Macaca speciosa) being exposed to either 2 or 9 ppm NO_2. These animals have now reached maturity, and some are middle aged. At least one of these monkeys appeared to have developed sufficient signs of chronic respiratory disease to warrant a pathological study. Several other animals also were sacrificed for comparative purposes. Half of the experimental animals, including some that were born in the exposure chambers while their parents were being exposed, are being investigated exhaustively.

The first five monkeys examined in this experiment comprised three unexposed control animals and one each from groups that had been exposed to either 2 or 9 ppm NO_2 for nine years. Included for comparison was one that had been exposed for more than one year to 0.9 ppm O_3. The findings suggest that

- The lesions produced by NO_2 and by O_3 in monkeys closely resemble those occurring in rats exposed to higher concentrations of NO_2 for up to two years and in rats exposed to 0.9 ppm O_3 for much shorter periods.

- The attenuation and disruption of peripheral alveolar structural tissue in one monkey that was exposed to 9 ppm NO_2 appear to be coincident with architectural changes in the smallest airways and associated proximal alveoli. The changes suggest that airflow between the peripheral alveoli and the bronchial airways was impeded by intervening features obstructive to airflow. The changes are both structural (due to hypertrophic epithelium and extracellular tissue in small airways and in adjacent alveolar walls) and inflammatory (due to free macrophages, other "inflammatory" cells, and organizing secretion and fibrinous exudate).

- The enlarged air spaces resulting from disruption and loss of alveolar tissue in the animal that was exposed to 9 ppm NO_2 is characteristic of chronic pulmonary emphysema.

- The morphological changes in the lungs of the monkey exposed to 2 ppm NO_2 for nine years are similar but much less advanced. There is no evidence of destruction of parenchyma.

- The effects of O_3 on pumonary tissue also are found mainly in the smallest airways, but the affected level occupies a slightly more peripheral segment of the airway system (the respiratory bronchiole and the alveolar duct) than do the effects of NO_2. This difference was seen also in the rat. The macrophage response

to injury of tissue by O_3 is more striking in the monkey than in the rat, although this may be attributable to large differences in relative dosage and duration of exposure.

- In three of five exposed animals, residual lung volumes were increased significantly over normal residual volumes.

The overall objective of these long-range experiments is to develop a perspective of the insidious and protracted development of chronic obstructive pulmonary disease in man. These investigations are a logical extension of those in which a parallel disease was induced during the relatively short lifetime of the rat through exposure to either NO_2 or O_3. The Macaca speciosa has an estimated life span intermediate between that of man and the rat; thus, we included this species to introduce temporal and genetic reality into the perspective of studies of chronic obstructive lung disease. The first compelling clinical evidence of developing pulmonary disease in the monkeys coincided with the administrative termination of the 1975 contract period.

Thus, in retrospect, we are describing threshold effects in terms of the total development of chronic obstructive pulmonary disease. Four monkeys, two of which were exposed to 9 ppm and two to 2 ppm NO_2, are now being compared with four similar animals that lived under parallel conditions in clean air. All four exposed animals developed similar morphological pathogenetic evidence of pulmonary disease, but only one had reached a state consistent with the early stage of emphysema seen in humans. These borderline but highly significant observations indicate the need for further specific definition of the roles of NO_2 and O_3 in the induction of the etiologically obscure and intractable chronic obstructive pulmonary disease.

Following completion of the investigation of half of the experimental animals, we will design a meaningful approach for studying the remaining monkeys. The unqualified raw data provided in earlier quarterly reports are being examined for validity and statistical significance. Such data include hematologic factors such as erythrocyte and leukocyte counts, differentials, hematocrits, hemoglobin levels, fragility tests, mean corpuscular volumes and mean corpuscular hemoglobin concentrations, and blood chemistry values for methemoglobin, ATP, 2,3-DPG, glutathione peroxidase and reductase, G-6-PDH, and red cell cholinesterase. Circulating blood gas levels are being reviewed, and vitamin E concentrations found portmortem in tissue, the lungs, the liver, and muscle are being evaluated also and will be reported.