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EFFECTS OF INHALED PARTICULATE MATTER

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ABSTRACT

Controlled laboratory studies were conducted utilizing two experimental animal models (Fischer-344 SPF male rats), one healthy and one impaired with elastase-induced emphysematous lung disease, to evaluate the nature and severity of responses to inhaled respirable aerosols (0.55 mg/m^3 to 3.0 mg/m^3) that were especially synthesized with selected realistic inorganic constituents to be typical of air pollution episodes in California, alone and in combination with ozone (0.4 ppm). Parallel comparative inhalation studies were performed with a respirable London-type aerosol (0.8 to 5.0 mg/m^3) typical of the the famous London smog episodes, alone and in combination with sulfur dioxide (1 to 21 ppm). Exposures were acute (3 day) or subchronic (30 day). The deleterious effects of the exposures were evaluated with selected lung biochemical measurements, measurements of lung particle clearance and permeability (using radiolabeled test aerosols), clinical signs of illness, and histological evaluation of lung inflammatory responses and structural alterations.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. In the 3-day studies, there were some significant increases in total lung DNA and protein content in rats exposed to the synthetic California-type aerosol compared to rats not exposed to this aerosol. In addition, small airway inflammation was observed in animals exposed to ozone, and this effect was significantly exacerbated by the inhalation of either aerosol and in impaired animals. There was no observed aerosol concentration dependence. In the 30-day studies, biochemical analyses showed increases in the lung content of hydroxyproline (indicative of collagen synthesis and potential lung fibrosis) in rats exposed to the California-type aerosol, and to London-type aerosol with SO_2 in impaired rats. Both aerosols tended to decrease the rate of tracheobronchial clearance of deposited test particles. There was no change in alveolar permeability. Exposure to ozone caused small airway lesions in the lungs and this was significantly exacerbated in impaired animals and by the inhalation of aerosols by impaired animals. Small airway fibrosis was caused by ozone, and this was significantly exacerbated by exposure to California-type aerosols. This study is apparently the first to demonstrate that aerosol/ozone interactions occur with atmospheres of composition and concentration similar to those found in the environment.

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DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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SUMMARY AND CONCLUSIONS

This project utilized two experimental animal models, one healthy and one impaired (Fischer-344 SPF male rats) in controlled laboratory studies to evaluate the nature and severity of responses to two types of inhaled particulate matter. One of these aerosols was synthesized so as to have constituents that are typical of California urban aerosols alone and in combination with 0.4 ppm ozone at levels that would be associated with episodes of elevated air pollutants. In addition, parallel comparative studies were performed with a London-type aerosol alone, and in combination with sulfur dioxide, associated with past London smog health effects. Both aerosols contained acidic sulfates. Half of the animals were impaired by instilling elastase intratracheally which resulted in a condition comparable to human emphysema with breakdown of lung alveolar septa and dilation of some air spaces. The exposures conducted in this project were designed to simulate acute air pollution episodes of unusually elevated concentrations that last for about three days. In addition, one 30-day subchronic episode was also designed to illustrate a worst case situation of a prolonged pollution episode, and to evaluate the consequences and progression of the acute effects. The aerosols consisted of mixtures of components formulated to provide synthetic, laboratory versions of the two types of pollutant atmospheres with appropriate respirable particle size distributions. The California-type aerosol consisted of ammonium sulfate, ammonium bisulfate, ammonium nitrate, graphitic carbon, natural clay mineral, and traces of lead, vanadium, nickel, and manganese. The London smog-type aerosol consisted of ammonium sulfate, ammonium bisulfate, coal fly ash, and carbon soot. Both aerosols were formulated to be acidic (pH = 1.8).

In developing the impaired rat lung model for these studies, an elastase dose of 25 U/100g body weight (instilled in the lung) appeared to be effective but generally non-lethal in producing alveolar emphysema-like lesions. The risk of developing post-instillation pneumonia in elastase treated rats was eliminated by the use of pathogen-free rats.

There were six 3-day exposures followed by a single 30-day exposure (23 hours per day), each utilizing a separate set of previously unexposed rats. All atmospheres utilized precleaned air at 80% relative humidity at 23°C. In the first 3-day study, rats were exposed to 0.55 mg/m^3 of California-type aerosol with and without 0.4 ppm ozone, to ozone alone, and with unexposed controls. In the second, the aerosol was increased to 1.45 mg/m^3, and in the third to 3 mg/m^3. The fourth acute exposure utilized 0.9 mg/m^3 of the London-type aerosol with and without 1.1 ppm of sulfur dioxide. The fifth exposure increased the aerosol concentration to 5 mg/m^3 with 21 ppm sulfur dioxide. The sixth exposure was a hybrid exposure utilizing 3 mg/m^3 of the London-type aerosol with 0.4 ppm of ozone. The seventh exposure was a continuous subchronic 30-day exposure of rats to 1 mg/m^3 California-type aerosol with and without 0.4 ppm ozone and 1.1 mg/m^3 of London-type aerosol with 1 ppm of sulfur dioxide.

The effects of the exposure were evaluated in several contrasting tests. Using nuclear medicine techniques, measurements were made of test particle lung clearance and lung permeability after brief inhalation of ^{99m}Tc-radiolabeled respirable test aerosols of DTPA (for lung permeability measurements) and iron oxide (for tracheobronchial particle clearance measurements). Histological observations were made of lung inflammatory responses and other structural alterations including small airway fibrosis. Observations were also made of clinical signs and symptoms of illness. In addition, measurements were made of specific lung biochemicals that are known to relate to potential health effects including, DNA, RNA, and protein content, and the content of hydroxyproline (indicative of collagen synthesis and potential lung fibrosis).

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. Overall, in 3-day studies, California-type aerosol exposure (1.45 mg/m^3) resulted in a significant increase in lung DNA and protein in exposed rats; while London type aerosol (5 mg/m^3), sulfur dioxide (21 ppm), and London type aerosol with sulfur dioxide yielded no observed significant biochemical or pathologic effects. Impaired rats had statistically significant increases in lung DNA and RNA, and this response was significantly exacerbated by exposure to London-type aerosol or

sulfur dioxide. Significant small-airway inflammation was observed in rats exposed to California-type aerosol (0.55 mg/m^3 or 1.45 mg/m^3) or ozone (0.4 ppm), and the combination of either California-type aerosol or London-type aerosol (3 mg/m^3) with ozone significantly exacerbated this response. Lung impairment also exacerbated the response. This augmentation of inflammation was not a quantifiable dose-response function of aerosol concentration but occurred at the lowest concentration studied (0.55 mg/m^3).

Likewise, in the 30-day study, neither aerosol was effective by itself in causing significant responses in healthy rats, but some aerosol effects were observed in association with ozone exposure or lung impairment. California-type aerosol (1 mg/m^3) exposures resulted in significant increases in lung hydroxyproline and decreases in lung RNA in exposed rats and showed a tendency to reduce the rate of tracheobronchial clearance of inhaled test particles. London-type aerosol (1 mg/m^3) with sulfur dioxide (1.1 ppm) showed a tendency to decrease the rate of tracheobronchial clearance and exacerbated increases in lung hydroxyproline observed in impaired rats. Both aerosol resulted in significant pigmentation of lung tissue that could be identified as deposited carbonaceous particles. Ozone (0.4 ppm) caused lung small-airway lesions, observable small airway fibrosis of the lung, and increases in rate of tracheobronchial clearance of inhaled test particles. Increased rates of tracheobronchial clearance also occurred when ozone and California-type aerosol were combined. There was exacerbation of the observed small airway fibrosis and synergistic exacerbation of the lung pigmentation by deposited particles by the combination of California-type aerosol and ozone. The combination of California-type aerosol and ozone in impaired rats led to a synergistic exacerbation of lung small airway lesions. London-type aerosol with sulfur dioxide significantly increased the lung content of hydroxyproline in impaired rats. Impaired rats had small-airway lesions associated with emphysema and increased lung DNA and hydroxyproline whether exposed to pollutants or not.

The acute and subchronic studies were similar in showing a somewhat lesser effectiveness for London-type aerosol, even with SO_2, than California-type aerosol in treatment-related significant biochemical or pathologic changes in exposed rats. Both showed that the combination of California-type aerosol and

ozone tends to exacerbate the small airway inflammation caused by ozone, but this was only apparent in impaired rats in the subchronic study. However, the observed lung fibrosis associated with ozone in the subchronic studies was exacerbated by exposure to California-type aerosol. The subchronic study showed significant increases for ozone and a tendency for both aerosols to decrease tracheobronchial clearance of test particles. Also, lung small airway lesions and remodeling were associated with ozone exposures in the subchronic study but not after acute 3-day exposures.

The following is an overview in semi-outline form of the whole project from the perspective of the initial objectives and goals of this investigation. There were five major objectives, all related to better understanding the effects of inhaled particulate matter present in California air.

The main objectives of the project and the results were as follows:

(1) Evaluation of acute and subchronic responses of the lung to inhaled London-type aerosol (LT) or California-type aerosol (CA), alone, and in combination with episodal levels of ozone (California) or sulfur dioxide (London), respectively.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. In the 3-day studies, there were some important observed differences in pulmonary response attributable to differences in the composition of the two aerosol types. Inhalation of California-type aerosol resulted in the appearance of statistically significant changes in the biochemistry of the lung in healthy rats in both the acute (increased lung DNA and protein) and subchronic studies (increased lung DNA and hydroxyproline and decreased RNA) that were not observed with the London-type aerosol. Both aerosols increased, to a similar extent, the acute small airway inflammation caused by ozone exposure. London-type aerosol interacted with elastase pretreatment (in impaired rats) to increase lung DNA content in a synergistic way in the acute studies while California-type aerosol did not.

(2) Quantification of specific responses that relate to health effects in human populations, including epithelial damage (measured as lung permeability), lung clearance impairment, biochemical responses (indicative of potential fibrosis and/or inflammation), inflammatory cell accumulation in the lung, and cellular level abnormalities in the lung.

Parameters which provided significant responses indicative of potential lung injury in the 3-day exposure studies included small-airway inflammation and lung biochemical changes in DNA, RNA and lung protein. No treatment-associated differences were detected in tracheobronchial clearance or lung permeability. For the 30-day exposure studies, the significant responses included treatment-associated changes in particle accumulation, small airway inflammation, structural changes in small airway walls, lung small airway fibrosis, tracheobronchial particle clearance, hydroxyproline synthesis and lung nucleic acid content.

(3) Comparison of responses between London-type aerosol (LT) (with and without sulfur dioxide) with California-type aerosol (CA) (with and without ozone); the purpose of this comparison is to relate the basis (London episodes) of current air pollution standards to appropriate standards in California.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some aerosol effects were observed in association with ozone exposure or lung impairment. In the 3-day studies, there were significant lung biochemical changes in rats exposed to the California-type aerosol were not observed with the London-type aerosol. Hence, there is an indication that the California-type aerosol may be somewhat more hazardous. It is clear that the effects and responses to California-type aerosol in urban air are inextricably linked to the simultaneous exposure to elevated ozone levels, just as the London smog episodes cannot be used to separately identify effects associated with particles from those associated with sulfur dioxide (USEPA, 1982). However, the combination of California-type aerosol and ozone was much more effective in causing a variety of significant detrimental pulmonary alterations than the combination of London-type aerosol and SO_2.

(4) Comparison between responses of impaired individuals (modeled by simulated emphysematous lung disease in elastase pretreated rats) versus healthy individuals.

An increase, sometimes significant, of several responses to air pollutants were demonstrated in impaired individual because of the lung impairement. Biochemical alterations in the lung induced by impairment where enhanced by exposure to London-type aerosol, sulfur dioxide, and ozone. Impaired rats were significantly more susceptible to small-airway disease and fibrosis elicited by aerosols in combination with ozone and were observed to have greater retention of lung deposits of particulate matter. The statistically signficant effects associated with inhalation of California-type aerosol were, in part, based upon the influence of lung impairment on the sensitivity of the exposed rats.

(5) Comparison of effects elicited by acute and subchronic exposures.

The subchronic exposures led to changes in tracheobronchial clearance that were not found in the acute studies. Also, increased hydroxyproline, quantifiable small airway lesions, and apparent accumulations of particles were found in the subchronic studies, but not the acute studies.

In summary, these findings indicate that the presence of particulate matter in California air may have a greater effect on health than the aerosol found in the London smog episodes of thirty or more years ago. Especially important is the promotional or synergistic effectiveness of inhaled aerosols of the California-type in exacerbating the injury known to be associated with the inhalation of environmental ozone.

The impaired rats with experimentally produced emphysema (elastase pretreated) had abnormal biochemical and morphological characteristics. Elastase pretreatment was found to significantly augment the inflammatory responses and lung structural remodeling (small airway lesions) observed for exposures to aerosols and ozone. These results suggest persons with impaired lungs are at greater risk for exposure to inhaled particulate matter both with and without ozone.

RECOMMENDATIONS

Airborne particulate matter in the respirable size range in California needs to be monitored and controlled to minimize the adverse responses in people, especially those with impaired lung function. Concern should address effects associated both with inhalation deposition and with the promotion and exacerbation of adverse responses from environmental ozone. A concentration of 0.55 mg/m^3 of California-type respirable aerosol exacerbated ozone-induced changes in the lungs of rats exposed for three days. Therefore, an ambient air inhalable (PM_{10}) standard of 0.05 mg/m^3 (based on a 24-hour sample), which is one-tenth of this concentration, appears both reasonable and prudent. Persons with emphysema or other pre-existing lung disease should be expected to be more susceptible to adverse responses to inhaled particulate matter based upon the results in impaired rats.

Future studies should consider further the altered tracheobronchial clearance of inhaled particles and the implications associated with the possible reduced lung clearance of various toxic or infectious agents inhaled in combination with particulate air pollutants. More information is also needed concerning the behavior of deep lung particle clearance associated with inhaled particulate matter. In addition, the mechanisms that lead to the exacerbation by inhaled airborne particulate material of the adverse responses associated with ozone need to studied in more detail. Since the aerosols used in these studies were acidic, the role of aerosol acidity in this promotional phenomena needs to be tested, verified, and quantified. Future studies should also include organic aerosols and vapors as well as inorganic constituents in the exposure atmospheres since these are also present in polluted air.

INTRODUCTION

Airborne particulate matter in association with pollutant gases has been implicated in observed health effects associated with serious pollution episodes such as those associated with the famous London smog episodes and suggested to be involved in potential health effects in California. Epidemiological studies show an inseparable correlation between responses and the concentrations in combination of sulfur dioxide and aerosol particulate matter. Elevated relative humidity is considered to be a contributing factor (U.S. EPA, 1982). This study was designed to elucidate the possible augmentation or synergism of systemic injury that might be associated with inhaled airborne particulate matter and specific gaseous pollutants. These are taken up by the respiratory airways of people if inhaled and may lead to a variety of undesirable biological responses including reduced lung clearance of inhaled particles that deposit in the lung, and exacerbation of existing respiratory abnormalities.

Current air pollution standards for airborne particulate matter are based primarily upon acute mortality and morbidity associated with episodes of high particle and sulfur dioxide pollution in London, England, in the 1950's and on other acute episodes of elevated levels of total suspended particulate material and sulfur dioxide in the United States and elsewhere in the world (U.S. EPA, 1982). In October, 1948, high levels of particulate matter (probably as high as 1 mg/m^3) and sulfur dioxide (estimated to be above 0.4 ppm) severely affected 8% of the population of Donora, Pennsylvania, and resulted in twenty deaths (Schrenk, et al., 1949). A four-day pollution episode also occurred on December 5-9, 1952, in London with average particulate matter concentrations measured at from 1.98 mg/m^3 to 2.65 mg/m^3 and average sulfur dioxide concentrations from 0.94 ppm to 1.26 ppm (Wilkins, 1954). Four thousand excess deaths were reported during this London smog episode and were readily correlated to elevated particulate matter (up to 4.46 mg/m^3) and sulfur dioxide (up to 1.34 ppm) concentrations (Logan, 1953). Other episodes in the U. S. in the 1950's and 1960's in Detroit (September, 1952), New York City (November, 1953; December, 1962; January, 1963; March, 1964) showed morbidity

and mortality relationships for particulate matter levels from in excess of 0.2 mg/m^3 to 0.88 mg/m^3 and sulfur dioxide levels from 0.4 to 1 ppm (U.S. EPA, 1982). Studies of mortality and morbidity in subchronic exposure over polluted months show a similar association between elevated sulfur dioxide and airborne particulate matter. Most deaths occurred with people having pre-existing illness, especially lung diseases (U.S. EPA, 1982).

The close correspondence between elevation of concentrations of sulfur dioxide and particulate matter have made it impossible to conclusively show that either of these pollutant entities alone was separately responsible for the observed serious health effects; consequently, air quality criteria have had to be established on the assumption that the elevation of either pollutant alone could have been the cause. This has led to separate standards that may have a measure of built-in safety. Hence, the national ambient air quality standards for acute exposure to these pollutants (24-hour total suspended particulate material levels of 0.26 mg/m^3, now replaced by 24-hour PM_{10} levels of 0.15 mg/m^3, for particles, and 0.5 ppm for sulfur dioxide) are based upon criteria that jointly consider these pollutants (U.S. EPA, 1982). The advent of size-selective sampling of particles that are inhalable (smaller than about 10 micrometer in aerodynamic equivalent diameter) has led to the new 24-hour ambient air quality particulate matter standard called PM_{10} that considers more appropriately the small inhalable particles. The California ambient air quality standards involve a degree of greater safety than the Federal standards, especially for effects in children, with the 24-hour standard for PM_{10} being 0.05 mg/m^3 for particulate matter and 0.05 ppm for sulfur dioxide.

The aerosols found in California air pollution are quite different in composition than those associated with the London smog and other serious pollution episodes noted above. In addition, California has little sulfur dioxide, but does have elevated levels of reactive oxidants (as ozone). These are probably more injurious to the deep lung tissue than sulfur dioxide, which is largely absorbed in the head and upper respiratory airways. Hence, there is a need to critically study the potential biological effects associated with California-type (CA) aerosols and compare these results to London-type (LT) aerosols, and to further compare the influence of the respective reactant

gases, ozone and sulfur dioxide. The differences in the constituents of the two types of aerosols (LT & CA) are discussed below. They provide a basis for establishing the appropriate constituents for synthetic versions of these two types of particulate matter used in this study.

The overall purpose of this project was to study the potential for systemic injury in both healthy and impaired laboratory animals (male rats) of a synthetic California-type aerosol and to compare those effects with responses to London-type aerosol. The California-type aerosol was administered to laboratory animals both with and without a typical elevated concentration of ozone. The counterpart gaseous pollutant for London-type aerosol was sulfur dioxide. Hence, the experimental design of this study was aimed at direct comparison of the dose response relationships for the two types of air pollution. In order to consider the strong indication that pre-existing disease is a major risk factor for people exposed to air pollution, both healthy and impaired animal models were studied. Since systemic injury to the lung is the principal response of concern, the biological evaluations focused on respiratory effects, while considering general health status as well. Key evaluations included histopathology of the lung, lung biochemistry, and lung permeability and particle clearance.

PROJECT OBJECTIVES

Utilizing two experimental animal models, one healthy and one impaired (Fischer 344 SPF male rats), this project tested the hypothesis that the inhalation of episodal levels of typical aerosols and gases associated with air pollution would result in adverse biological responses including systemic injury to the respiratory tract and impairment of lung clearance of potentially toxic particles. Both acute (3-day) and subchronic (30-day) exposures were tested.

Specific objectives included:

(1) Evaluation of acute and subchronic responses of the lung to inhaled London-type aerosol (LT) or California-type aerosol (CA) alone and in combination with episodal levels of ozone (California) or sulfur dioxide (London).

(2) Quantification of specific responses that relate to health effects in human populations including epithelial damage (measured as lung permeability), lung clearance impairment, biochemical responses (indicative of potential fibrosis and/or inflammation), inflammatory cell accumulation in the lung, and cellular level abnormalities in the lung.

(3) Comparison of responses between London-type aerosol (LT) with and without sulfur dioxide with California-type aerosol (CA) with and without ozone for the purpose of relating the basis (London episodes) of current air pollution standards to appropriate standards in California.

(4) Comparison of responses in impaired individuals (simulated emphysematous lung disease in rats pretreated with elastase) to healthy individuals.

(5) Comparison of effects elicited by acute and subchronic exposures.

TECHNICAL PLAN

Overall Design.

In acute exposure studies, Fischer-344 rats were exposed for twenty-three hours daily for three days to simulate a peak air pollution episode at (a) the chosen concentration of from 0.55 mg/m3 to 5 mg/m^3 of either the London-type aerosol (LT) or California-type aerosol (CA), (b) the same level of aerosol in combination with 0.40 ppm ozone (CA) or from 1 to 21 ppm sulfur dioxide (LT), and (c) clean air, in four 4 m^3 stainless steel and glass exposure chambers capable of accommodating 24 rats in a monolayer. Of the rats in each chamber (with associated pollutant environment), 12 were healthy (H) and 12 were impaired (I) with induced alveolar emphysema caused by intratracheally instilled elastase. Additional healthy rats in each chamber were used as sentinels for viral screening performed at the conclusion of the exposures to complement pre-exposure screening. Of the 12 rats of each type in each chamber 6 went first to lung particle-clearance evaluation after sacrifice and then to biochemistry, while the other six went to pathological evaluation and clinical chemistry. This experimental design for the 3-day studies is illustrated schematically in Figures 1 and 2. Because these studies were conducted to contrast California-type aerosols with London-type aerosols, the exposures were designated by the prefix CL, e.g., CL-1 was the first 3 day exposure series. There were six acute exposure studies, CL-1 through CL-6.

In the original design there were to be three exposures to different concentrations of each of the two aerosol types shown in Figures 1 & 2, respectively. The third London-type aerosol exposure was changed to include ozone at 0.4 ppm instead of sulfur dioxide after it was found that there were no responses even with 21 ppm SO_2. This provided a means to find out if London-type aerosol interacted with ozone in a manner similar to the California-type aerosol.

In the subchronic exposure study (CL-7), 30-day, 23 hour per day, subchronic exposures were conducted utilizing 5 chambers, each with 12 healthy and 12 impaired male rats. An key pathologic response in the acute studies was associated with the 0.4 ppm ozone. Since this concentration of ozone is a plausible episodal level and the only one tested in the acute studies, the ozone was kept at 0.4 ppm for the subchronic studies. Aerosol concentrations were chosen at a high but not exceptional 1 mg/m3, and sulfur dioxide was targeted at 1 ppm. Sulfur dioxide was not be tested without London-type aerosol in the subchronic study. The layout of the subchronic 30-day exposure is shown in Figure 3. The purpose of the subchronic 30-day exposures was to compare the effects of the two types of aerosols under subchronic conditions where certain serious longer term effects may become apparent which would not have been observed in the acute studies. Pulmonary fibrosis is an example.

The nominal constituents of the synthetic London-type aerosol (LT) and the synthetic California-type aerosol (CA) are summarized in Table 1. Emphasis in this study was upon inorganic constituents since they predominate and are most likely to be the cause of systemic injury to the lung. These representative aerosols were formulated to incorporate key features of ambient airborne particles from the respective regions and pollution conditions. For example, though both have free inorganic carbon, the London type aerosol has larger lamp-black type particles associated with unburned carbon in coal fly ash, while the California-type aerosol has primarily submicronic graphitic carbon with high surface area. California-type aerosol (CA) has about 60% as much carbon as does London smog-type aerosol (LT) (ARB, 1982). In both cases, fine particles were studied that would be represented by PM_{10}. Since humid conditions are usually implicated in air pollution episodes, a relatively high relative humidity of 80% was chosen for these studies, but one that would not lead to excessive condensation in the chambers.

According to ARB reports, nitrate compounds are one and one-half times as common in the California air than sulfates, so this relationship was included in the CA constituents. Lead and manganese sulfate represents the contribution of lead and manganese in fuel additives to current pollution levels. The vanadyl and nickel sulfate constituents represent trace metals contributed by

oil-fired power plants (Kimble, Raabe, and Silberman, 1982). The natural clay represents the common dirt and dust so prevalent in many areas of California. The London-type aerosol (LT) has a large contribution of coal fly ash from coal burning and acid ammonium bisulfate to simulate the acid sulfates identified in the London smog episode.

TABLE 1: Composition of Synthetic Pollutant Aerosols

California-Type Aerosol (CA)		London-Type Aerosol (LT)	
Temperature 23°C		Temperature 23°C	
Relative Humidity 80%		Relative Humidity 80%	
Size: 1 micrometer MMAD		Size: 1.5 micrometer MMAD	
O_3: 0.4 ppm		SO_2: 1 or 21 ppm	
NH_4HSO_4	15%	NH_4HSO_4	20%
$(NH_4)_2SO_4$	15%	$(NH_4)_2SO_4$	20
NH_4NO_3	25%	Coal Fly Ash	30%
Carbon	18%	Carbon	30%
$VOSO_4$	0.01%		
$MnSO_4$	0.03%		
$NiSO_4$	0.01%		
$PbSO_4$	0.04%		
Natural Clay	27%		

[%= mass fraction]

Figure 1: Acute 3-day exposure experimental design and evaluation plan for the California-type aerosol studies with and without 0.4 ppm ozone.

CALIFORNIA TYPE AEROSOL ACUTE STUDIES

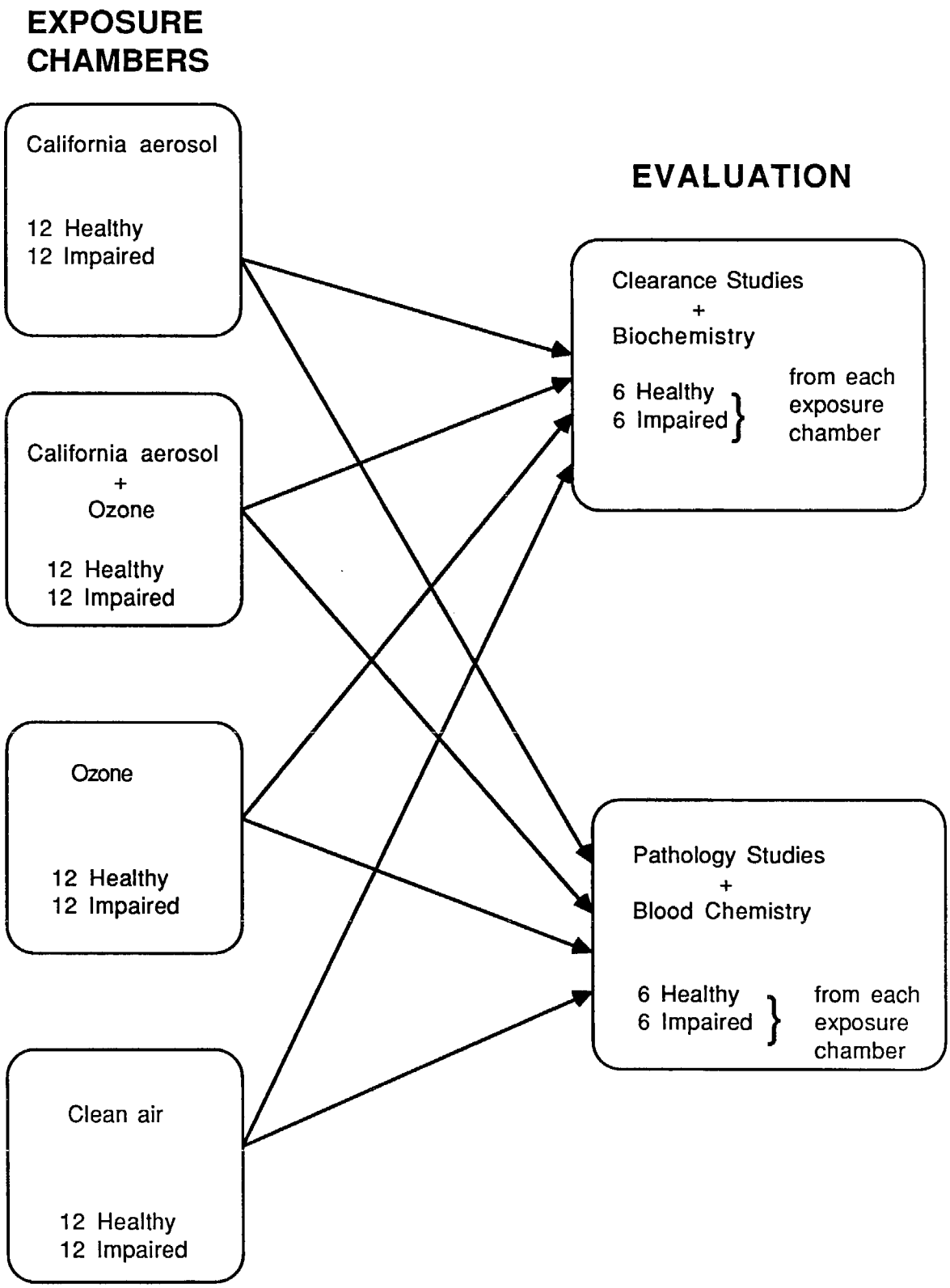


Figure 2: Acute 3-day exposure experimental design and evaluation plan for the London-type aerosol studies with and without 1 ppm to 20 ppm sulfur dioxide.

LONDON TYPE AEROSOL ACUTE STUDIES

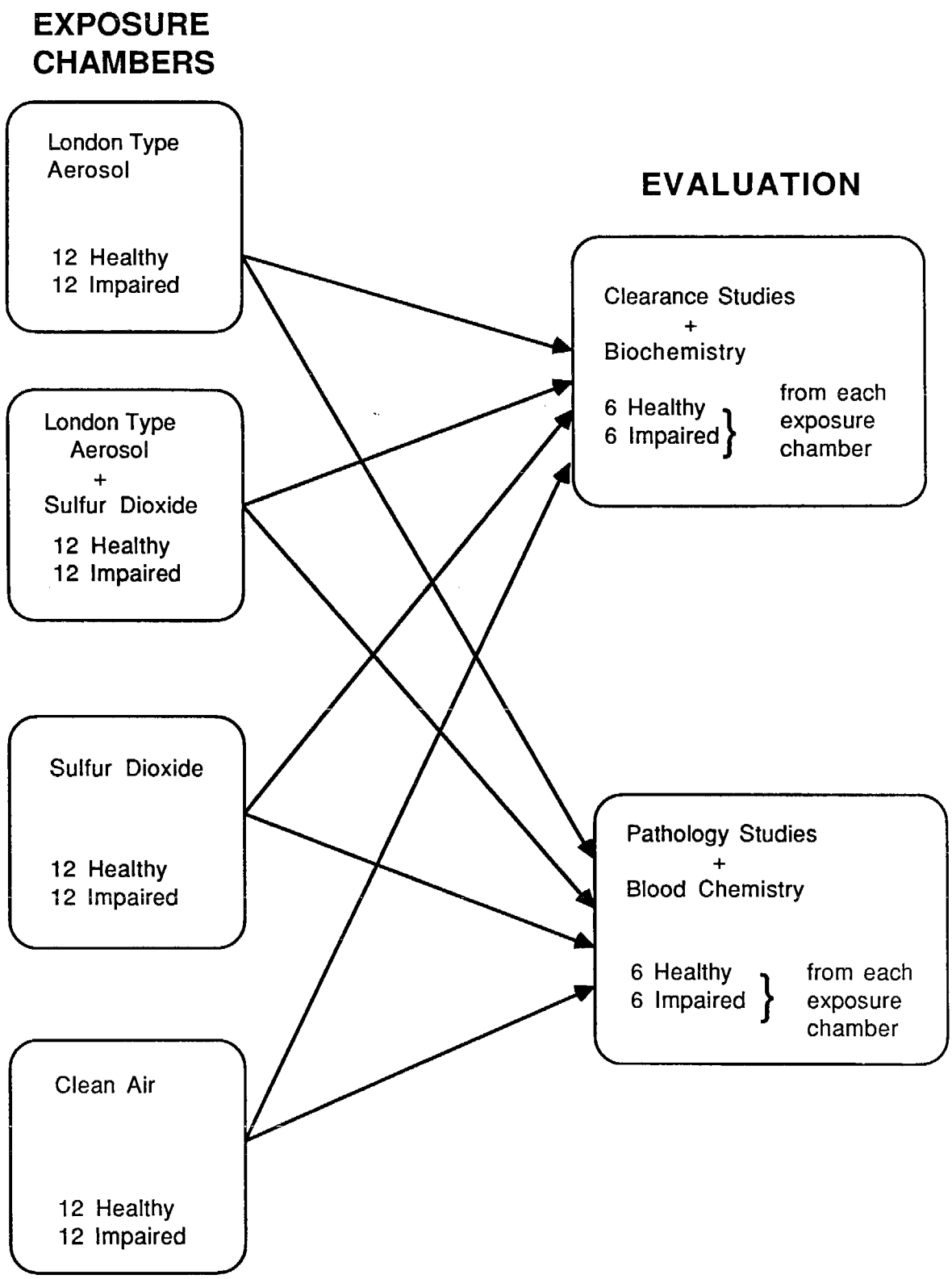
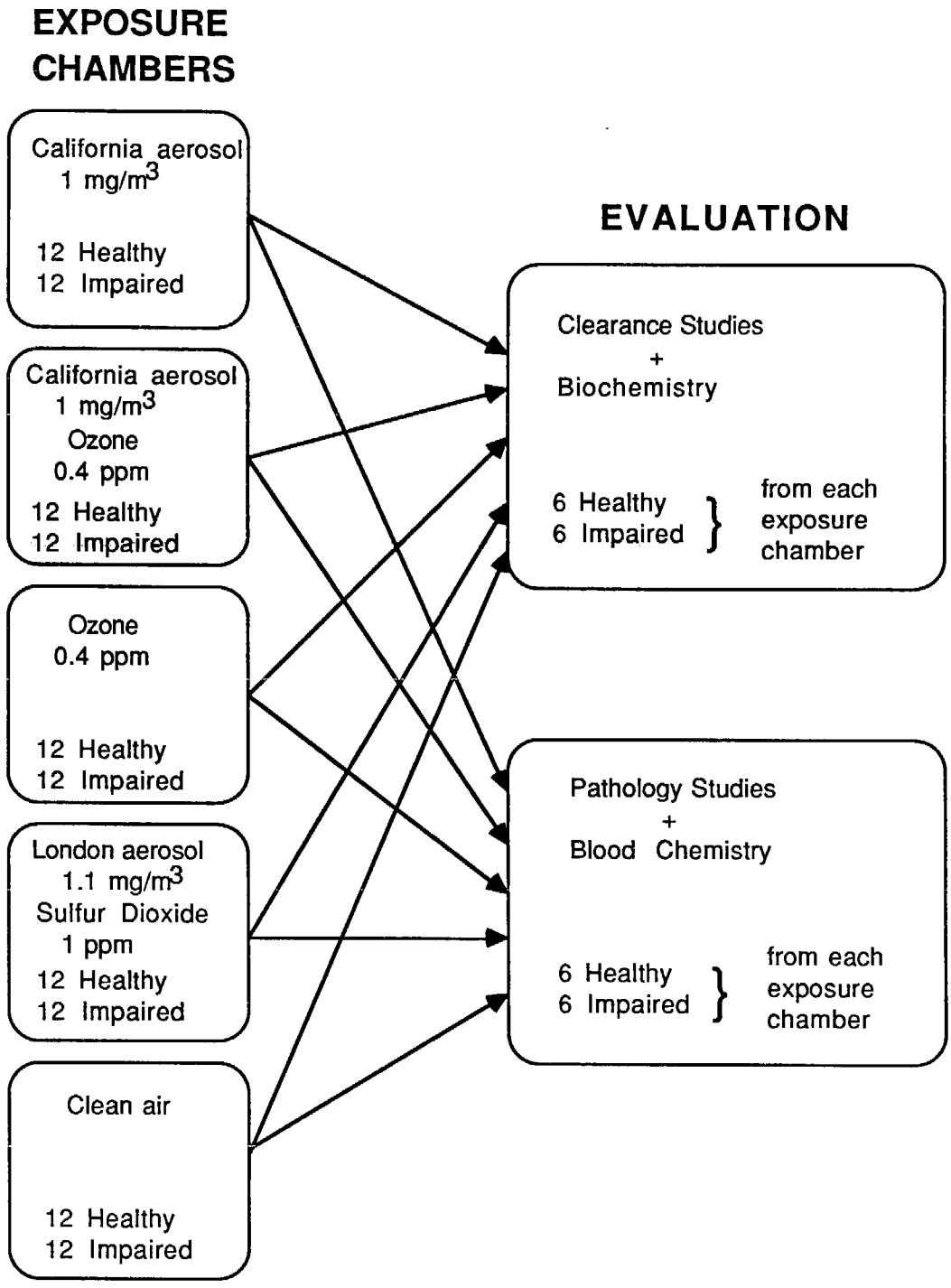


Figure 3: Subchronic 30-day exposure experimental design and evaluation plan for the London-type aerosol with sulfur dioxide and California-type aerosol studies with and without 0.4 ppm ozone.

LONDON TYPE AND CALIFORNIA TYPE SUBCHRONIC AEROSOL STUDIES



METHODS

Animal Exposures.

Overall, 750 respiratory disease-free Fischer 344 SPF 70 day old rats were used in these studies. Fifty-four representative animals were screened for both bacterial and viral infections prior to, during (in the subchronic 30-day study), and after the inhalation exposures. All animals for a given exposure series were ordered and received together and randomly selected for treatment so that statistical analyses and comparisons among the groups would be valid.

Each rat was ear-tagged with a unique identification number. Prior to beginning of exposures, all rats were weighed, and the weights were used for stratified selection and assignment. Assignments were made to the exposure groups as discussed in the statistical design section. During whole body exposure in large 4 cubic-meter stainless-steel and glass chambers rats were maintained individually in stainless steel wire mesh cages placed in a monolayer planar array perpendicular to the direction of aerosol flow. Aerosols, gases, steam, and clean incoming air were mixed at the chamber inlet. Chamber exhaust was filtered to remove contaminants prior to release. Chamber flow rates were maintained at 2100 L/min.

As discussed in the previous section, the experiments consisted of seven sets of exposures. Three were modeled after a California urban atmosphere (Figure 1) and three were modeled after a London-type atmosphere (Figure 2). One exception was made in London-type aerosol exposure with ozone being substituted for sulfur dioxide. These first six sets of exposures were three days long for 23 hours a day (a total, 69 hours). The last set of exposures was thirty days long and used both types of atmospheres (a total, 690 hours; Figure 3). The exposures are summarized in Figure 4.

Figure 4: Chart of the pollutant concentrations used for the six acute 3-day exposure (CL-1 to CL-6) and for the subchronic 30-day exposure (CL-7) for the California-type aerosol and the London-type aerosol generated in this study of the effects of airborne particulate matter.

CONCENTRATION OF POLLUTANTS

Phase I, Exposures CL1-6 (3 days @ 23 Hrs/day)

Phase II, Exposure CL7 (30 days @ 23 hrs/day)

Exposure # / Chamber Atmosphere	CL1	CL2	CL3	CL4	CL5	CL6	CL7
Ca Type Aerosol	0.53	1.42	2.99				1.05
Ca Type Aerosol + Ozone	0.58 + 0.41	1.47 + 0.39	2.96 + 0.41				1.02 + 0.37
Ozone	0.40	0.40	0.40			0.39	0.38
London type Aerosol				0.83	4.68	3.21	
London type Aerosol + SO_2				0.99 + 1.07	5.02 + 21.89		1.10 + 0.96
SO_2				1.07	20.98		
London type Aerosol + Ozone						2.64 + 0.39	

Aerosol concentration = mg/m^3

O_3 & SO_2 concentration = ppm

Biostatistical Design.

Both parametric and non-parametric methods were used for evaluation of data, and modern analysis of variance (ANOVA) methods were used to evaluate observed frequencies of changes and responses. The experimental design was established to facilitate these data comparisons.

The 3-day study design (Figures 1 & 2) was as follows:

		HEALTH STATUS	
		H	I
EXPOSURE ATMOSPHERE	AEROSOL	12	12
	AEROSOL + GAS	12	12
	GAS	12	12
	CONTROL	12	12

Here AEROSOL was either LT or CA and GAS was either SO_2 or O_3, respectively. The number of rats per cell was 12. However, of the 12 rats per cell, the assignments used in tests were as follows:

(1) 6 rats were randomly selected (stratified by weight) for lung clearance studies (total of 12 per chamber atmosphere).

(2) These 6 rats were also used for lung biochemistry studies (12 per chamber atmosphere)

(3) The remaining 6 rats were used for pathology and blood chemistry (12 per chamber atmosphere).

The results were analyzed using a three-way Analysis of Variance (ANOVA), fixed effects model. We used two levels of contrasting responses. A statistically significant result was assumed for a significance level of $p \leq 0.05$. In addition, an important tendency was assumed for a nearly

significant level of $p \leq 0.1$. The experimental design shown above is that of a factorial with Health Status at two levels and exposure atmospheres at 4 levels. The statistical main effects (health status and exposure atmosphere) and the cross interactions were tested.

Consider alpha=0.05 as the level of significance (Type I error) and beta=0.20 (Type II error); power= 80% with d the true difference from the mean of the controls and s the estimate of the true standard error per experimental unit. With 6 rats per cell we should have been able to detect true differences (given the accompanying standard errors, expressed as percentage coefficients of variation or relative standard error): d= 10%, s= 6%; d= 15%, s= 9%; d= 20%, s=12%; d= 25%, s= 16%; and d= 30%, s= 18%. We have used a one-tail test because we believe that the treatments used was deleterious and never beneficial.

We were dealing with a variety of variables, and their standard errors relative to their means were not known precisely. The observed data were sometimes skewed, so the relative standard errors were large. In addition, there were clear responders to treatment, whereas others had little or no response, or the response was opposite from expectation. This led to the use of non-parametric tests in some cases, such as for the results of the lung particle-clearance studies. These included the Mann-Whitney and Kruskal-Wallis analysis of variance tests. Morphometric data and inflammatory cell counts were analyzed by a general linear model analysis of variance computer program (Numbercruncher Statistical Analysis System version 5.0). Lesion scores in subjective evaluations were analyzed by the Mann-Whitney test.

RANDOMIZATION AND STRATIFICATION: It was extremely important that the rats be assigned to treatment randomly. However, because the body weights of rats covered a range of values, it was necessary to insure that the average weights and variance of weights in the treatment cells matched as well as possible. It was necessary, therefore, to stratify the rats so that this occurs, while at the same time allowing for random assignment.

This was done by:

(1) Weighing each rat individually and ordering the rats as to body weight from smallest to largest.

(2) The first two (smallest) rats were randomly assigned to be intact (Healthy, H) or elastase instilled (Impaired, I). Succeeding pairs of rats were similarly assigned.

(3) The four smallest rats in each group (H versus I) were randomly assigned to the four different exposure atmospheres. Succeeding groups of 4 H and 4 I rats were similarly assigned.

Design of the subchronic 30-day study (Figure 3); this study compared five exposure atmospheres:

		HEALTH STATUS	
		H	I
EXPOSURE ATMOSPHERE	CA	12	12
	CA + OZONE	12	12
	LT + SO_2	12	12
	OZONE	12	12
	CONTROL	12	12

Exposures in the subchronic 30-day study were 23 hours per day for 30 days. There were 12 rats per cell (24 per aerosol). Each of the classes of biologic tests (lung clearance, pathology, biochemistry, blood chemistry) was performed on 6 rats per cell (a total of 60 rats per test). The main statistical effects were health status and exposure atmospheres. The significance of the cross interactions was tested. The methods were the same as described for the 3-day studies. The power of these tests slightly exceeded that of the acute studies because there are more degrees of freedom for the estimate of error.

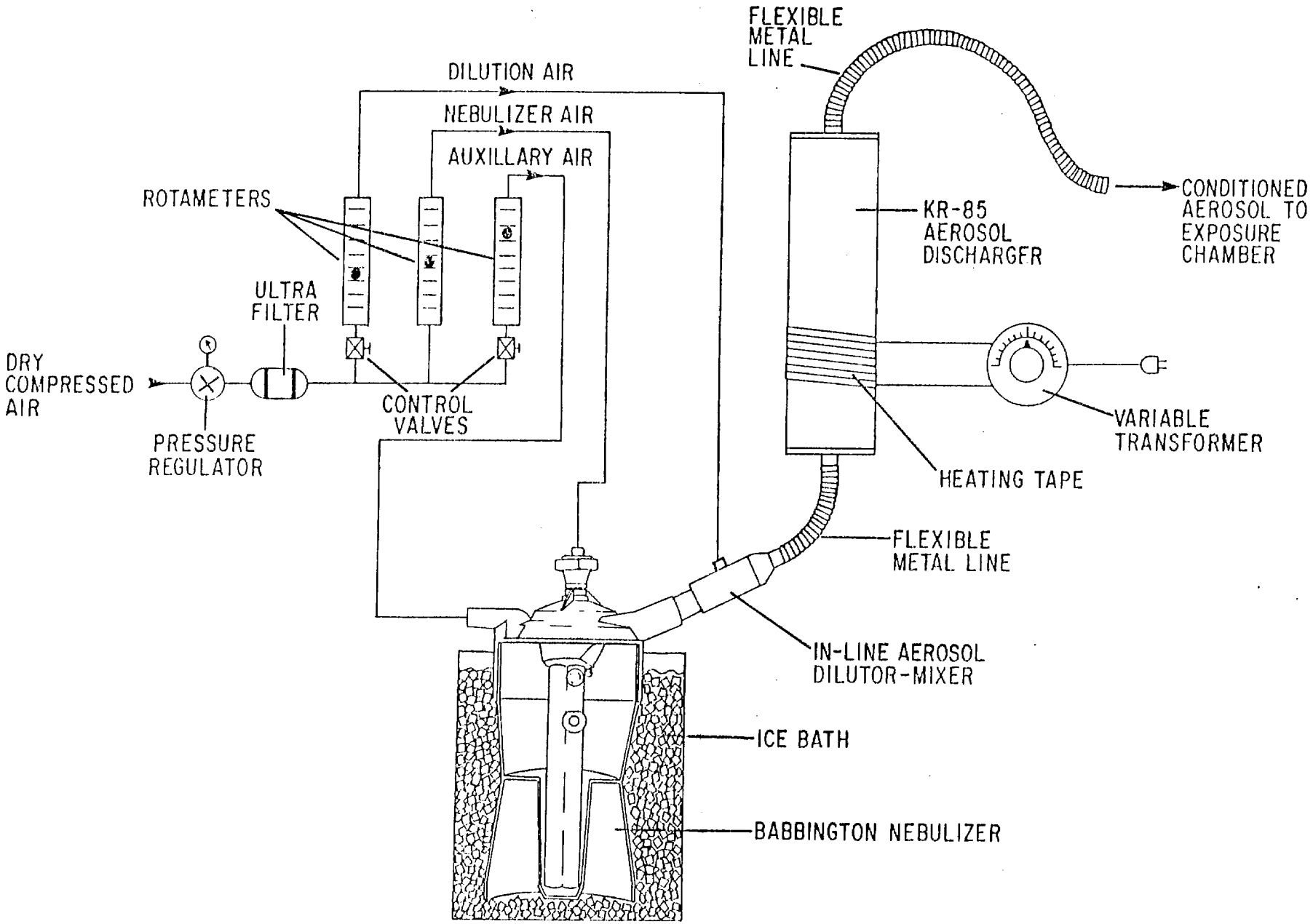
Aerosol Generation

The two separate types of aerosol mixes were generated using nebulization of liquids for the inorganic salts and using the Wright dust feeder for dry dust dispersion. These aerosols were conditioned and mixed with the main 2100 L/min of air flow to each 4 m^3 aerosol exposure chamber. Steam was also mixed with the main inlet flow to yield a nominal relative humidity of 80%. This system produced aerosols similar to the particulate air pollutants of concern. They were in the respirable range (<3.5 micrometer in aerodynamic diameter) with a significant portion being smaller than one micrometer in aerodynamic diameter.

Aerosols of the inorganic salts were generated together from aqueous solutions containing the appropriate chemical constituents using a Babington-type Solosphere (CL-1) or multiple jet version Hydrosphere nebulizer (American Hospital Supply, Irvine California) as shown in Figure 5. The liquid aerosol was passed through a diluter to accelerate drying of the droplets and then through a heated cylinder containing a sealed krypton-85 (^{85}Kr) radioactive source (10 mCi). This arrangement was used to reduce the aerosol electrostatic charge to Boltzmann equilibrium. The quantity of aerosol produced by the nebulizers was adjusted by altering four factors: (1) the concentration of the solution, (2) the Hydrosphere generator changeable elements (these include the ball and flow pressure restrictor), (3) pressure supplied to the generator and (4) the flow of the auxiliary air. The nebulizer was modified by removing the air intake mechanism which was on the stock model and replacing it with a cap having a series of holes that were set to produce different amounts of air to mix with the nebulized aerosol. A plug with two "O" rings was inserted into the intake air inlet, and a metered flow of filtered compressed air was used to adjust the amount of make up air. The operating characteristics of the Babington nebulizer are summarized in

Appendix A along with the chemical constituents of the nebulized aqueous solutions for the surrogate California aerosols. For exposure set CL-2 through CL-7 a Hydrosphere was used for nebulizing solutions. These have outputs similar to Solosphere, with increased output capability achieved by

Figure 5: Schematic illustration of the nebulizer system used in the study to generate aerosols of inorganic salts from aqueous solutions.

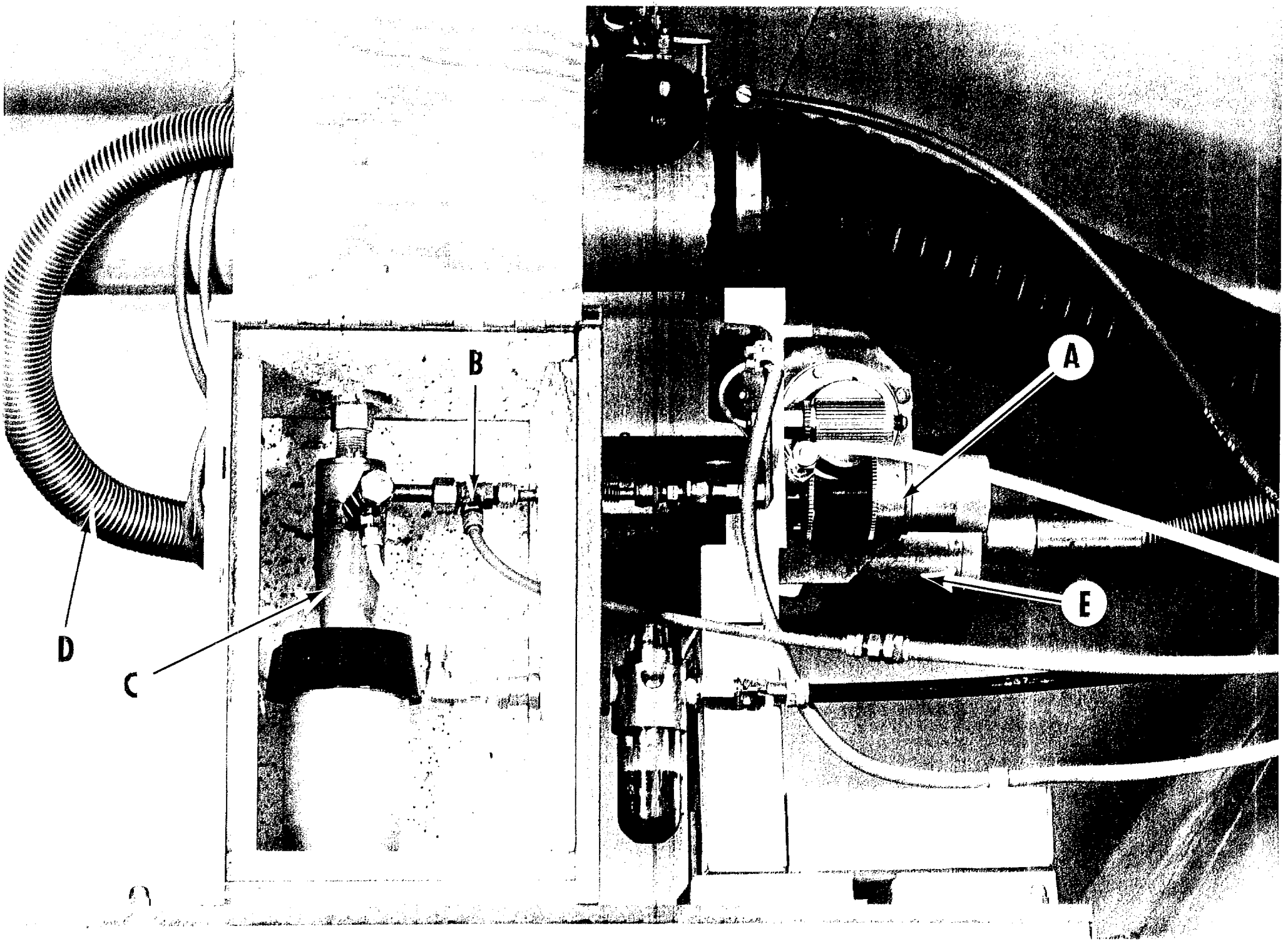

FLEXIBLE
METAL
LINE
DILUTION AIR
NEBULIZER AIR
AUXILLARY AIR
ROTAMETERS
CONDITIONED
AEROSOL TO
EXPOSURE
CHAMBER
KR-85
AEROSOL
DISCHARGER
ULTRA
FILTER
DRY
COMPRESSED
AIR
CONTROL
VALVES
VARIABLE
TRANSFORMER
PRESSURE
REGULATOR
HEATING TAPE
FLEXIBLE
METAL LINE
IN-LINE AEROSOL
DILUTOR-MIXER
ICE BATH
BABBINGTON NEBULIZER

adding generating jets and increasing the flow through the unit. The Solosphere and Hydrosphere nebulizers have similar designs and operating characteristics.

The acidity of all of the inorganic salt solutions was adjusted to pH 1.8 with sulfuric acid to maintain the balance between sulfate and bisulfate. The concentrations of samples of the nebulizer solutions were verified by ion chromatography for quantification of the sulfate and nitrate cations and by atomic absorption spectrophotometry for the metals. Chemical analyses data for typical exposure solutions are given in Appendix A. Aerosol samples were collected with filters and analyzed to verify the airborne chemical constituents of the aerosols; typical data are shown in Appendix A. Separate aerosolization tests, using only the nebulized aerosols, were performed with filter samples and cascade impactor samples to verify the uniformity of the chemical composition with respect to particle size.

The lamp black plus fly ash used in the London-type aerosols, or carbon plus natural clay, used in the California type aerosols, were generated as dry powder utilizing a Wright dust feed (WDF) generator (Mod #180, Messrs. L. Adams Ltd., 22 Minerva Rd., London) in combination with a miniature cyclone separator and a krypton-85 (^{85}Kr) discharger by the method of Raabe (1979) as shown in Figure 6. For the WDF method the mixture of powders was packed into stainless steel cups and resuspended by scraping the dust off by means of a blade and blowing it off with compressed air. The amount of dust generated by this method was set by a series of gear drives which changed the speed at which a circular blade scrapes off the cake packed into the cup. The dust was blown off the cake and was impacted into a metal baffle to help deagglomerate the packed powder. The dust then was passed through a cyclone collector, with a 250 milliliter cup below the unit to collect the dust particles larger than the median cut point of 2.6 micrometers aerodynamic size. The dust was mixed with the other aerosol (from the nebulizer) and piped into a large volume ^{85}Kr discharger (Teague, et al., 1978) to reduce the electrostatic charge on the particles to Boltzmann equilibrium. This aerosol was mixed with the filtered and conditioned air and the gas was metered in at the proper rate. The humidity was adjusted to 80% by adjusting the steam pressure which passed

Figure 6: Photograph of the system used for generating aerosols of dry dusts (carbon plus clay or lamp-black plus fly ash mixtures); (A) is the Wright dust feed, (B) the diluting air connection, (C) the cyclone separator, (D) the aerosol line from the cyclone separator to the discharger, and (E) the ^{85}Kr discharger.


A
B
C
D
E

into a small orifice placed in the line immediately before the air entrance to each chamber. The experimental arrangement of the dry dust generation system in which aerosol was discharged into the inlet flow of an exposure chamber, is shown schematically in Figure 7.

The California-type aerosol dust mixture was prepared with equal amounts of fine carbon (Asbury Graphite Mills, Inc., Asbury, NJ) and natural montmorillonite clay (Southern Clay Products, Gonzales, Texas). The latter was processed with sodium to provide a fine powder. The London-type dust was prepared with equal amounts of lamp black and respirable coal fly ash. The coal fly ash was identical to that described by Raabe, et al. (1979). These respective dry dusts were thoroughly mixed and loaded under firm pressure to form a cake in the stainless steel dust cup of the Wright Dust Feed (WDF) using a special pressure-regulated hydraulic ram, shown schematically in Figure 8. It was capable of pressures of up to 10 tons. Below is the extension tube used to pack the cake using one pack. This method was preferred because in eliminated the interfaces caused by the multiple pack method.

Ozone was generated by silent arc discharge in pure oxygen. Sulfur dioxide was generated from dilute mixtures in nitrogen stored in a compressed gas cylinder. Both gases were measured automatically with instrumental detection equipment. The sampling lines, filter holder, and solenoid valve of each monitor were made of teflon. The ozone was measured with a Dasibi ozone analyzer that was calibrated against a Dasibi UV photometer model 1008PC, which was in turn calibrated against a National Bureau of Standards standard reference photometer (serial #4) located at the California Air Resources Board Quality Assurance Standards Laboratory. The sampling interval for ozone was every 10 minutes, providing concentrations every 5 chamber volume changes. The sulfur dioxide was monitored in one-hour averaged blocks by a Meloy Laboratories FPD Sulfur analyzer model SA 285 that was calibrated using the dynamic dilution system. This process used a known 49.2$\pm$1 ppm sulfur dioxide in air (cylinder No. CC49614) traceable to National Bureau of Standards SRM 1693.

Figure 7: Schematic illustration of the system used to generate aerosols of dry dusts including mixtures of carbon and clay or lamp-black and fly ash. (R1 and R2 are regulators; V1, V2, V3 and V4 are valves; F1 and F2 are flowmeters; and P1 and P2 are pressure gages.)

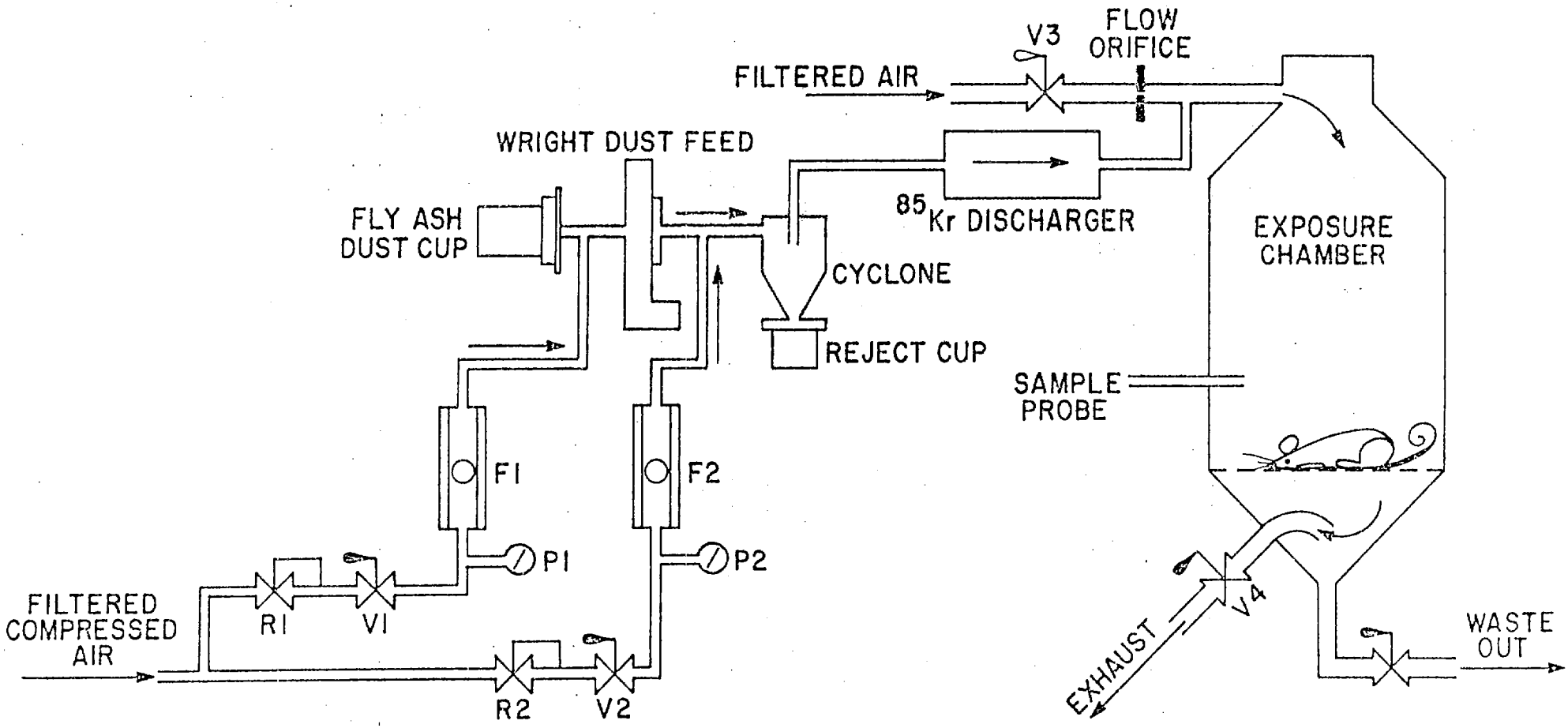
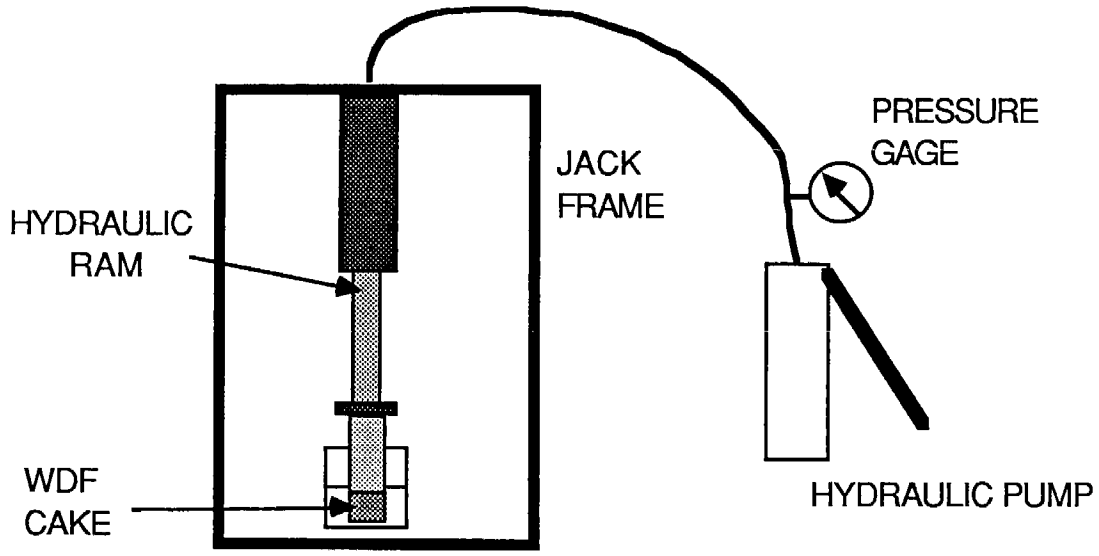
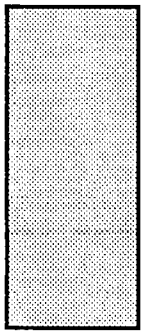
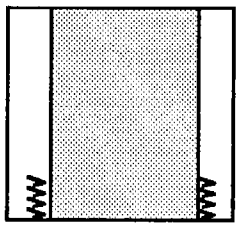

FLOW
ORIFICE
V3
FILTERED AIR
WRIGHT DUST FEED
FLY ASH
DUST CUP
85Kr DISCHARGER
EXPOSURE
CHAMBER
CYCLONE
REJECT CUP
SAMPLE
PROBE
F1
F2
P1
P2
FILTERED
COMPRESSED
AIR
R1
V1
R2
V2
V4
EXHAUST
WASTE
OUT

Figure 8: Illustration of the hydraulic ram system used in this study to pack the stainless steel dust cup of the Wright dust feed with dry mixtures of either carbon and clay or lamp-black and fly ash. The top figure shows the press used to pack the stainless steel cups with the dust. Below is the extension tube used to pack the cake using one pack.

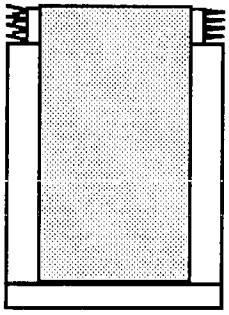




PLUNGER
ROD



CUP
ADAPTOR
EXTENSION



SS CUP

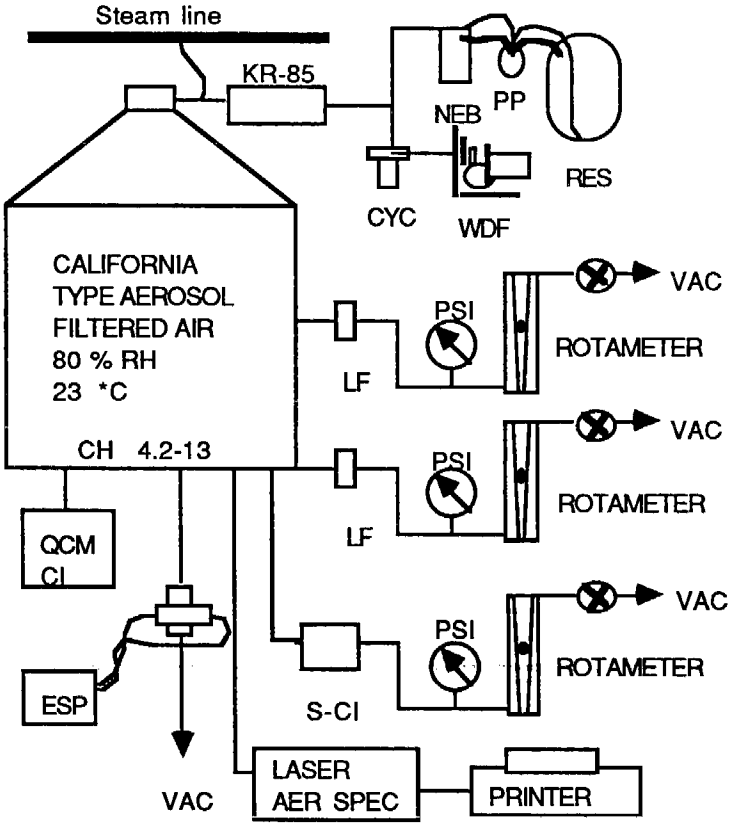
Relative humidity for the chambers was controlled by steam ejection at the top of each exposure chamber through a small orifice device located in the air stream. Control of the relative humidity was determined by steam pressure and orifice hole size. This was added to the relative humidity of the conditioned air going into the chambers which was controlled at 50% RH to achieve a relative humidity of 80% at 23^{o}C for the exposures.

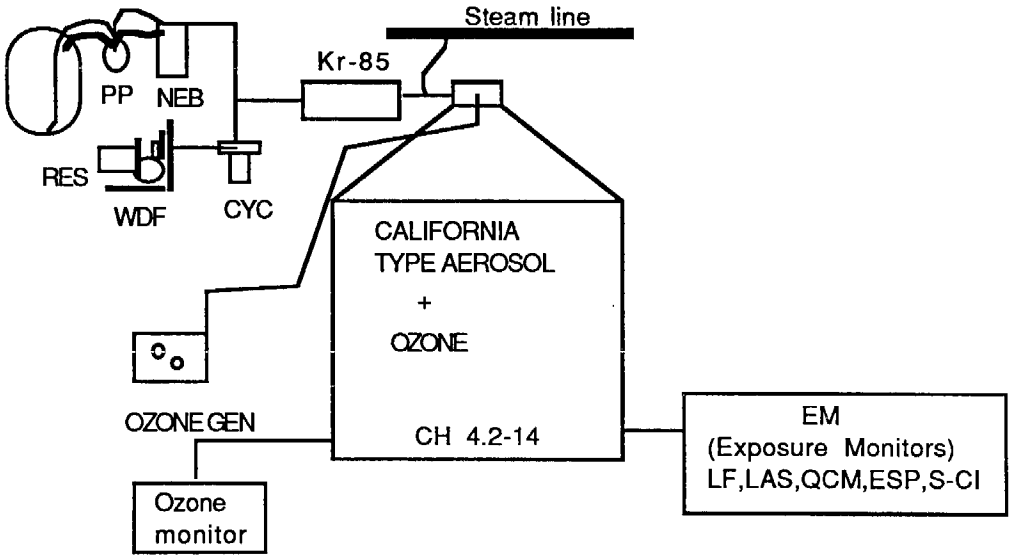
All air used in the generation of aerosols as well as that used to dilute aerosol mixtures was filtered for both particulate and organic contaminants utilizing absolute and activated charcoal filters. All effluent from exposure chambers was collected prior to discharge and filtered to prevent environmental releases. Potentially toxic wastes, including animal tissues, were disposed of using procedures in effect on the Campus, as administered by the Office of Environmental Health and Safety.

Experiment CL-1 with California-type aerosol was the first exposure and was conducted at a concentration of 0.55 mg/m^{3} for three days (Figure 9). Two Wright dust feeds and two Solospheres were used; these were placed on a working platform above the large exposure chamber doors. Steam entered from a steam line located above the top of the chamber producing the near 80% Relative Humidity (RH). The main airstream through the chamber was conditioned and filtered air at 23^{o} C. The cups were packed with a mixture of 40% carbon and 60% clay and packed with a single pack at 800 PSI with a special packing extension (Figure 8). The Wright dust feeds both operated well for the first experiment although not at equal efficiency. To compensate for the difference between the dust feeds, the exhaust from the cyclone separators was switched between the two aerosol chambers at the midway point of the three-day exposure. Each of the nebulizers was fed by a constant supply of cold solution by pumping from one of two 4.5 liter containers placed in a refrigerator unit. The temperature was maintained around 6^{o} C for all the exposures. The nebulizers were placed in a small refrigerator unit with a special top constructed of styrofoam placed over them to reduce heat loss. The pumping system consisted of a peristaltic pump, controlled by a variable speed motor, with four heads and multiple plastic feed tubes. This system assured that the corrosive

Figure 9: Generator configuration for CL-1 showing two separate generation arrangements for the two chambers located on the working platform above the chamber doors. Labels shown are: WDF (Wright dust feed generator); CYC (cyclone separator); RES (cooled liquid reservoir); PP (peristaltic pump); Neb (nebulizer unit); KR-85 (Krypton discharger unit); EM (Exposure chamber monitors).

GENERATOR CONFIGURATION
CL-1





solution was not contaminated by contact with the metallic components. Constant recirculation of a large volume of generator solution helped minimize the effects of increasing concentration due to evaporation extended periods of nebulizer operation. The duct carrying the nebulized material to the chamber was heated to prevent condensation before the aerosol was mixed with dry air and the dust. Air quality in the chambers was sampled using two 47 mm diameter Versapore 0.2 micrometer filters and a Sierra cascade impactor operating continuously for 8 hours, point-to plane electrostatic precipitators, a laser aerosol light-scattering particle counter with printer, and relative humidity and temperature monitors.

For experiments CL-2 and CL-3 the aerosol concentration was increased (Fig. 10). To produce this higher output of material we had to use a larger Babington-type nebulizer, a hydrosphere with one ball for the CL-2 and two-ball configuration for the CL-3 exposure. The nebulizers were also put on a shelf constructed between the two aerosol exposure chambers and stainless steel lines located in a vertical position to facilitate drying of the increased volume of nebulized solution. These lines were also wrapped with heating tape and insulated to achieve the heat input required to completely dry the aerosol. The dried nebulized portion of the aerosol was mixed with the dust portion from the Wright dust feeds downstream of the cyclone separator and before the aerosol dischargers. This vertical configuration worked much better than the horizontal configuration used in CL-1 and was used for the rest of the exposures (Figures 11-13).

Aerosol Characterization

Aerosol characterization included multiple and repeated cascade impactor samples with a Mercer-style impactor (Raabe, 1977) and a real time quartz crystal microbalance, QCM, impactor (Berkeley Instruments, Berkeley, CA) to determine the aerodynamic size distribution of each aerosol. In addition, numerous and repeated filter samples collected in the breathing zone of the animals were used to provide quantification of the mass and chemical concentration of the aerosols. These breathing zone samples were collected through a metallic probe inserted into the chambers through sampling ports

Figure 10: Generator configuration for CL-2 and CL-3 showing modification of system incorporating one large reservoir for cooling container. The lines to the nebulizer were made of stainless steel, heated and located in a vertical position below the Wright dust feed (WDF) system. (NEB = (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

GENERATOR CONFIGURATION
CL-2 and 3

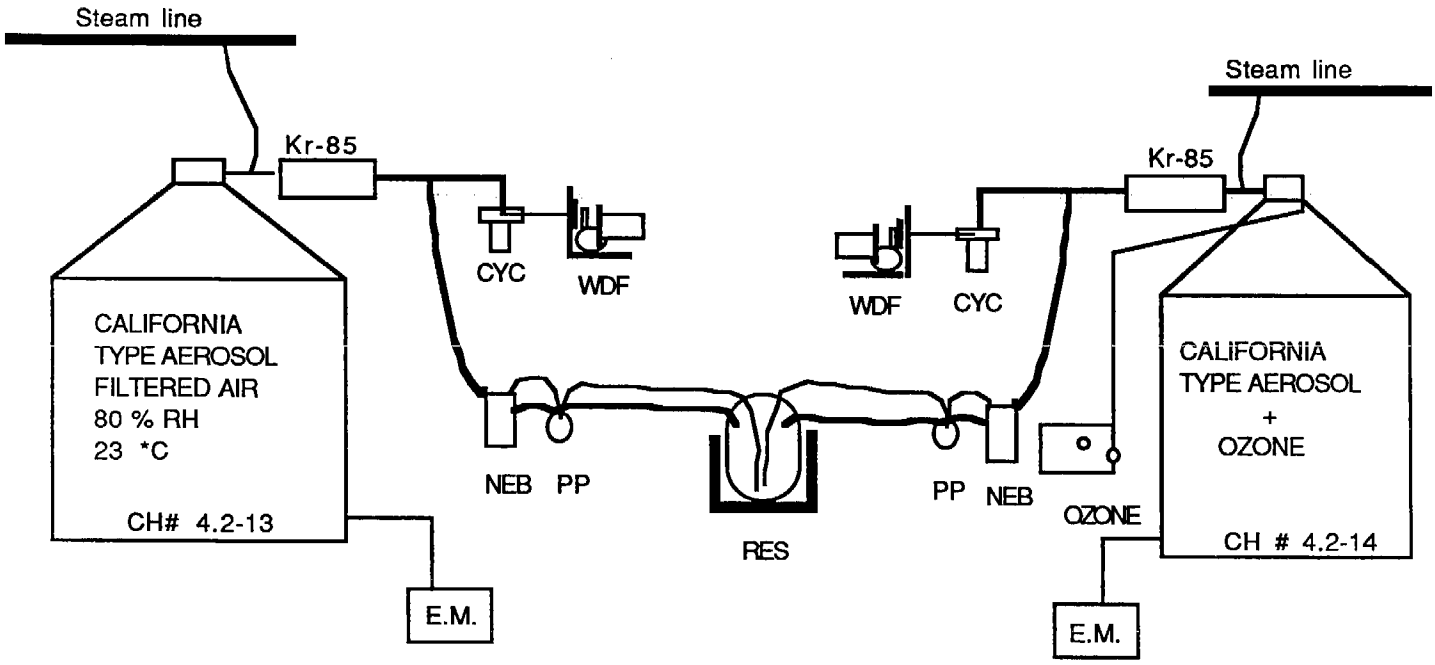


Figure 11: Generator configuration for CL-4 to CL-5 showing changes in the Wright dust feed, using one unit to supply the two chambers. By using a venturi split tee, equal quantities of material was distributed to two exposure chambers. (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

GENERATOR CONFIGURATION
CL-4 and 5

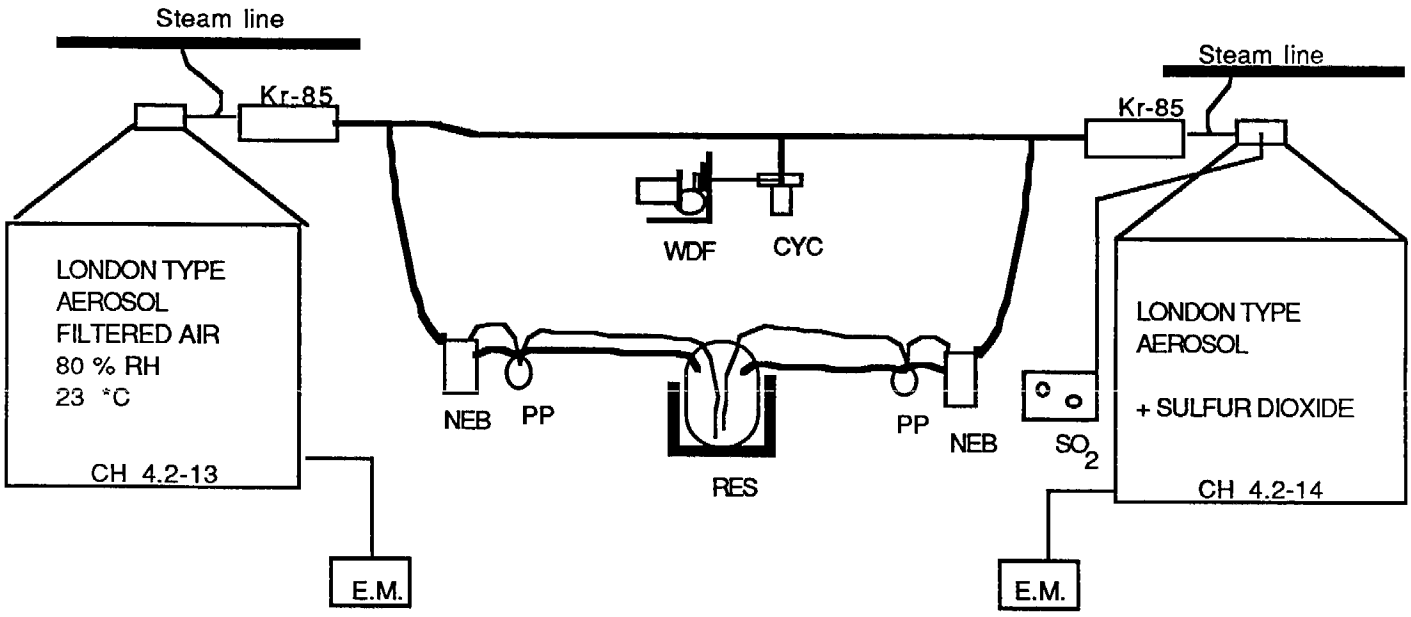


Figure 12: Generator configuration for CL-6 showing the use of ozone in place of sulfur dioxide with the London-type aerosol. (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

GENERATOR CONFIGURATION
CL-6

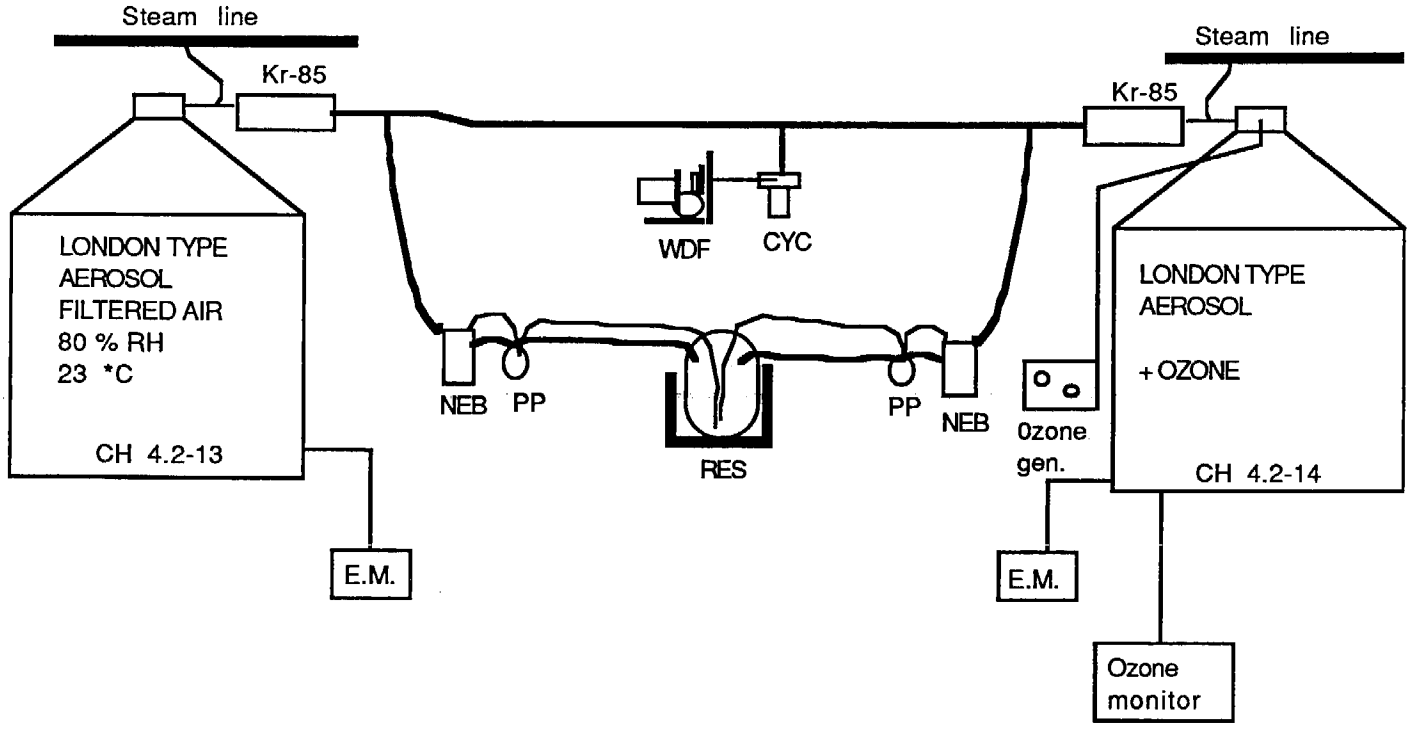


Figure 13: Generator configuration for CL-7 showing the use of two Wright dust feed systems. One dust feed and one nebulizer supplied two chambers for the California-type aerosol (one with and one without ozone) and another dust feed and nebulizer for the London-type aerosol with sulfur dioxide. (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

UCD-472-508

EFFECTS OF INHALED PARTICULATE MATTER

Final Report
to the California Air Resources Board
CARB Contract No. A4-133-33
Project Term: 1 September 1985 to 31 May 1987

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15 January 1989

ABSTRACT

Controlled laboratory studies were conducted utilizing two experimental animal models (Fischer-344 SPF male rats), one healthy and one impaired with elastase-induced emphysematous lung disease, to evaluate the nature and severity of responses to inhaled respirable aerosols (0.55 mg/m^3 to 3.0 mg/m^3) that were especially synthesized with selected realistic inorganic constituents to be typical of air pollution episodes in California, alone and in combination with ozone (0.4 ppm). Parallel comparative inhalation studies were performed with a respirable London-type aerosol (0.8 to 5.0 mg/m^3) typical of the the famous London smog episodes, alone and in combination with sulfur dioxide (1 to 21 ppm). Exposures were acute (3 day) or subchronic (30 day). The deleterious effects of the exposures were evaluated with selected lung biochemical measurements, measurements of lung particle clearance and permeability (using radiolabeled test aerosols), clinical signs of illness, and histological evaluation of lung inflammatory responses and structural alterations.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. In the 3-day studies, there were some significant increases in total lung DNA and protein content in rats exposed to the synthetic California-type aerosol compared to rats not exposed to this aerosol. In addition, small airway inflammation was observed in animals exposed to ozone, and this effect was significantly exacerbated by the inhalation of either aerosol and in impaired animals. There was no observed aerosol concentration dependence. In the 30-day studies, biochemical analyses showed increases in the lung content of hydroxyproline (indicative of collagen synthesis and potential lung fibrosis) in rats exposed to the California-type aerosol, and to London-type aerosol with SO_2 in impaired rats. Both aerosols tended to decrease the rate of tracheobronchial clearance of deposited test particles. There was no change in alveolar permeability. Exposure to ozone caused small airway lesions in the lungs and this was significantly exacerbated in impaired animals and by the inhalation of aerosols by impaired animals. Small airway fibrosis was caused by ozone, and this was significantly exacerbated by exposure to California-type aerosols. This study is apparently the first to demonstrate that aerosol/ozone interactions occur with atmospheres of composition and concentration similar to those found in the environment.

ACKNOWLEDGEMENTS

The principal technical staff who contributed to this project are listed on the title page. Additional technical support was received from Martha Conard (histology), Victor Pietrzak (computer systems), Charles Baty and Pam Carroll (clerical support), Cecilia Bauernhuber (administration) and Diane Schroeder (financial services).

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DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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SUMMARY AND CONCLUSIONS

This project utilized two experimental animal models, one healthy and one impaired (Fischer-344 SPF male rats) in controlled laboratory studies to evaluate the nature and severity of responses to two types of inhaled particulate matter. One of these aerosols was synthesized so as to have constituents that are typical of California urban aerosols alone and in combination with 0.4 ppm ozone at levels that would be associated with episodes of elevated air pollutants. In addition, parallel comparative studies were performed with a London-type aerosol alone, and in combination with sulfur dioxide, associated with past London smog health effects. Both aerosols contained acidic sulfates. Half of the animals were impaired by instilling elastase intratracheally which resulted in a condition comparable to human emphysema with breakdown of lung alveolar septa and dilation of some air spaces. The exposures conducted in this project were designed to simulate acute air pollution episodes of unusually elevated concentrations that last for about three days. In addition, one 30-day subchronic episode was also designed to illustrate a worst case situation of a prolonged pollution episode, and to evaluate the consequences and progression of the acute effects. The aerosols consisted of mixtures of components formulated to provide synthetic, laboratory versions of the two types of pollutant atmospheres with appropriate respirable particle size distributions. The California-type aerosol consisted of ammonium sulfate, ammonium bisulfate, ammonium nitrate, graphitic carbon, natural clay mineral, and traces of lead, vanadium, nickel, and manganese. The London smog-type aerosol consisted of ammonium sulfate, ammonium bisulfate, coal fly ash, and carbon soot. Both aerosols were formulated to be acidic (pH = 1.8).

In developing the impaired rat lung model for these studies, an elastase dose of 25 U/100g body weight (instilled in the lung) appeared to be effective but generally non-lethal in producing alveolar emphysema-like lesions. The risk of developing post-instillation pneumonia in elastase treated rats was eliminated by the use of pathogen-free rats.

There were six 3-day exposures followed by a single 30-day exposure (23 hours per day), each utilizing a separate set of previously unexposed rats. All atmospheres utilized precleaned air at 80% relative humidity at 23°C. In the first 3-day study, rats were exposed to 0.55 mg/m^3 of California-type aerosol with and without 0.4 ppm ozone, to ozone alone, and with unexposed controls. In the second, the aerosol was increased to 1.45 mg/m^3, and in the third to 3 mg/m^3. The fourth acute exposure utilized 0.9 mg/m^3 of the London-type aerosol with and without 1.1 ppm of sulfur dioxide. The fifth exposure increased the aerosol concentration to 5 mg/m^3 with 21 ppm sulfur dioxide. The sixth exposure was a hybrid exposure utilizing 3 mg/m^3 of the London-type aerosol with 0.4 ppm of ozone. The seventh exposure was a continuous subchronic 30-day exposure of rats to 1 mg/m^3 California-type aerosol with and without 0.4 ppm ozone and 1.1 mg/m^3 of London-type aerosol with 1 ppm of sulfur dioxide.

The effects of the exposure were evaluated in several contrasting tests. Using nuclear medicine techniques, measurements were made of test particle lung clearance and lung permeability after brief inhalation of ^{99m}Tc-radiolabeled respirable test aerosols of DTPA (for lung permeability measurements) and iron oxide (for tracheobronchial particle clearance measurements). Histological observations were made of lung inflammatory responses and other structural alterations including small airway fibrosis. Observations were also made of clinical signs and symptoms of illness. In addition, measurements were made of specific lung biochemicals that are known to relate to potential health effects including, DNA, RNA, and protein content, and the content of hydroxyproline (indicative of collagen synthesis and potential lung fibrosis).

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. Overall, in 3-day studies, California-type aerosol exposure (1.45 mg/m^3) resulted in a significant increase in lung DNA and protein in exposed rats; while London type aerosol (5 mg/m^3), sulfur dioxide (21 ppm), and London type aerosol with sulfur dioxide yielded no observed significant biochemical or pathologic effects. Impaired rats had statistically significant increases in lung DNA and RNA, and this response was significantly exacerbated by exposure to London-type aerosol or

sulfur dioxide. Significant small-airway inflammation was observed in rats exposed to California-type aerosol (0.55 mg/m^3 or 1.45 mg/m^3) or ozone (0.4 ppm), and the combination of either California-type aerosol or London-type aerosol (3 mg/m^3) with ozone significantly exacerbated this response. Lung impairment also exacerbated the response. This augmentation of inflammation was not a quantifiable dose-response function of aerosol concentration but occurred at the lowest concentration studied (0.55 mg/m^3).

Likewise, in the 30-day study, neither aerosol was effective by itself in causing significant responses in healthy rats, but some aerosol effects were observed in association with ozone exposure or lung impairment. California-type aerosol (1 mg/m^3) exposures resulted in significant increases in lung hydroxyproline and decreases in lung RNA in exposed rats and showed a tendency to reduce the rate of tracheobronchial clearance of inhaled test particles. London-type aerosol (1 mg/m^3) with sulfur dioxide (1.1 ppm) showed a tendency to decrease the rate of tracheobronchial clearance and exacerbated increases in lung hydroxyproline observed in impaired rats. Both aerosol resulted in significant pigmentation of lung tissue that could be identified as deposited carbonaceous particles. Ozone (0.4 ppm) caused lung small-airway lesions, observable small airway fibrosis of the lung, and increases in rate of tracheobronchial clearance of inhaled test particles. Increased rates of tracheobronchial clearance also occurred when ozone and California-type aerosol were combined. There was exacerbation of the observed small airway fibrosis and synergistic exacerbation of the lung pigmentation by deposited particles by the combination of California-type aerosol and ozone. The combination of California-type aerosol and ozone in impaired rats led to a synergistic exacerbation of lung small airway lesions. London-type aerosol with sulfur dioxide significantly increased the lung content of hydroxyproline in impaired rats. Impaired rats had small-airway lesions associated with emphysema and increased lung DNA and hydroxyproline whether exposed to pollutants or not.

The acute and subchronic studies were similar in showing a somewhat lesser effectiveness for London-type aerosol, even with SO_2, than California-type aerosol in treatment-related significant biochemical or pathologic changes in exposed rats. Both showed that the combination of California-type aerosol and

ozone tends to exacerbate the small airway inflammation caused by ozone, but this was only apparent in impaired rats in the subchronic study. However, the observed lung fibrosis associated with ozone in the subchronic studies was exacerbated by exposure to California-type aerosol. The subchronic study showed significant increases for ozone and a tendency for both aerosols to decrease tracheobronchial clearance of test particles. Also, lung small airway lesions and remodeling were associated with ozone exposures in the subchronic study but not after acute 3-day exposures.

The following is an overview in semi-outline form of the whole project from the perspective of the initial objectives and goals of this investigation. There were five major objectives, all related to better understanding the effects of inhaled particulate matter present in California air.

The main objectives of the project and the results were as follows:

(1) Evaluation of acute and subchronic responses of the lung to inhaled London-type aerosol (LT) or California-type aerosol (CA), alone, and in combination with episodal levels of ozone (California) or sulfur dioxide (London), respectively.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. In the 3-day studies, there were some important observed differences in pulmonary response attributable to differences in the composition of the two aerosol types. Inhalation of California-type aerosol resulted in the appearance of statistically significant changes in the biochemistry of the lung in healthy rats in both the acute (increased lung DNA and protein) and subchronic studies (increased lung DNA and hydroxyproline and decreased RNA) that were not observed with the London-type aerosol. Both aerosols increased, to a similar extent, the acute small airway inflammation caused by ozone exposure. London-type aerosol interacted with elastase pretreatment (in impaired rats) to increase lung DNA content in a synergistic way in the acute studies while California-type aerosol did not.

(2) Quantification of specific responses that relate to health effects in human populations, including epithelial damage (measured as lung permeability), lung clearance impairment, biochemical responses (indicative of potential fibrosis and/or inflammation), inflammatory cell accumulation in the lung, and cellular level abnormalities in the lung.

Parameters which provided significant responses indicative of potential lung injury in the 3-day exposure studies included small-airway inflammation and lung biochemical changes in DNA, RNA and lung protein. No treatment-associated differences were detected in tracheobronchial clearance or lung permeability. For the 30-day exposure studies, the significant responses included treatment-associated changes in particle accumulation, small airway inflammation, structural changes in small airway walls, lung small airway fibrosis, tracheobronchial particle clearance, hydroxyproline synthesis and lung nucleic acid content.

(3) Comparison of responses between London-type aerosol (LT) (with and without sulfur dioxide) with California-type aerosol (CA) (with and without ozone); the purpose of this comparison is to relate the basis (London episodes) of current air pollution standards to appropriate standards in California.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some aerosol effects were observed in association with ozone exposure or lung impairment. In the 3-day studies, there were significant lung biochemical changes in rats exposed to the California-type aerosol were not observed with the London-type aerosol. Hence, there is an indication that the California-type aerosol may be somewhat more hazardous. It is clear that the effects and responses to California-type aerosol in urban air are inextricably linked to the simultaneous exposure to elevated ozone levels, just as the London smog episodes cannot be used to separately identify effects associated with particles from those associated with sulfur dioxide (USEPA, 1982). However, the combination of California-type aerosol and ozone was much more effective in causing a variety of significant detrimental pulmonary alterations than the combination of London-type aerosol and SO_2.

(4) Comparison between responses of impaired individuals (modeled by simulated emphysematous lung disease in elastase pretreated rats) versus healthy individuals.

An increase, sometimes significant, of several responses to air pollutants were demonstrated in impaired individual because of the lung impairement. Biochemical alterations in the lung induced by impairment where enhanced by exposure to London-type aerosol, sulfur dioxide, and ozone. Impaired rats were significantly more susceptible to small-airway disease and fibrosis elicited by aerosols in combination with ozone and were observed to have greater retention of lung deposits of particulate matter. The statistically signficant effects associated with inhalation of California-type aerosol were, in part, based upon the influence of lung impairment on the sensitivity of the exposed rats.

(5) Comparison of effects elicited by acute and subchronic exposures.

The subchronic exposures led to changes in tracheobronchial clearance that were not found in the acute studies. Also, increased hydroxyproline, quantifiable small airway lesions, and apparent accumulations of particles were found in the subchronic studies, but not the acute studies.

In summary, these findings indicate that the presence of particulate matter in California air may have a greater effect on health than the aerosol found in the London smog episodes of thirty or more years ago. Especially important is the promotional or synergistic effectiveness of inhaled aerosols of the California-type in exacerbating the injury known to be associated with the inhalation of environmental ozone.

The impaired rats with experimentally produced emphysema (elastase pretreated) had abnormal biochemical and morphological characteristics. Elastase pretreatment was found to significantly augment the inflammatory responses and lung structural remodeling (small airway lesions) observed for exposures to aerosols and ozone. These results suggest persons with impaired lungs are at greater risk for exposure to inhaled particulate matter both with and without ozone.

RECOMMENDATIONS

Airborne particulate matter in the respirable size range in California needs to be monitored and controlled to minimize the adverse responses in people, especially those with impaired lung function. Concern should address effects associated both with inhalation deposition and with the promotion and exacerbation of adverse responses from environmental ozone. A concentration of 0.55 mg/m^3 of California-type respirable aerosol exacerbated ozone-induced changes in the lungs of rats exposed for three days. Therefore, an ambient air inhalable (PM_{10}) standard of 0.05 mg/m^3 (based on a 24-hour sample), which is one-tenth of this concentration, appears both reasonable and prudent. Persons with emphysema or other pre-existing lung disease should be expected to be more susceptible to adverse responses to inhaled particulate matter based upon the results in impaired rats.

Future studies should consider further the altered tracheobronchial clearance of inhaled particles and the implications associated with the possible reduced lung clearance of various toxic or infectious agents inhaled in combination with particulate air pollutants. More information is also needed concerning the behavior of deep lung particle clearance associated with inhaled particulate matter. In addition, the mechanisms that lead to the exacerbation by inhaled airborne particulate material of the adverse responses associated with ozone need to studied in more detail. Since the aerosols used in these studies were acidic, the role of aerosol acidity in this promotional phenomena needs to be tested, verified, and quantified. Future studies should also include organic aerosols and vapors as well as inorganic constituents in the exposure atmospheres since these are also present in polluted air.

INTRODUCTION

Airborne particulate matter in association with pollutant gases has been implicated in observed health effects associated with serious pollution episodes such as those associated with the famous London smog episodes and suggested to be involved in potential health effects in California. Epidemiological studies show an inseparable correlation between responses and the concentrations in combination of sulfur dioxide and aerosol particulate matter. Elevated relative humidity is considered to be a contributing factor (U.S. EPA, 1982). This study was designed to elucidate the possible augmentation or synergism of systemic injury that might be associated with inhaled airborne particulate matter and specific gaseous pollutants. These are taken up by the respiratory airways of people if inhaled and may lead to a variety of undesirable biological responses including reduced lung clearance of inhaled particles that deposit in the lung, and exacerbation of existing respiratory abnormalities.

Current air pollution standards for airborne particulate matter are based primarily upon acute mortality and morbidity associated with episodes of high particle and sulfur dioxide pollution in London, England, in the 1950's and on other acute episodes of elevated levels of total suspended particulate material and sulfur dioxide in the United States and elsewhere in the world (U.S. EPA, 1982). In October, 1948, high levels of particulate matter (probably as high as 1 mg/m^3) and sulfur dioxide (estimated to be above 0.4 ppm) severely affected 8% of the population of Donora, Pennsylvania, and resulted in twenty deaths (Schrenk, et al., 1949). A four-day pollution episode also occurred on December 5-9, 1952, in London with average particulate matter concentrations measured at from 1.98 mg/m^3 to 2.65 mg/m^3 and average sulfur dioxide concentrations from 0.94 ppm to 1.26 ppm (Wilkins, 1954). Four thousand excess deaths were reported during this London smog episode and were readily correlated to elevated particulate matter (up to 4.46 mg/m^3) and sulfur dioxide (up to 1.34 ppm) concentrations (Logan, 1953). Other episodes in the U. S. in the 1950's and 1960's in Detroit (September, 1952), New York City (November, 1953; December, 1962; January, 1963; March, 1964) showed morbidity

and mortality relationships for particulate matter levels from in excess of 0.2 mg/m^3 to 0.88 mg/m^3 and sulfur dioxide levels from 0.4 to 1 ppm (U.S. EPA, 1982). Studies of mortality and morbidity in subchronic exposure over polluted months show a similar association between elevated sulfur dioxide and airborne particulate matter. Most deaths occurred with people having pre-existing illness, especially lung diseases (U.S. EPA, 1982).

The close correspondence between elevation of concentrations of sulfur dioxide and particulate matter have made it impossible to conclusively show that either of these pollutant entities alone was separately responsible for the observed serious health effects; consequently, air quality criteria have had to be established on the assumption that the elevation of either pollutant alone could have been the cause. This has led to separate standards that may have a measure of built-in safety. Hence, the national ambient air quality standards for acute exposure to these pollutants (24-hour total suspended particulate material levels of 0.26 mg/m^3, now replaced by 24-hour PM_{10} levels of 0.15 mg/m^3, for particles, and 0.5 ppm for sulfur dioxide) are based upon criteria that jointly consider these pollutants (U.S. EPA, 1982). The advent of size-selective sampling of particles that are inhalable (smaller than about 10 micrometer in aerodynamic equivalent diameter) has led to the new 24-hour ambient air quality particulate matter standard called PM_{10} that considers more appropriately the small inhalable particles. The California ambient air quality standards involve a degree of greater safety than the Federal standards, especially for effects in children, with the 24-hour standard for PM_{10} being 0.05 mg/m^3 for particulate matter and 0.05 ppm for sulfur dioxide.

The aerosols found in California air pollution are quite different in composition than those associated with the London smog and other serious pollution episodes noted above. In addition, California has little sulfur dioxide, but does have elevated levels of reactive oxidants (as ozone). These are probably more injurious to the deep lung tissue than sulfur dioxide, which is largely absorbed in the head and upper respiratory airways. Hence, there is a need to critically study the potential biological effects associated with California-type (CA) aerosols and compare these results to London-type (LT) aerosols, and to further compare the influence of the respective reactant

gases, ozone and sulfur dioxide. The differences in the constituents of the two types of aerosols (LT & CA) are discussed below. They provide a basis for establishing the appropriate constituents for synthetic versions of these two types of particulate matter used in this study.

The overall purpose of this project was to study the potential for systemic injury in both healthy and impaired laboratory animals (male rats) of a synthetic California-type aerosol and to compare those effects with responses to London-type aerosol. The California-type aerosol was administered to laboratory animals both with and without a typical elevated concentration of ozone. The counterpart gaseous pollutant for London-type aerosol was sulfur dioxide. Hence, the experimental design of this study was aimed at direct comparison of the dose response relationships for the two types of air pollution. In order to consider the strong indication that pre-existing disease is a major risk factor for people exposed to air pollution, both healthy and impaired animal models were studied. Since systemic injury to the lung is the principal response of concern, the biological evaluations focused on respiratory effects, while considering general health status as well. Key evaluations included histopathology of the lung, lung biochemistry, and lung permeability and particle clearance.

PROJECT OBJECTIVES

Utilizing two experimental animal models, one healthy and one impaired (Fischer 344 SPF male rats), this project tested the hypothesis that the inhalation of episodal levels of typical aerosols and gases associated with air pollution would result in adverse biological responses including systemic injury to the respiratory tract and impairment of lung clearance of potentially toxic particles. Both acute (3-day) and subchronic (30-day) exposures were tested.

Specific objectives included:

(1) Evaluation of acute and subchronic responses of the lung to inhaled London-type aerosol (LT) or California-type aerosol (CA) alone and in combination with episodal levels of ozone (California) or sulfur dioxide (London).

(2) Quantification of specific responses that relate to health effects in human populations including epithelial damage (measured as lung permeability), lung clearance impairment, biochemical responses (indicative of potential fibrosis and/or inflammation), inflammatory cell accumulation in the lung, and cellular level abnormalities in the lung.

(3) Comparison of responses between London-type aerosol (LT) with and without sulfur dioxide with California-type aerosol (CA) with and without ozone for the purpose of relating the basis (London episodes) of current air pollution standards to appropriate standards in California.

(4) Comparison of responses in impaired individuals (simulated emphysematous lung disease in rats pretreated with elastase) to healthy individuals.

(5) Comparison of effects elicited by acute and subchronic exposures.

TECHNICAL PLAN

Overall Design.

In acute exposure studies, Fischer-344 rats were exposed for twenty-three hours daily for three days to simulate a peak air pollution episode at (a) the chosen concentration of from 0.55 mg/m3 to 5 mg/m^3 of either the London-type aerosol (LT) or California-type aerosol (CA), (b) the same level of aerosol in combination with 0.40 ppm ozone (CA) or from 1 to 21 ppm sulfur dioxide (LT), and (c) clean air, in four 4 m^3 stainless steel and glass exposure chambers capable of accommodating 24 rats in a monolayer. Of the rats in each chamber (with associated pollutant environment), 12 were healthy (H) and 12 were impaired (I) with induced alveolar emphysema caused by intratracheally instilled elastase. Additional healthy rats in each chamber were used as sentinels for viral screening performed at the conclusion of the exposures to complement pre-exposure screening. Of the 12 rats of each type in each chamber 6 went first to lung particle-clearance evaluation after sacrifice and then to biochemistry, while the other six went to pathological evaluation and clinical chemistry. This experimental design for the 3-day studies is illustrated schematically in Figures 1 and 2. Because these studies were conducted to contrast California-type aerosols with London-type aerosols, the exposures were designated by the prefix CL, e.g., CL-1 was the first 3 day exposure series. There were six acute exposure studies, CL-1 through CL-6.

In the original design there were to be three exposures to different concentrations of each of the two aerosol types shown in Figures 1 & 2, respectively. The third London-type aerosol exposure was changed to include ozone at 0.4 ppm instead of sulfur dioxide after it was found that there were no responses even with 21 ppm SO_2. This provided a means to find out if London-type aerosol interacted with ozone in a manner similar to the California-type aerosol.

In the subchronic exposure study (CL-7), 30-day, 23 hour per day, subchronic exposures were conducted utilizing 5 chambers, each with 12 healthy and 12 impaired male rats. An key pathologic response in the acute studies was associated with the 0.4 ppm ozone. Since this concentration of ozone is a plausible episodal level and the only one tested in the acute studies, the ozone was kept at 0.4 ppm for the subchronic studies. Aerosol concentrations were chosen at a high but not exceptional 1 mg/m3, and sulfur dioxide was targeted at 1 ppm. Sulfur dioxide was not be tested without London-type aerosol in the subchronic study. The layout of the subchronic 30-day exposure is shown in Figure 3. The purpose of the subchronic 30-day exposures was to compare the effects of the two types of aerosols under subchronic conditions where certain serious longer term effects may become apparent which would not have been observed in the acute studies. Pulmonary fibrosis is an example.

The nominal constituents of the synthetic London-type aerosol (LT) and the synthetic California-type aerosol (CA) are summarized in Table 1. Emphasis in this study was upon inorganic constituents since they predominate and are most likely to be the cause of systemic injury to the lung. These representative aerosols were formulated to incorporate key features of ambient airborne particles from the respective regions and pollution conditions. For example, though both have free inorganic carbon, the London type aerosol has larger lamp-black type particles associated with unburned carbon in coal fly ash, while the California-type aerosol has primarily submicronic graphitic carbon with high surface area. California-type aerosol (CA) has about 60% as much carbon as does London smog-type aerosol (LT) (ARB, 1982). In both cases, fine particles were studied that would be represented by PM_{10}. Since humid conditions are usually implicated in air pollution episodes, a relatively high relative humidity of 80% was chosen for these studies, but one that would not lead to excessive condensation in the chambers.

According to ARB reports, nitrate compounds are one and one-half times as common in the California air than sulfates, so this relationship was included in the CA constituents. Lead and manganese sulfate represents the contribution of lead and manganese in fuel additives to current pollution levels. The vanadyl and nickel sulfate constituents represent trace metals contributed by

oil-fired power plants (Kimble, Raabe, and Silberman, 1982). The natural clay represents the common dirt and dust so prevalent in many areas of California. The London-type aerosol (LT) has a large contribution of coal fly ash from coal burning and acid ammonium bisulfate to simulate the acid sulfates identified in the London smog episode.

TABLE 1: Composition of Synthetic Pollutant Aerosols

California-Type Aerosol (CA)		London-Type Aerosol (LT)	
Temperature 23°C		Temperature 23°C	
Relative Humidity 80%		Relative Humidity 80%	
Size: 1 micrometer MMAD		Size: 1.5 micrometer MMAD	
O_3: 0.4 ppm		SO_2: 1 or 21 ppm	
NH_4HSO_4	15%	NH_4HSO_4	20%
$(NH_4)_2SO_4$	15%	$(NH_4)_2SO_4$	20
NH_4NO_3	25%	Coal Fly Ash	30%
Carbon	18%	Carbon	30%
$VOSO_4$	0.01%		
$MnSO_4$	0.03%		
$NiSO_4$	0.01%		
$PbSO_4$	0.04%		
Natural Clay	27%		

[%= mass fraction]

Figure 1: Acute 3-day exposure experimental design and evaluation plan for the California-type aerosol studies with and without 0.4 ppm ozone.

CALIFORNIA TYPE AEROSOL ACUTE STUDIES

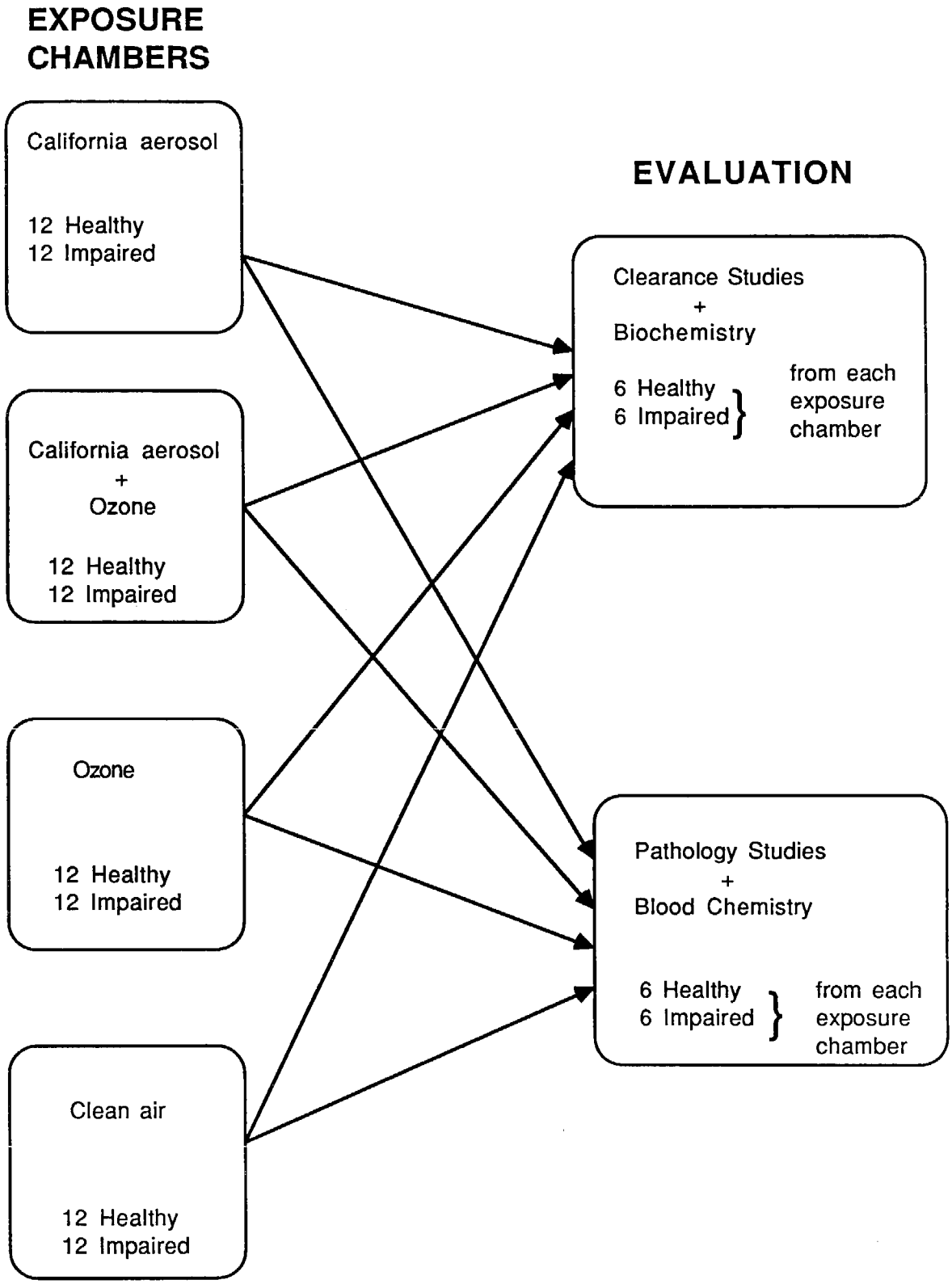


Figure 2: Acute 3-day exposure experimental design and evaluation plan for the London-type aerosol studies with and without 1 ppm to 20 ppm sulfur dioxide.

LONDON TYPE AEROSOL ACUTE STUDIES

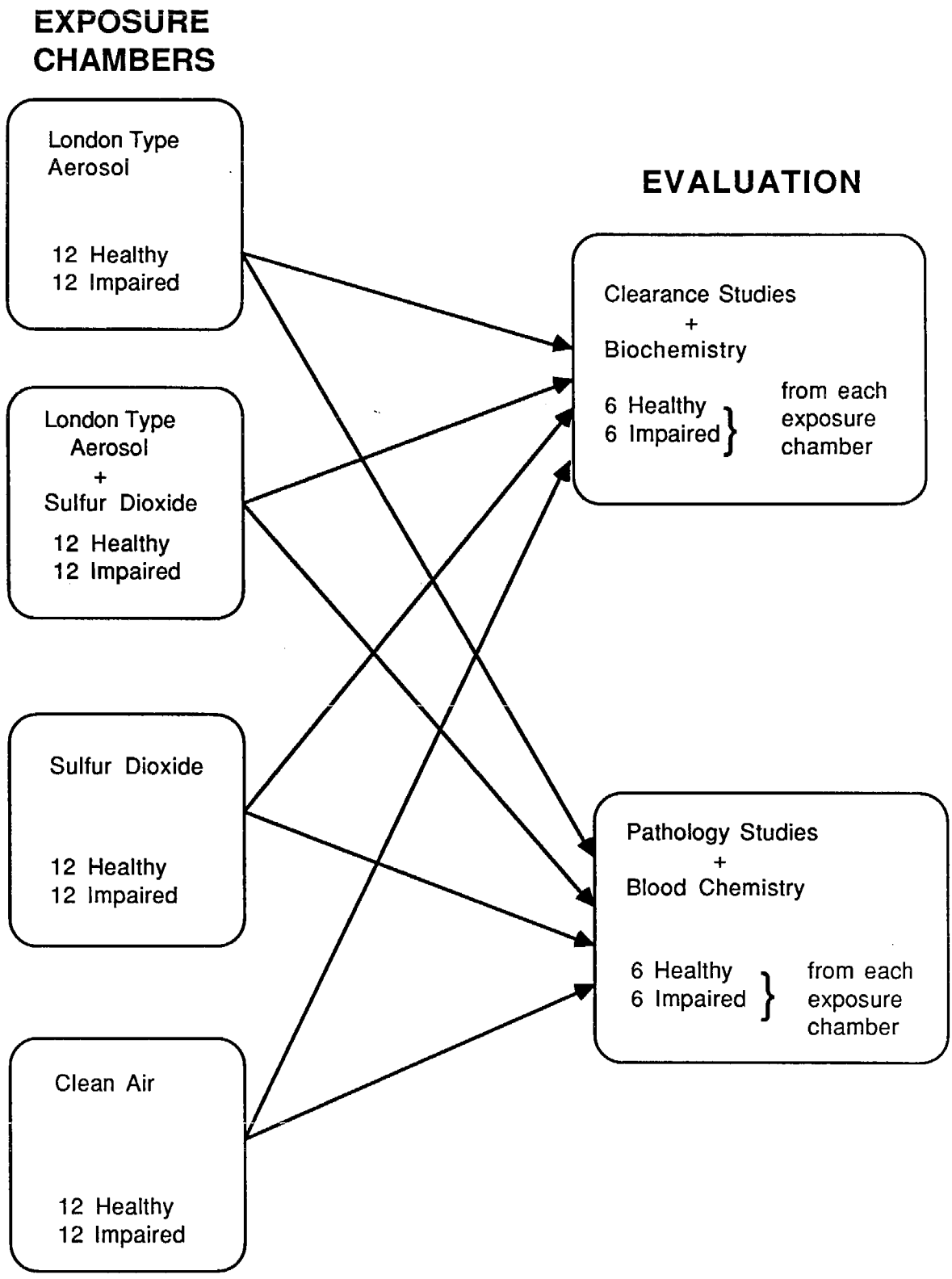
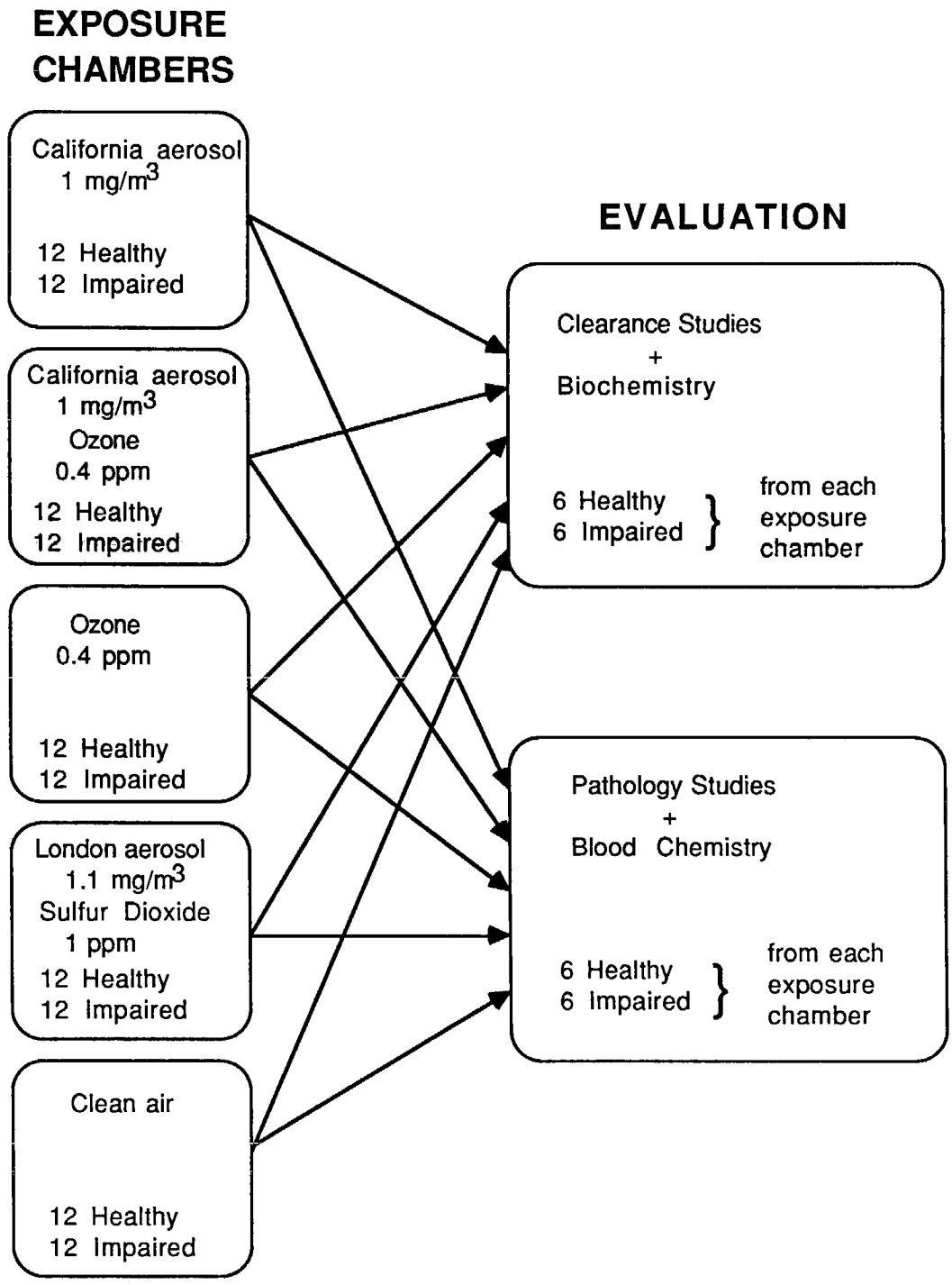


Figure 3: Subchronic 30-day exposure experimental design and evaluation plan for the London-type aerosol with sulfur dioxide and California-type aerosol studies with and without 0.4 ppm ozone.

LONDON TYPE AND CALIFORNIA TYPE SUBCHRONIC AEROSOL STUDIES



METHODS

Animal Exposures.

Overall, 750 respiratory disease-free Fischer 344 SPF 70 day old rats were used in these studies. Fifty-four representative animals were screened for both bacterial and viral infections prior to, during (in the subchronic 30-day study), and after the inhalation exposures. All animals for a given exposure series were ordered and received together and randomly selected for treatment so that statistical analyses and comparisons among the groups would be valid.

Each rat was ear-tagged with a unique identification number. Prior to beginning of exposures, all rats were weighed, and the weights were used for stratified selection and assignment. Assignments were made to the exposure groups as discussed in the statistical design section. During whole body exposure in large 4 cubic-meter stainless-steel and glass chambers rats were maintained individually in stainless steel wire mesh cages placed in a monolayer planar array perpendicular to the direction of aerosol flow. Aerosols, gases, steam, and clean incoming air were mixed at the chamber inlet. Chamber exhaust was filtered to remove contaminants prior to release. Chamber flow rates were maintained at 2100 L/min.

As discussed in the previous section, the experiments consisted of seven sets of exposures. Three were modeled after a California urban atmosphere (Figure 1) and three were modeled after a London-type atmosphere (Figure 2). One exception was made in London-type aerosol exposure with ozone being substituted for sulfur dioxide. These first six sets of exposures were three days long for 23 hours a day (a total, 69 hours). The last set of exposures was thirty days long and used both types of atmospheres (a total, 690 hours; Figure 3). The exposures are summarized in Figure 4.

Figure 4: Chart of the pollutant concentrations used for the six acute 3-day exposure (CL-1 to CL-6) and for the subchronic 30-day exposure (CL-7) for the California-type aerosol and the London-type aerosol generated in this study of the effects of airborne particulate matter.

CONCENTRATION OF POLLUTANTS

Phase I, Exposures CL1-6 (3 days @ 23 Hrs/day)

Phase II, Exposure CL7 (30 days @ 23 hrs/day)

Exposure # / Chamber Atmosphere	CL1	CL2	CL3	CL4	CL5	CL6	CL7
Ca Type Aerosol	0.53	1.42	2.99				1.05
Ca Type Aerosol + Ozone	0.58 + 0.41	1.47 + 0.39	2.96 + 0.41				1.02 + 0.37
Ozone	0.40	0.40	0.40			0.39	0.38
London type Aerosol				0.83	4.68	3.21	
London type Aerosol + SO_2				0.99 + 1.07	5.02 + 21.89		1.10 + 0.96
SO_2				1.07	20.98		
London type Aerosol + Ozone						2.64 + 0.39	

Aerosol concentration = mg/m^3

O_3 & SO_2 concentration = ppm

Biostatistical Design.

Both parametric and non-parametric methods were used for evaluation of data, and modern analysis of variance (ANOVA) methods were used to evaluate observed frequencies of changes and responses. The experimental design was established to facilitate these data comparisons.

The 3-day study design (Figures 1 & 2) was as follows:

		HEALTH STATUS	
		H	I
EXPOSURE ATMOSPHERE	AEROSOL	12	12
	AEROSOL + GAS	12	12
	GAS	12	12
	CONTROL	12	12

Here AEROSOL was either LT or CA and GAS was either SO_2 or O_3, respectively. The number of rats per cell was 12. However, of the 12 rats per cell, the assignments used in tests were as follows:

(1) 6 rats were randomly selected (stratified by weight) for lung clearance studies (total of 12 per chamber atmosphere).

(2) These 6 rats were also used for lung biochemistry studies (12 per chamber atmosphere)

(3) The remaining 6 rats were used for pathology and blood chemistry (12 per chamber atmosphere).

The results were analyzed using a three-way Analysis of Variance (ANOVA), fixed effects model. We used two levels of contrasting responses. A statistically significant result was assumed for a significance level of $p \leq 0.05$. In addition, an important tendency was assumed for a nearly

significant level of $p \leq 0.1$. The experimental design shown above is that of a factorial with Health Status at two levels and exposure atmospheres at 4 levels. The statistical main effects (health status and exposure atmosphere) and the cross interactions were tested.

Consider alpha=0.05 as the level of significance (Type I error) and beta=0.20 (Type II error); power= 80% with d the true difference from the mean of the controls and s the estimate of the true standard error per experimental unit. With 6 rats per cell we should have been able to detect true differences (given the accompanying standard errors, expressed as percentage coefficients of variation or relative standard error): d= 10%, s= 6%; d= 15%, s= 9%; d= 20%, s=12%; d= 25%, s= 16%; and d= 30%, s= 18%. We have used a one-tail test because we believe that the treatments used was deleterious and never beneficial.

We were dealing with a variety of variables, and their standard errors relative to their means were not known precisely. The observed data were sometimes skewed, so the relative standard errors were large. In addition, there were clear responders to treatment, whereas others had little or no response, or the response was opposite from expectation. This led to the use of non-parametric tests in some cases, such as for the results of the lung particle-clearance studies. These included the Mann-Whitney and Kruskal-Wallis analysis of variance tests. Morphometric data and inflammatory cell counts were analyzed by a general linear model analysis of variance computer program (Numbercruncher Statistical Analysis System version 5.0). Lesion scores in subjective evaluations were analyzed by the Mann-Whitney test.

RANDOMIZATION AND STRATIFICATION: It was extremely important that the rats be assigned to treatment randomly. However, because the body weights of rats covered a range of values, it was necessary to insure that the average weights and variance of weights in the treatment cells matched as well as possible. It was necessary, therefore, to stratify the rats so that this occurs, while at the same time allowing for random assignment.

This was done by:

(1) Weighing each rat individually and ordering the rats as to body weight from smallest to largest.

(2) The first two (smallest) rats were randomly assigned to be intact (Healthy, H) or elastase instilled (Impaired, I). Succeeding pairs of rats were similarly assigned.

(3) The four smallest rats in each group (H versus I) were randomly assigned to the four different exposure atmospheres. Succeeding groups of 4 H and 4 I rats were similarly assigned.

Design of the subchronic 30-day study (Figure 3); this study compared five exposure atmospheres:

		HEALTH STATUS	
		H	I
EXPOSURE ATMOSPHERE	CA	12	12
	CA + OZONE	12	12
	LT + SO_2	12	12
	OZONE	12	12
	CONTROL	12	12

Exposures in the subchronic 30-day study were 23 hours per day for 30 days. There were 12 rats per cell (24 per aerosol). Each of the classes of biologic tests (lung clearance, pathology, biochemistry, blood chemistry) was performed on 6 rats per cell (a total of 60 rats per test). The main statistical effects were health status and exposure atmospheres. The significance of the cross interactions was tested. The methods were the same as described for the 3-day studies. The power of these tests slightly exceeded that of the acute studies because there are more degrees of freedom for the estimate of error.

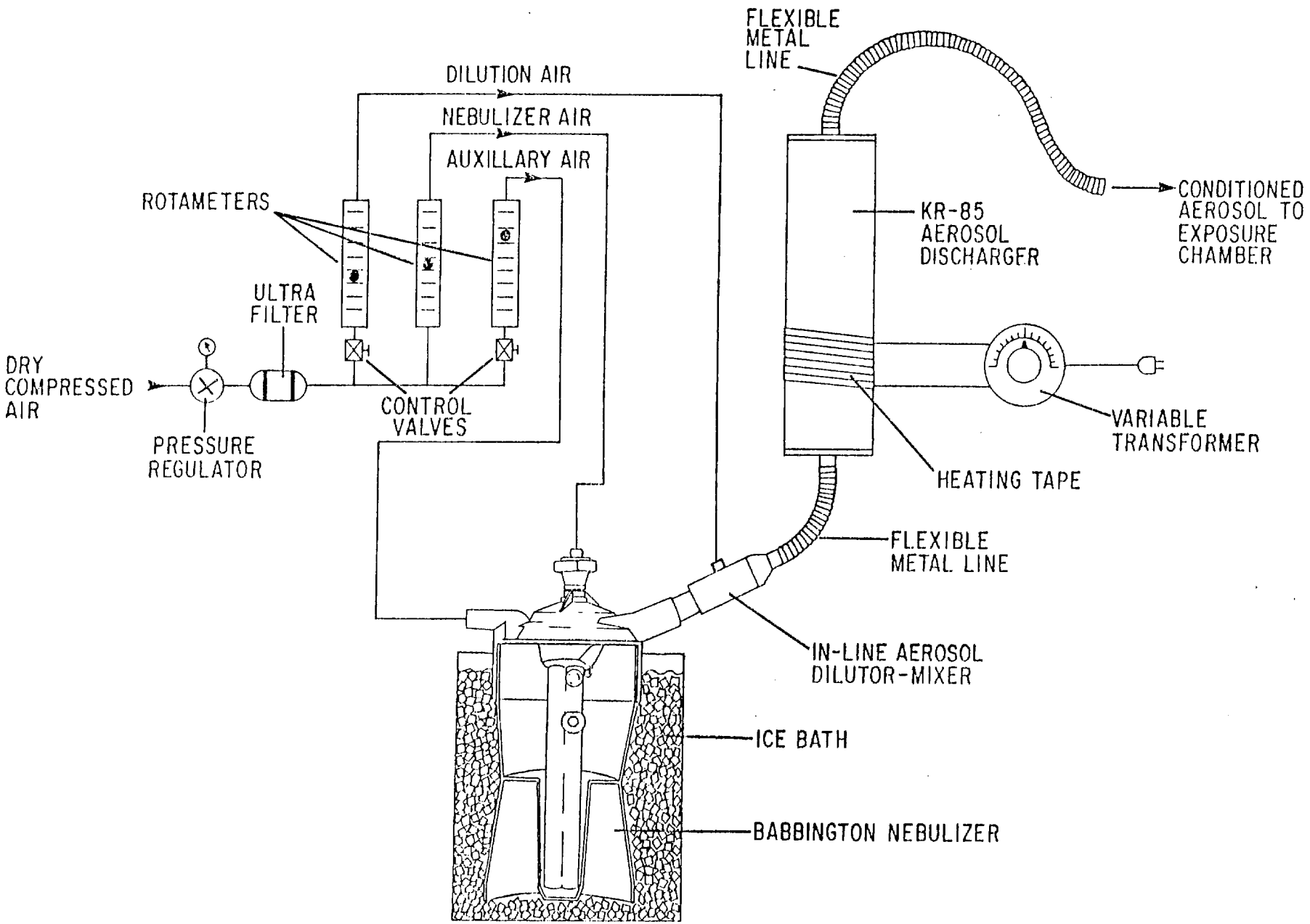
Aerosol Generation

The two separate types of aerosol mixes were generated using nebulization of liquids for the inorganic salts and using the Wright dust feeder for dry dust dispersion. These aerosols were conditioned and mixed with the main 2100 L/min of air flow to each 4 m^3 aerosol exposure chamber. Steam was also mixed with the main inlet flow to yield a nominal relative humidity of 80%. This system produced aerosols similar to the particulate air pollutants of concern. They were in the respirable range (<3.5 micrometer in aerodynamic diameter) with a significant portion being smaller than one micrometer in aerodynamic diameter.

Aerosols of the inorganic salts were generated together from aqueous solutions containing the appropriate chemical constituents using a Babington-type Solosphere (CL-1) or multiple jet version Hydrosphere nebulizer (American Hospital Supply, Irvine California) as shown in Figure 5. The liquid aerosol was passed through a diluter to accelerate drying of the droplets and then through a heated cylinder containing a sealed krypton-85 (^{85}Kr) radioactive source (10 mCi). This arrangement was used to reduce the aerosol electrostatic charge to Boltzmann equilibrium. The quantity of aerosol produced by the nebulizers was adjusted by altering four factors: (1) the concentration of the solution, (2) the Hydrosphere generator changeable elements (these include the ball and flow pressure restrictor), (3) pressure supplied to the generator and (4) the flow of the auxiliary air. The nebulizer was modified by removing the air intake mechanism which was on the stock model and replacing it with a cap having a series of holes that were set to produce different amounts of air to mix with the nebulized aerosol. A plug with two "O" rings was inserted into the intake air inlet, and a metered flow of filtered compressed air was used to adjust the amount of make up air. The operating characteristics of the Babington nebulizer are summarized in

Appendix A along with the chemical constituents of the nebulized aqueous solutions for the surrogate California aerosols. For exposure set CL-2 through CL-7 a Hydrosphere was used for nebulizing solutions. These have outputs similar to Solosphere, with increased output capability achieved by

Figure 5: Schematic illustration of the nebulizer system used in the study to generate aerosols of inorganic salts from aqueous solutions.

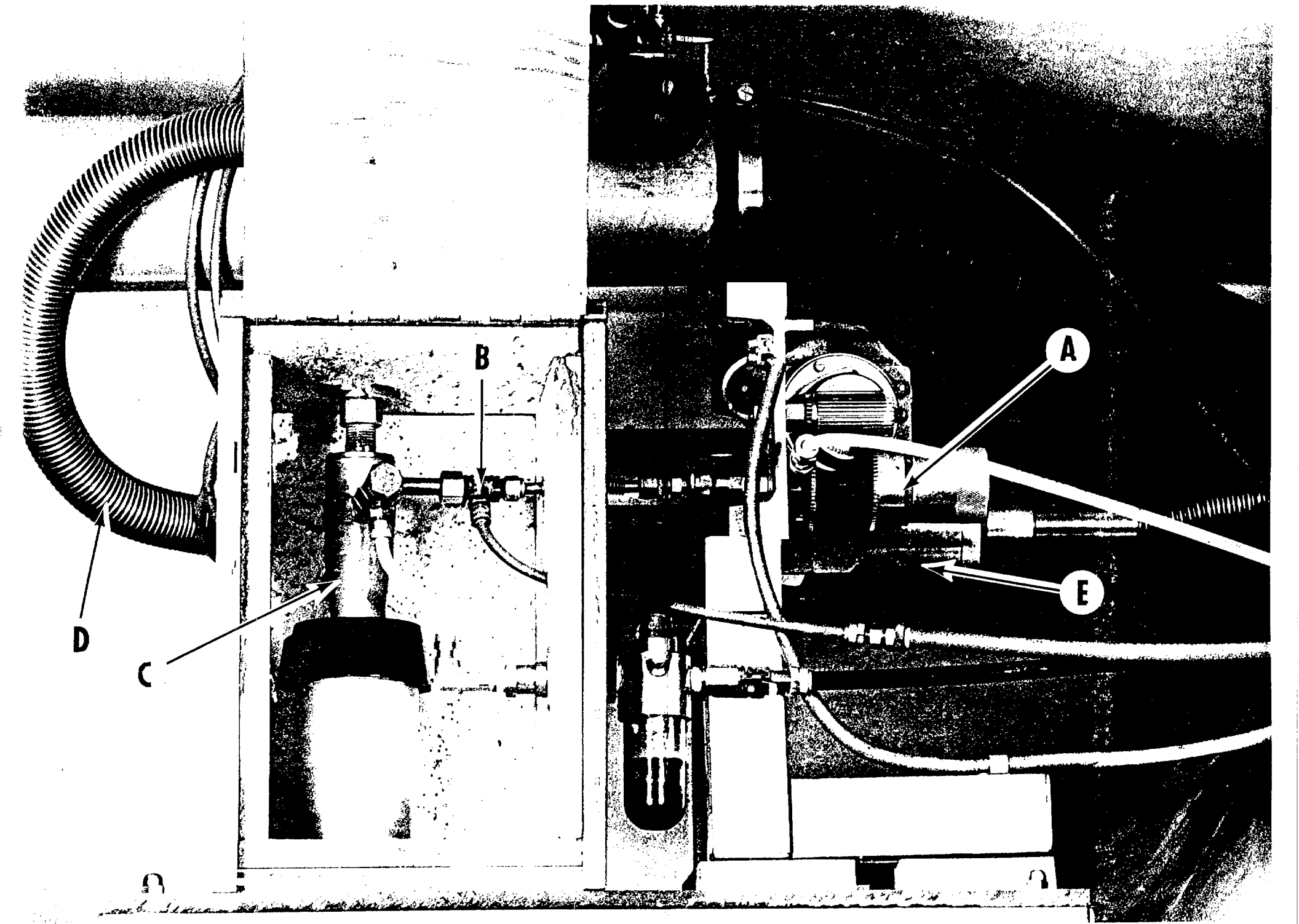

FLEXIBLE
METAL
LINE
DILUTION AIR
NEBULIZER AIR
AUXILLARY AIR
ROTAMETERS
CONDITIONED
AEROSOL TO
EXPOSURE
CHAMBER
KR-85
AEROSOL
DISCHARGER
ULTRA
FILTER
DRY
COMPRESSED
AIR
CONTROL
VALVES
VARIABLE
TRANSFORMER
PRESSURE
REGULATOR
HEATING TAPE
FLEXIBLE
METAL LINE
IN-LINE AEROSOL
DILUTOR-MIXER
ICE BATH
BABBINGTON NEBULIZER

adding generating jets and increasing the flow through the unit. The Solosphere and Hydrosphere nebulizers have similar designs and operating characteristics.

The acidity of all of the inorganic salt solutions was adjusted to pH 1.8 with sulfuric acid to maintain the balance between sulfate and bisulfate. The concentrations of samples of the nebulizer solutions were verified by ion chromatography for quantification of the sulfate and nitrate cations and by atomic absorption spectrophotometry for the metals. Chemical analyses data for typical exposure solutions are given in Appendix A. Aerosol samples were collected with filters and analyzed to verify the airborne chemical constituents of the aerosols; typical data are shown in Appendix A. Separate aerosolization tests, using only the nebulized aerosols, were performed with filter samples and cascade impactor samples to verify the uniformity of the chemical composition with respect to particle size.

The lamp black plus fly ash used in the London-type aerosols, or carbon plus natural clay, used in the California type aerosols, were generated as dry powder utilizing a Wright dust feed (WDF) generator (Mod #180, Messrs. L. Adams Ltd., 22 Minerva Rd., London) in combination with a miniature cyclone separator and a krypton-85 (^{85}Kr) discharger by the method of Raabe (1979) as shown in Figure 6. For the WDF method the mixture of powders was packed into stainless steel cups and resuspended by scraping the dust off by means of a blade and blowing it off with compressed air. The amount of dust generated by this method was set by a series of gear drives which changed the speed at which a circular blade scrapes off the cake packed into the cup. The dust was blown off the cake and was impacted into a metal baffle to help deagglomerate the packed powder. The dust then was passed through a cyclone collector, with a 250 milliliter cup below the unit to collect the dust particles larger than the median cut point of 2.6 micrometers aerodynamic size. The dust was mixed with the other aerosol (from the nebulizer) and piped into a large volume ^{85}Kr discharger (Teague, et al., 1978) to reduce the electrostatic charge on the particles to Boltzmann equilibrium. This aerosol was mixed with the filtered and conditioned air and the gas was metered in at the proper rate. The humidity was adjusted to 80% by adjusting the steam pressure which passed

Figure 6: Photograph of the system used for generating aerosols of dry dusts (carbon plus clay or lamp-black plus fly ash mixtures); (A) is the Wright dust feed, (B) the diluting air connection, (C) the cyclone separator, (D) the aerosol line from the cyclone separator to the discharger, and (E) the ^{85}Kr discharger.


A
B
C
D
E

into a small orifice placed in the line immediately before the air entrance to each chamber. The experimental arrangement of the dry dust generation system in which aerosol was discharged into the inlet flow of an exposure chamber, is shown schematically in Figure 7.

The California-type aerosol dust mixture was prepared with equal amounts of fine carbon (Asbury Graphite Mills, Inc., Asbury, NJ) and natural montmorillonite clay (Southern Clay Products, Gonzales, Texas). The latter was processed with sodium to provide a fine powder. The London-type dust was prepared with equal amounts of lamp black and respirable coal fly ash. The coal fly ash was identical to that described by Raabe, et al. (1979). These respective dry dusts were thoroughly mixed and loaded under firm pressure to form a cake in the stainless steel dust cup of the Wright Dust Feed (WDF) using a special pressure-regulated hydraulic ram, shown schematically in Figure 8. It was capable of pressures of up to 10 tons. Below is the extension tube used to pack the cake using one pack. This method was preferred because in eliminated the interfaces caused by the multiple pack method.

Ozone was generated by silent arc discharge in pure oxygen. Sulfur dioxide was generated from dilute mixtures in nitrogen stored in a compressed gas cylinder. Both gases were measured automatically with instrumental detection equipment. The sampling lines, filter holder, and solenoid valve of each monitor were made of teflon. The ozone was measured with a Dasibi ozone analyzer that was calibrated against a Dasibi UV photometer model 1008PC, which was in turn calibrated against a National Bureau of Standards standard reference photometer (serial #4) located at the California Air Resources Board Quality Assurance Standards Laboratory. The sampling interval for ozone was every 10 minutes, providing concentrations every 5 chamber volume changes. The sulfur dioxide was monitored in one-hour averaged blocks by a Meloy Laboratories FPD Sulfur analyzer model SA 285 that was calibrated using the dynamic dilution system. This process used a known 49.2$\pm$1 ppm sulfur dioxide in air (cylinder No. CC49614) traceable to National Bureau of Standards SRM 1693.

Figure 7: Schematic illustration of the system used to generate aerosols of dry dusts including mixtures of carbon and clay or lamp-black and fly ash. (R1 and R2 are regulators; V1, V2, V3 and V4 are valves; F1 and F2 are flowmeters; and P1 and P2 are pressure gages.)

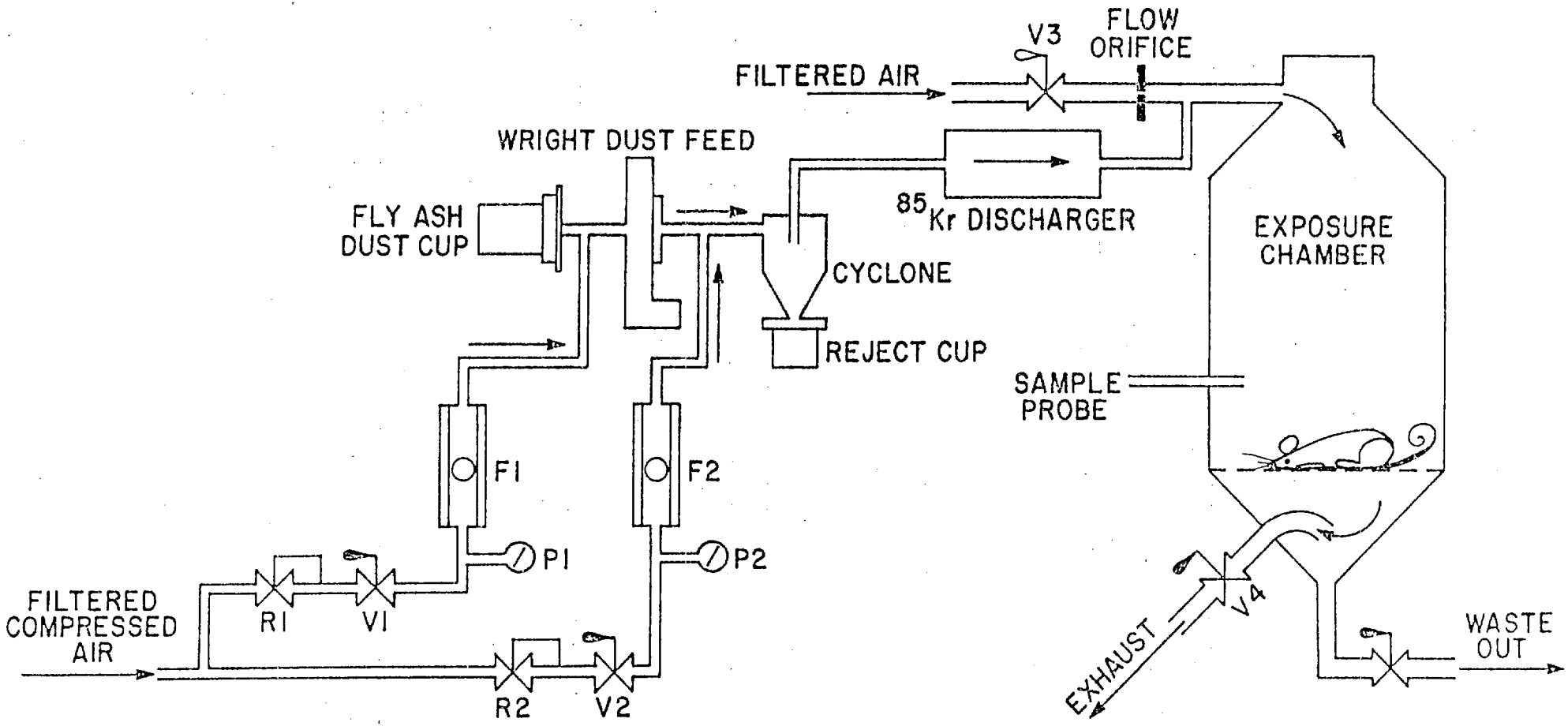
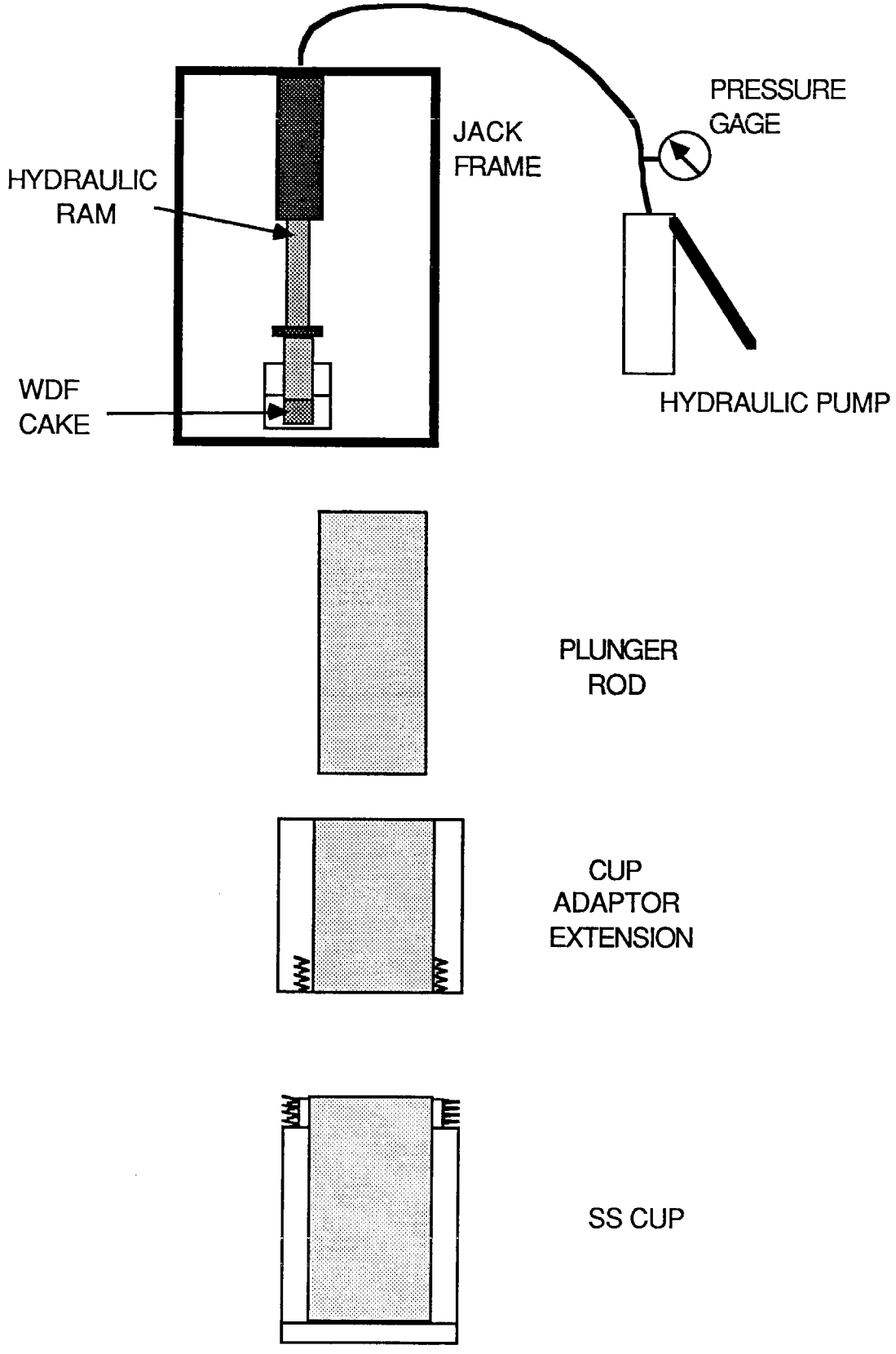

V3
FLOW ORIFICE
FILTERED AIR
WRIGHT DUST FEED
FLY ASH DUST CUP
85Kr DISCHARGER
EXPOSURE CHAMBER
CYCLONE
REJECT CUP
SAMPLE PROBE
F1
F2
P1
P2
FILTERED COMPRESSED AIR
R1
V1
R2
V2
V4
EXHAUST
WASTE OUT

Figure 8: Illustration of the hydraulic ram system used in this study to pack the stainless steel dust cup of the Wright dust feed with dry mixtures of either carbon and clay or lamp-black and fly ash. The top figure shows the press used to pack the stainless steel cups with the dust. Below is the extension tube used to pack the cake using one pack.


PRESSURE
GAGE
JACK
FRAME
HYDRAULIC
RAM
WDF
CAKE
HYDRAULIC PUMP
PLUNGER
ROD
CUP
ADAPTOR
EXTENSION
SS CUP

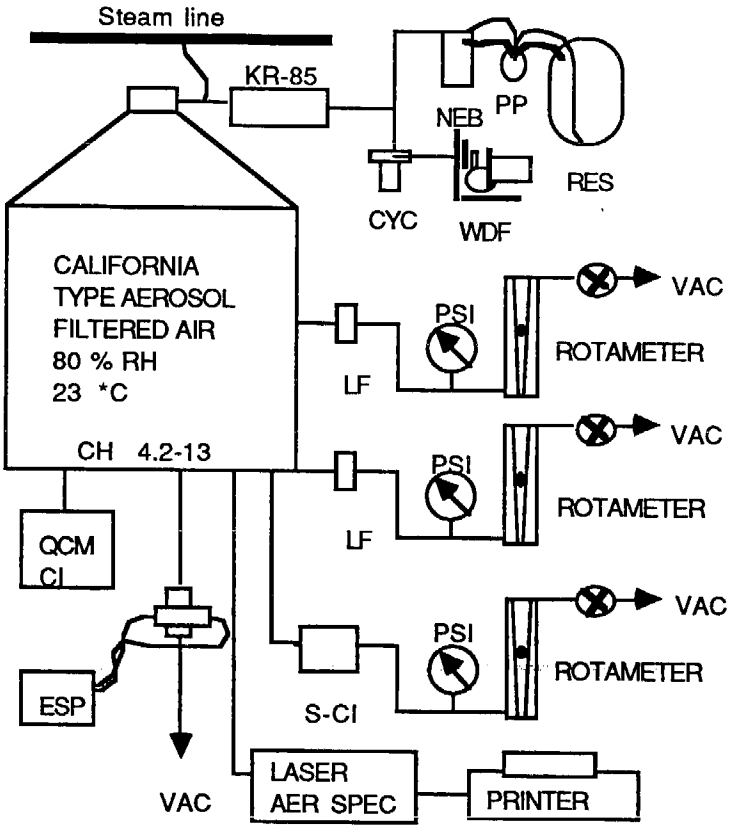
Relative humidity for the chambers was controlled by steam ejection at the top of each exposure chamber through a small orifice device located in the air stream. Control of the relative humidity was determined by steam pressure and orifice hole size. This was added to the relative humidity of the conditioned air going into the chambers which was controlled at 50% RH to achieve a relative humidity of 80% at 23^{o}C for the exposures.

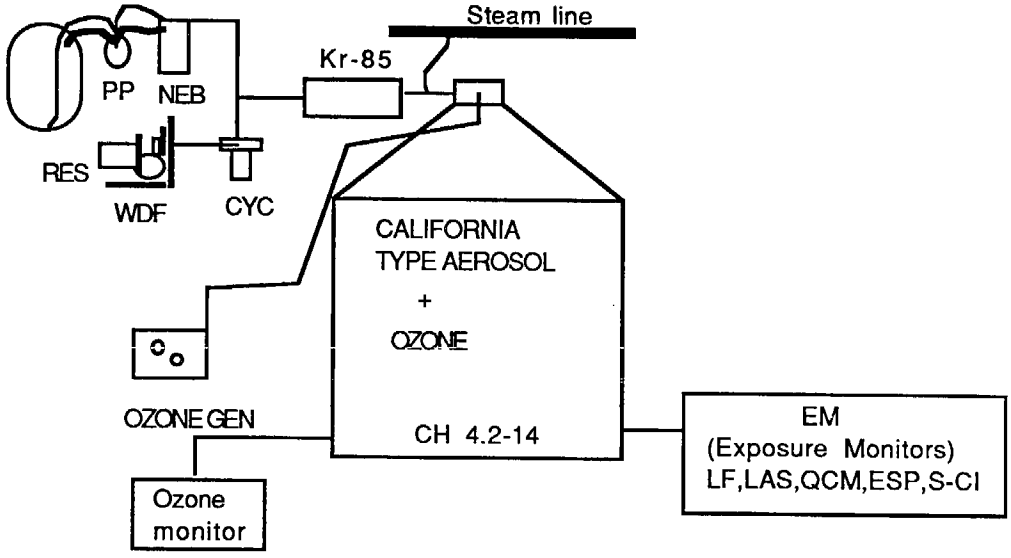
All air used in the generation of aerosols as well as that used to dilute aerosol mixtures was filtered for both particulate and organic contaminants utilizing absolute and activated charcoal filters. All effluent from exposure chambers was collected prior to discharge and filtered to prevent environmental releases. Potentially toxic wastes, including animal tissues, were disposed of using procedures in effect on the Campus, as administered by the Office of Environmental Health and Safety.

Experiment CL-1 with California-type aerosol was the first exposure and was conducted at a concentration of 0.55 mg/m^{3} for three days (Figure 9). Two Wright dust feeds and two Solospheres were used; these were placed on a working platform above the large exposure chamber doors. Steam entered from a steam line located above the top of the chamber producing the near 80% Relative Humidity (RH). The main airstream through the chamber was conditioned and filtered air at 23^{o} C. The cups were packed with a mixture of 40% carbon and 60% clay and packed with a single pack at 800 PSI with a special packing extension (Figure 8). The Wright dust feeds both operated well for the first experiment although not at equal efficiency. To compensate for the difference between the dust feeds, the exhaust from the cyclone separators was switched between the two aerosol chambers at the midway point of the three-day exposure. Each of the nebulizers was fed by a constant supply of cold solution by pumping from one of two 4.5 liter containers placed in a refrigerator unit. The temperature was maintained around 6^{o} C for all the exposures. The nebulizers were placed in a small refrigerator unit with a special top constructed of styrofoam placed over them to reduce heat loss. The pumping system consisted of a peristaltic pump, controlled by a variable speed motor, with four heads and multiple plastic feed tubes. This system assured that the corrosive

Figure 9: Generator configuration for CL-1 showing two separate generation arrangements for the two chambers located on the working platform above the chamber doors. Labels shown are: WDF (Wright dust feed generator); CYC (cyclone separator); RES (cooled liquid reservoir); PP (peristaltic pump); Neb (nebulizer unit); KR-85 (Krypton discharger unit); EM (Exposure chamber monitors).

GENERATOR CONFIGURATION
CL-1





solution was not contaminated by contact with the metallic components. Constant recirculation of a large volume of generator solution helped minimize the effects of increasing concentration due to evaporation extended periods of nebulizer operation. The duct carrying the nebulized material to the chamber was heated to prevent condensation before the aerosol was mixed with dry air and the dust. Air quality in the chambers was sampled using two 47 mm diameter Versapore 0.2 micrometer filters and a Sierra cascade impactor operating continuously for 8 hours, point-to plane electrostatic precipitators, a laser aerosol light-scattering particle counter with printer, and relative humidity and temperature monitors.

For experiments CL-2 and CL-3 the aerosol concentration was increased (Fig. 10). To produce this higher output of material we had to use a larger Babington-type nebulizer, a hydrosphere with one ball for the CL-2 and two-ball configuration for the CL-3 exposure. The nebulizers were also put on a shelf constructed between the two aerosol exposure chambers and stainless steel lines located in a vertical position to facilitate drying of the increased volume of nebulized solution. These lines were also wrapped with heating tape and insulated to achieve the heat input required to completely dry the aerosol. The dried nebulized portion of the aerosol was mixed with the dust portion from the Wright dust feeds downstream of the cyclone separator and before the aerosol dischargers. This vertical configuration worked much better than the horizontal configuration used in CL-1 and was used for the rest of the exposures (Figures 11-13).

Aerosol Characterization

Aerosol characterization included multiple and repeated cascade impactor samples with a Mercer-style impactor (Raabe, 1977) and a real time quartz crystal microbalance, QCM, impactor (Berkeley Instruments, Berkeley, CA) to determine the aerodynamic size distribution of each aerosol. In addition, numerous and repeated filter samples collected in the breathing zone of the animals were used to provide quantification of the mass and chemical concentration of the aerosols. These breathing zone samples were collected through a metallic probe inserted into the chambers through sampling ports

Figure 10: Generator configuration for CL-2 and CL-3 showing modification of system incorporating one large reservoir for cooling container. The lines to the nebulizer were made of stainless steel, heated and located in a vertical position below the Wright dust feed (WDF) system. (NEB = (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

GENERATOR CONFIGURATION
CL-2 and 3

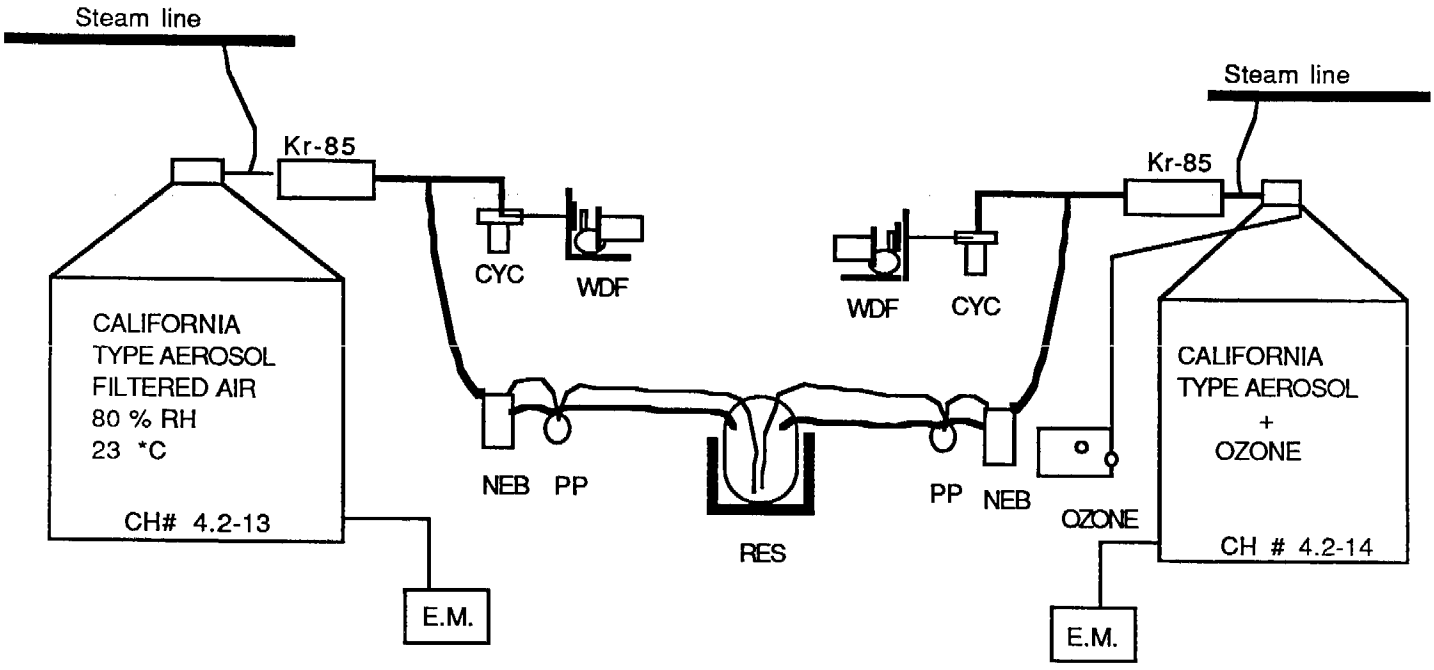


Figure 11: Generator configuration for CL-4 to CL-5 showing changes in the Wright dust feed, using one unit to supply the two chambers. By using a venturi split tee, equal quantities of material was distributed to two exposure chambers. (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

GENERATOR CONFIGURATION
CL-4 and 5

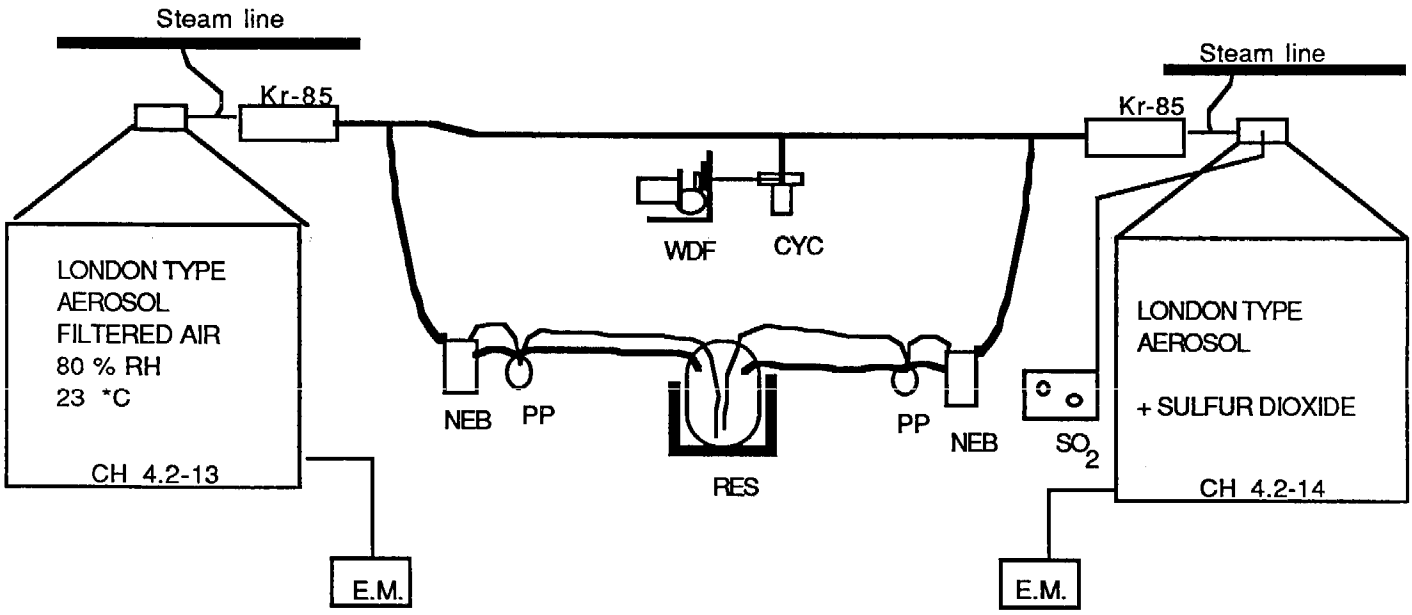


Figure 12: Generator configuration for CL-6 showing the use of ozone in place of sulfur dioxide with the London-type aerosol. (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

GENERATOR CONFIGURATION
CL-6

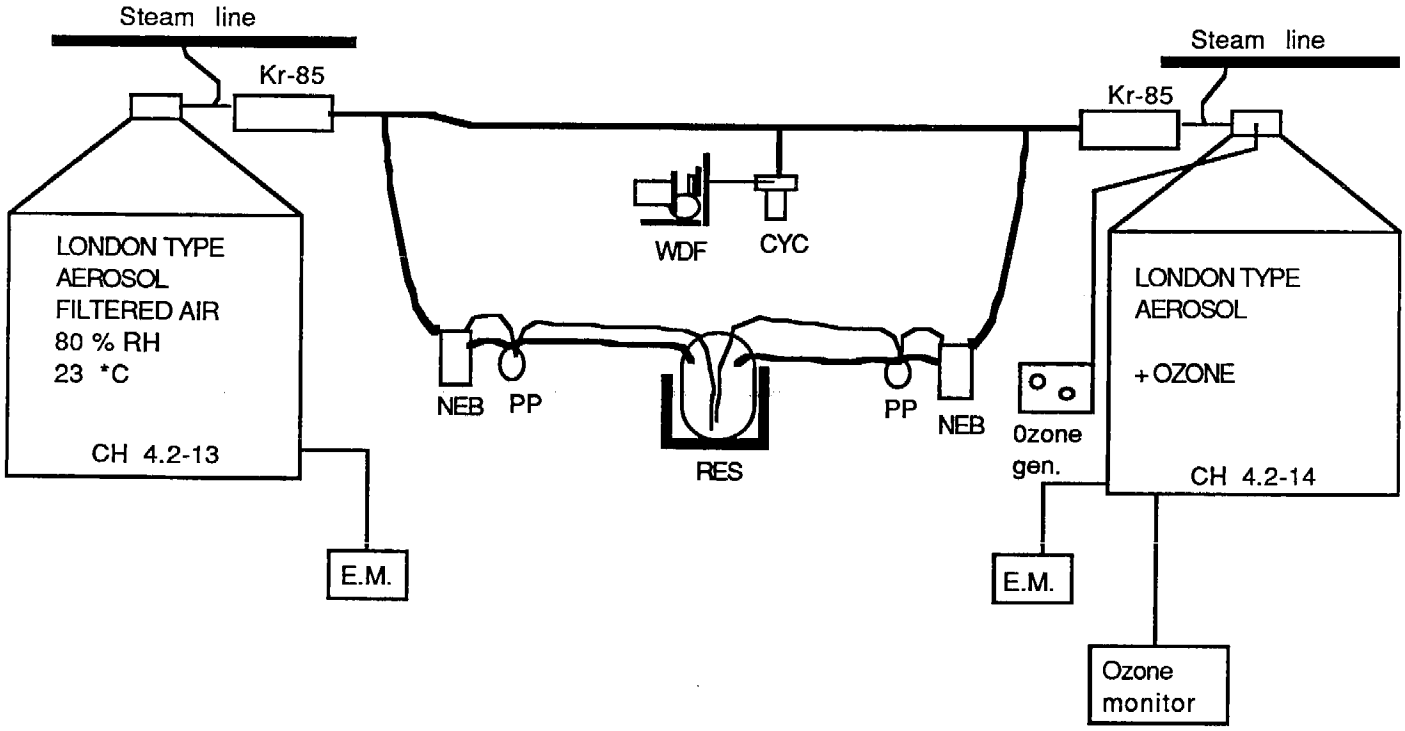
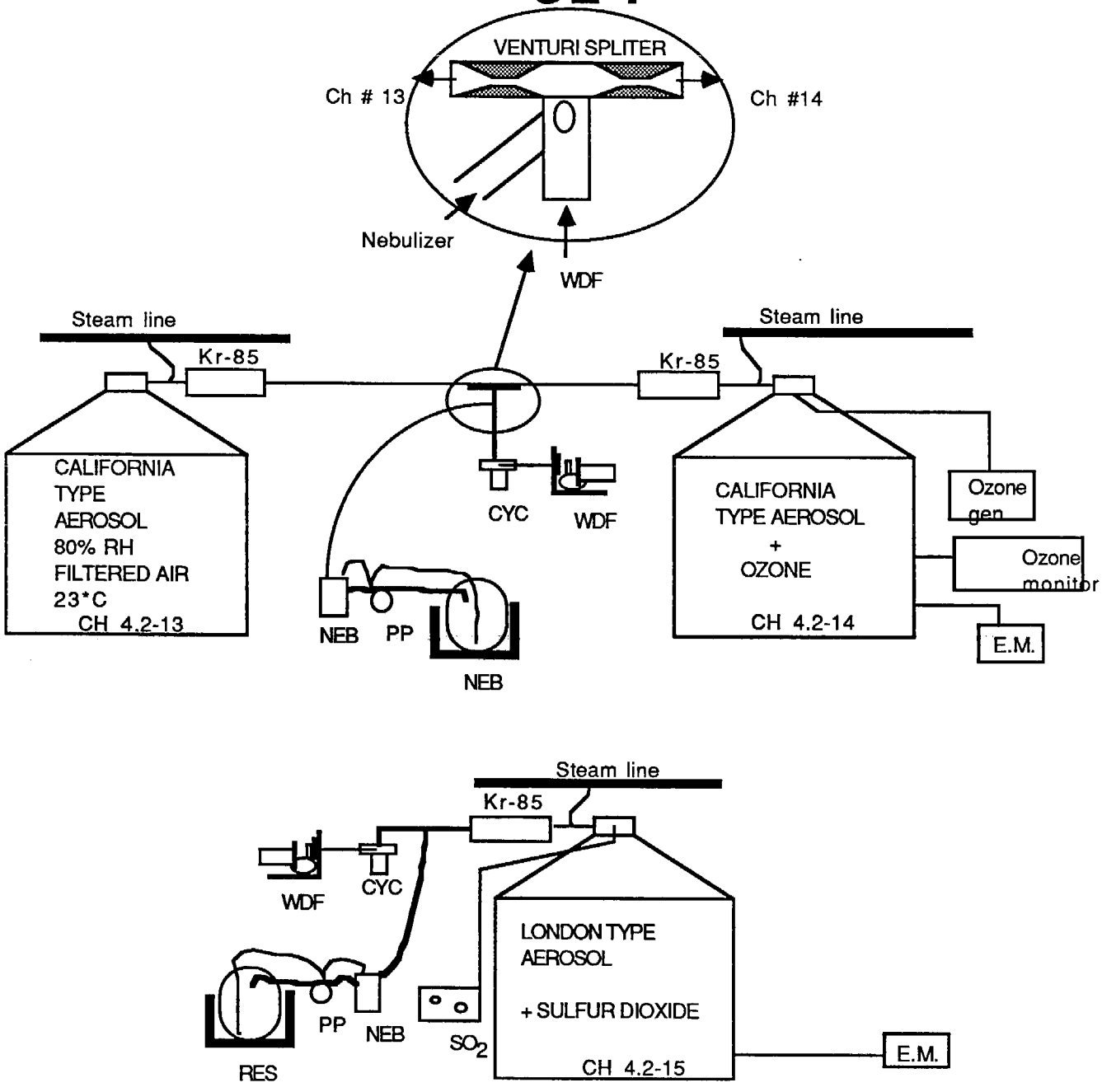


Figure 13: Generator configuration for CL-7 showing the use of two Wright dust feed systems. One dust feed and one nebulizer supplied two chambers for the California-type aerosol (one with and one without ozone) and another dust feed and nebulizer for the London-type aerosol with sulfur dioxide. (NEB = nebulizer; RES = reservoir; CYC = cyclone; E.M. = exhaust manifold; PP = peristaltic pump)

GENERATOR CONFIGURATION

CL-7



(shown schematically in Fig. 7). A Particle Data laser light-scattering particle counter and a Royco laser light-scattering particle counter were used to measure the concentrations of the aerosols in the breathing zone of the animals continually throughout the exposures and recorded to provide a permanent record of the uniformity of of the aerosols throughout the exposures and among successive exposures.

During operation the overall system was monitored and checked every four hours of continuous operation for up to 30 days in the case of CL-7. This included checks of the level of solution in the reservoirs, operation of WDF's and nebulizers, temperature in the nebulizer lines, pressure readings and verification of proper flow to sampling devices. Relative humidity was measured by moving the humidity sensor from one chamber to the other and recording the readings. Ozone level was recorded continuously using an automatic sampling and and computer-based data accumulation system (see Appendix A) for the chambers having ozone. The laser particle counter was connected to the two chambers, with the animals being exposed to the aerosol. Automatic valves were switched every thirty minutes, alternating between the chambers.

Every 4-8 hours the sample filters were changed and/or sampler flow rates were adjusted to compensate for the pressure increase caused by deposition of material on the filters. Print-page of the particle counts gave an indication of the level of dust in the chambers relative to earlier observations and also described the size distribution in preset size intervals. Continuous filter samples from the chambers were measured gravimetrically to evaluate the concentration during the exposures and were changed at frequent intervals. Flow rates were adjusted periodically to maintain proper sampling even though filter loading tended to lower flows. During the experiments where there was heavy loading of the filters, an average between the starting flow and the final flow was used for calculations. The filters were allowed to equilibrate with the ambient air in the room before weighing because the elevated relative humidity of the chamber could cause filters to weigh more than the preweighed amount and artificially inflate the apparent particulate weights of the aerosol collected.

Sierra impactor samples were taken occasionally from the different chambers to determine aerodynamic particle size distribution. Samples of the aerosol from the chambers using a quartz crystal impactor (QCM) were taken in some of the experiments to give an additional measure of aerodynamic particle size distribution for the aerosols in these experiments. Point-to-plane electrostatic precipitator samples were taken to collect particles on electron microscope grids for characterization using transmission and scanning electron microscopes.

Scanning electron micrographs of the California-type aerosol and synthetic London-type aerosol are shown in Figures 14 and 15, respectively. The large flake-like particles in the California-type aerosol (Fig. 14) are primarily single and agglomerated crystals of nebulized salts; the smaller flake like and compact particles are clay and graphitic carbon. The small spherical particles in the London-type aerosol (Fig. 15) are the coal fly ash particles, the finer flake like particles are carbon black, and the larger flake-like particles are single and agglomerated salt crystals from the nebulized salts. Representative aerosol size distribution data are illustrated in Figures 16, 17 and 18.

General Observations.

Between exposures, rats were maintained individually in the exposure chambers. Food and water were provided ad lib between exposures. All animals were cared for and observed daily. There were no unexpected fatalities; no rats died spontaneously during exposure or at any other time during this study.

During exposures and for two hours immediately after exposures the rats were observed for signs of sensory irritation. Qualitative observations of respiratory function were made and recorded. A veterinarian was involved in cases of severe distress, if observed. Each animal was weighed before and after exposure, and the changes in weight that were observed provided part of the basis for diagnosis of adverse responses from inhalation exposures.

Figure 14: Scanning electron micrograph (SEM) of synthetic California-type aerosol sampled from exposure chamber onto a Nuclepore filter during exposure of rats.

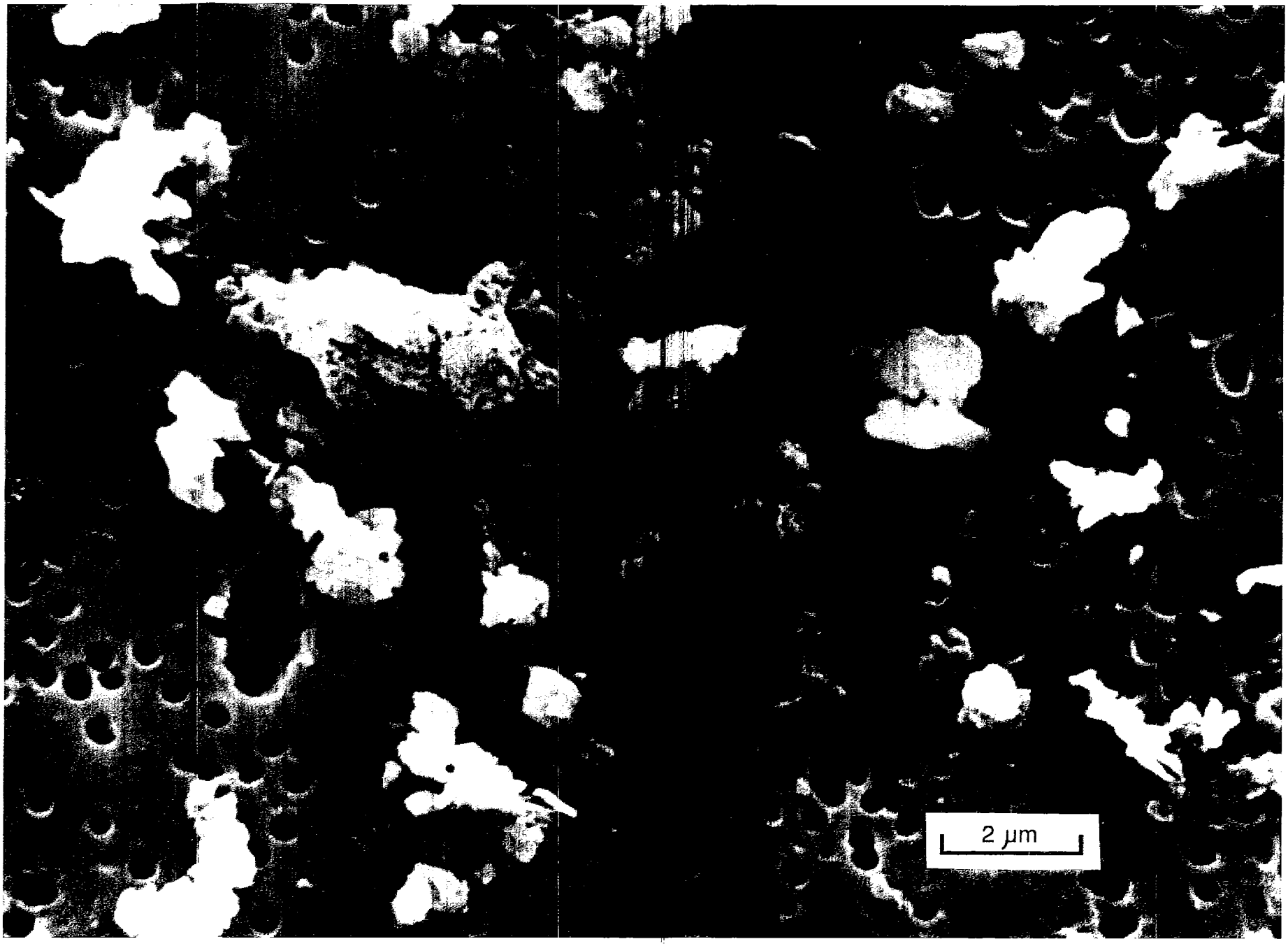

2 µm

Figure 15

Figure 15: Scanning electron micrograph (SEM) of synthetic London-type aerosol taken from exposure chamber onto a Nuclepore filter during exposure of rats.

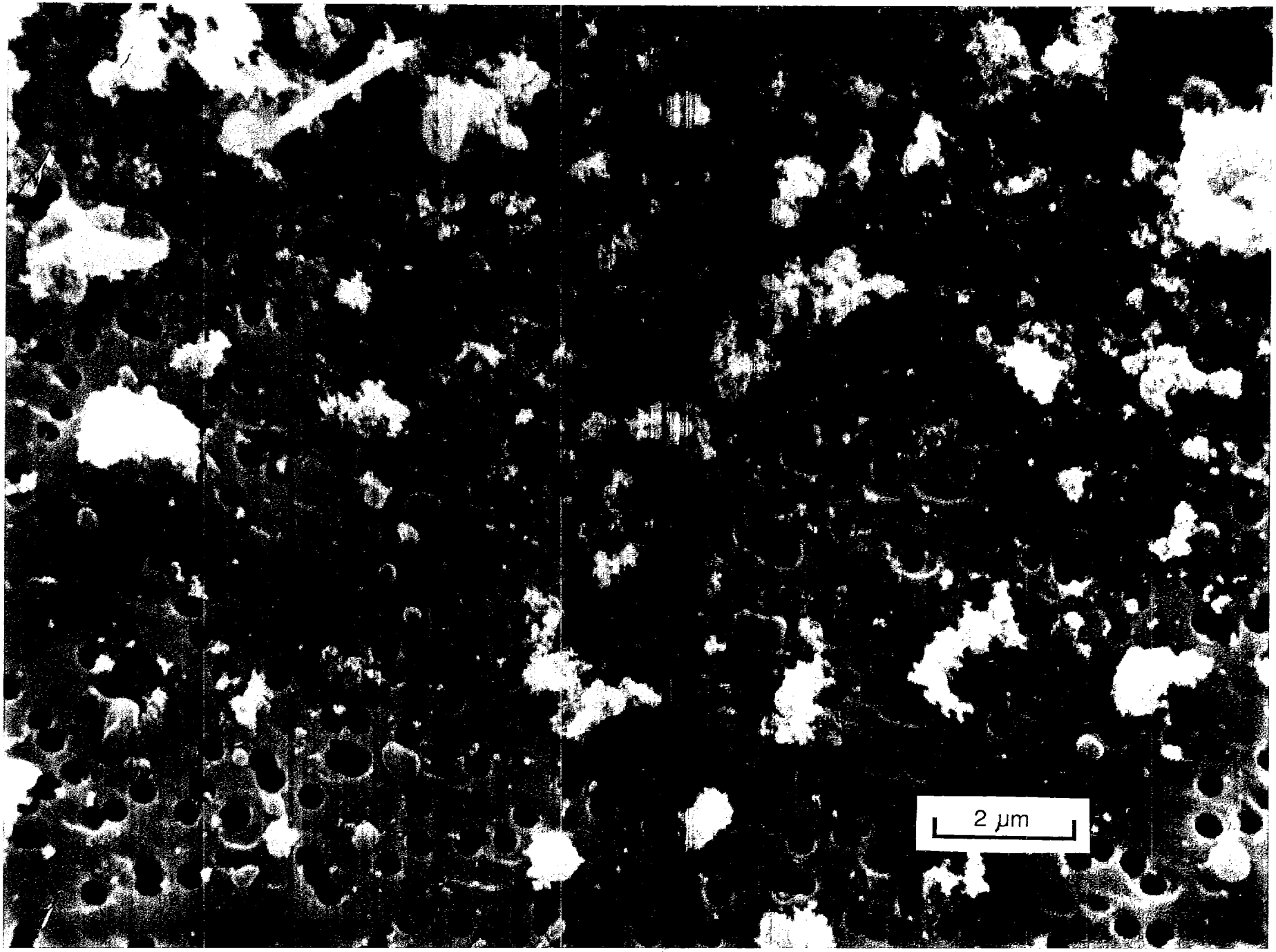
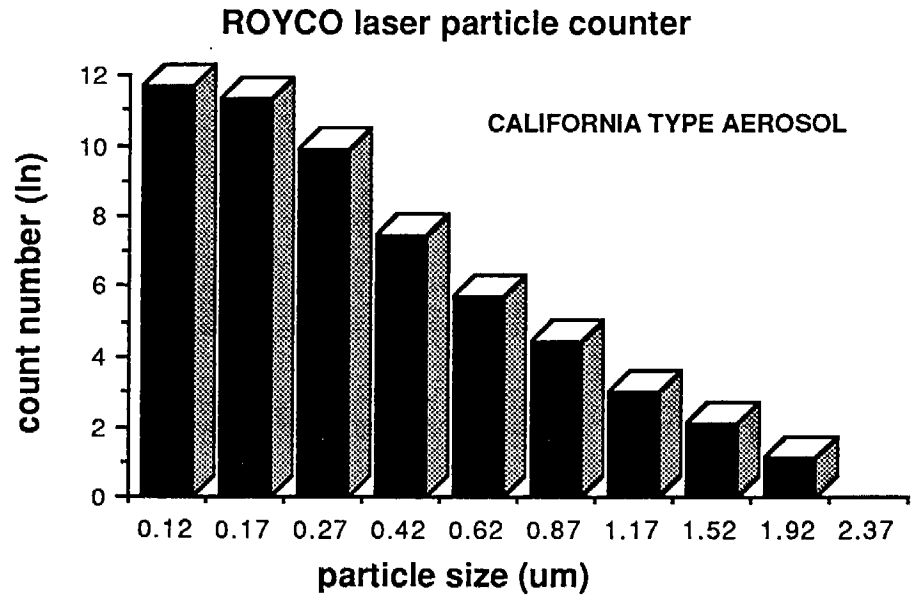

2 µm

Figure 16

Figure 16: Examples of particle size distributions, based upon the number of particles in ranges described by the physical diameters, for separate representative samples of the two synthetic aerosols in this study. These are based upon samples collected from chambers during exposures with the Royco Model 236 laser light-scattering particle counter. The physical size intervals are those established by the counter based upon independent calibration. The histograms show the relationships of the logarithms of particle count number in each size interval for the synthetic California-type aerosol and the synthetic London-type aerosol.


ROYCO laser particle counter
CALIFORNIA TYPE AEROSOL
count number (ln)
12
10
8
6
4
2
0
0.12 0.17 0.27 0.42 0.62 0.87 1.17 1.52 1.92 2.37
particle size (um)

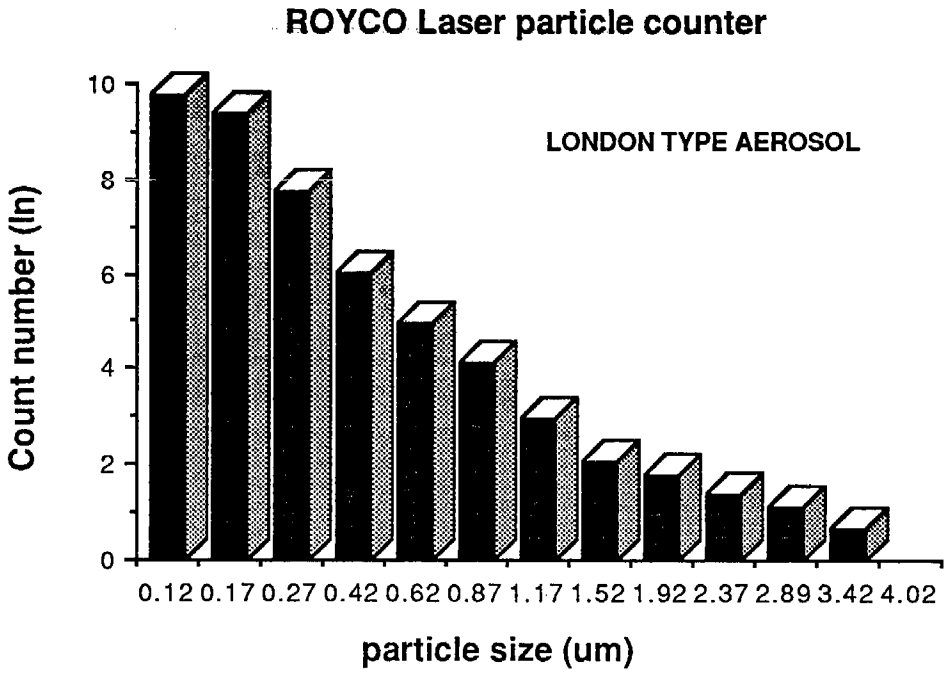
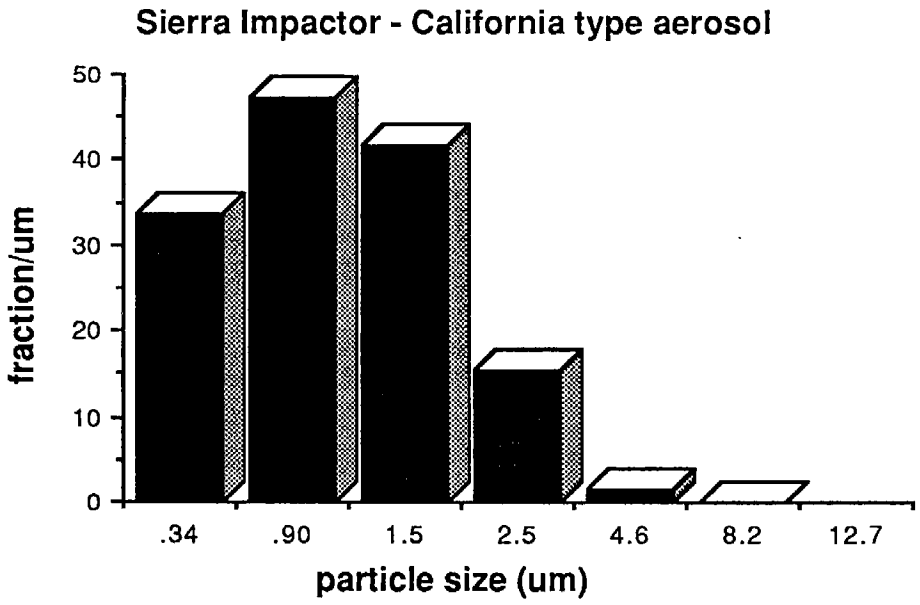

ROYCO Laser particle counter
LONDON TYPE AEROSOL
Count number (ln)
10
8
6
4
2
0
0.12 0.17 0.27 0.42 0.62 0.87 1.17 1.52 1.92 2.37 2.89 3.42 4.02
particle size (um)

Figure 17: Examples of particle size distributions, for particle mass with respect to aerodynamic size, for separate representative samples of the two synthetic aerosols in this study. These are based upon samples collected from chambers during exposures with the Sierra Instruments radial-slot cascade impactor. The aerodynamic size intervals are those established by the impactor based upon independent calibration. The histograms show the relationships of the probability density function (percentage of aerosol mass per micrometer of size interval) of particle mass in each size interval for the synthetic California-type aerosol and the synthetic London-type aerosol.


Sierra Impactor - California type aerosol
fraction/um
50
40
30
20
10
0
.34
.90
1.5
2.5
4.6
8.2
12.7
particle size (um)

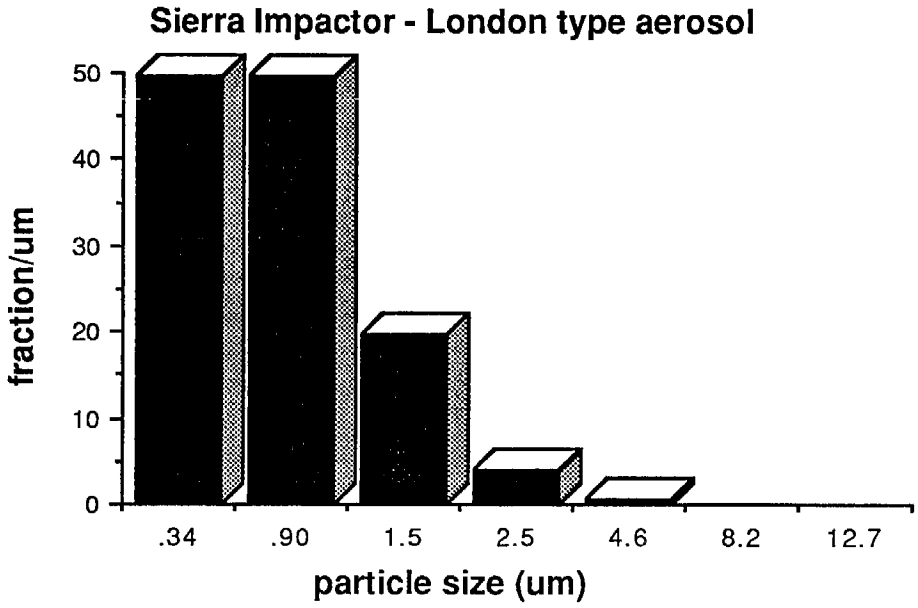
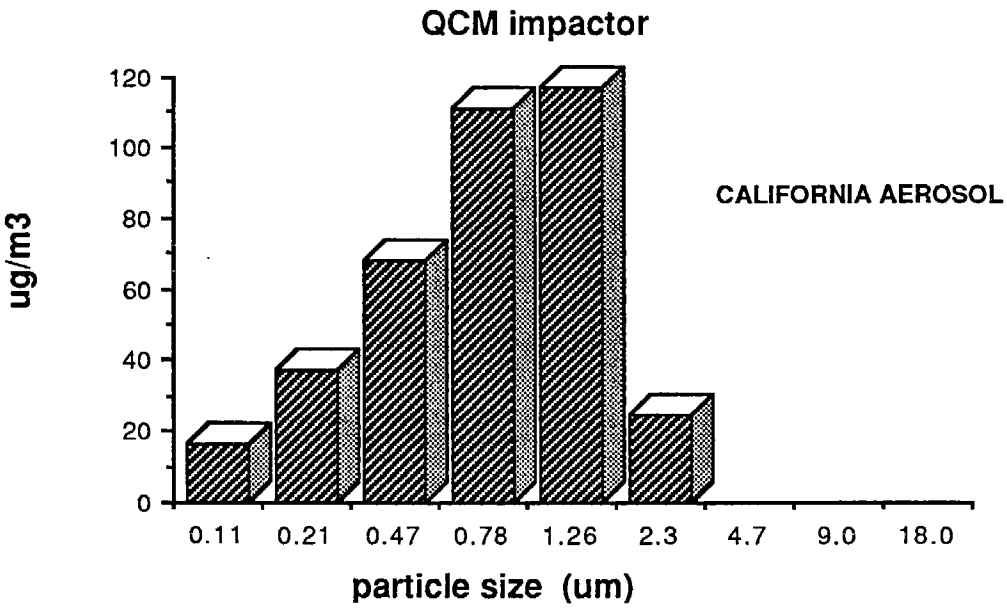
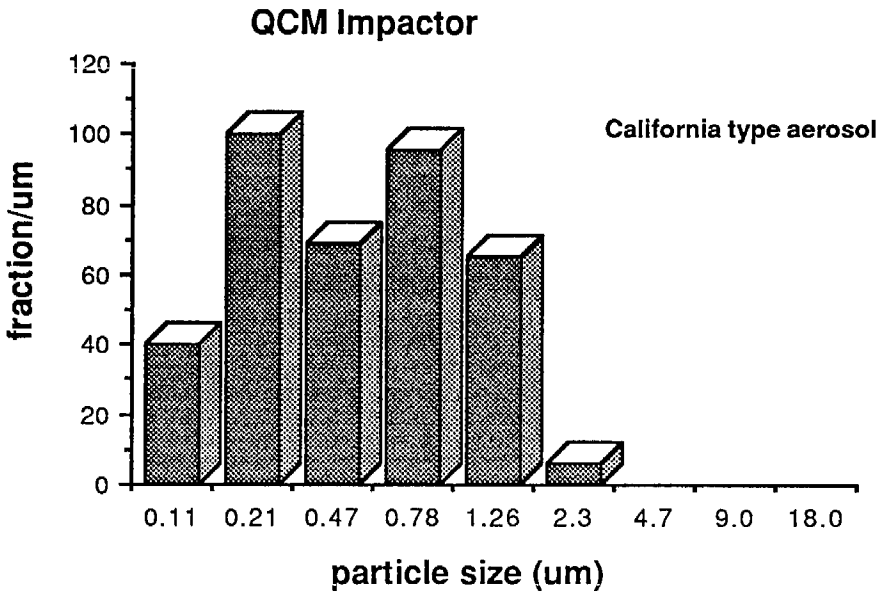

Sierra Impactor - London type aerosol
fraction/um
50
40
30
20
10
0
.34
.90
1.5
2.5
4.6
8.2
12.7
particle size (um)

Figure 18: Example particle mass aerodynamic size distributions of aerosols in this study based upon separate representative samples collected from chambers during exposures with the QCM Quartz Crystal Microbalance cascade impactor. The aerodynamic size intervals are those established by the impactor based upon independent calibration. The histograms show the mass concentration in each size interval (upper) and relationships of the probability density function (fraction per micrometer of size interval) of particle mass in each size interval (lower) for the synthetic California-type aerosol. Similar samples were collected for the London-type aerosol studies.


QCM impactor
ug/m3
120
100
80
60
40
20
0
0.11
0.21
0.47
0.78
1.26
2.3
4.7
9.0
18.0
particle size (um)
CALIFORNIA AEROSOL


QCM Impactor
fraction/um
120
100
80
60
40
20
0
0.11
0.21
0.47
0.78
1.26
2.3
4.7
9.0
18.0
particle size (um)
California type aerosol

Hematology and Clinical Chemistry.

Hematological and blood chemical indicators of illness are useful in evaluation of the health of the experimental animals and relating the clinical signs to similar health effects that are observed in people. For example, anemia or abnormal levels of serum enzymes can indicate cell damage in major organs induced by exposure to air pollutants that affects the whole body, not just the respiratory tract. Therefore, routine hematological and clinical chemical determinations were made on blood samples from two randomly selected animals per group of in these studies.

Hematological measurements included white blood count, red blood count, hemoglobin, hematocrit, mean cell volume, platelets, expanded sedimentation rate, and complete white cell differential counts. The Technicon SMA-12 was

utilized to determine serum glutamic oxalacetic transaminase, glutamic pyruvic transaminase, lactic dehyrogenase, alkaline phosphatase, total bilirubin, blood urea nitrogen, glucose, cholesterol, inorganic phosphorous, calcium, albumin, and total protein. Steroids were also measured.

Biochemical Studies

Lungs from six exposed rats and from six control rats, including both healthy and impaired animals from each exposure group, were analyzed for selected biochemicals.

DNA, RNA, and Protein Assays: DNA, RNA, and Protein were measured in the lungs of six rats per group (total of 48-60 animals per study) by the methods of Wannemacher et al., 1965 (DNA and RNA), and Lowry et al., 1951 (protein). Briefly, the frozen right lung was homogenized individually in 0.9% NaCl solution, and one ml of the homogenate was treated with 0.25 ml of 50% trichloroacetic acid to precipitate the protein. The sample was centrifuged at 3000 g for 10 minutes and the pellet was serially extracted with two solvent systems. These systems were (a) 10 ml of 95% ethanol saturated with sodium

acetate, and (b) 10 ml of ethanol:ethyl ether mixture(3:1). The pellet was dissolved in 4 ml of 0.3 N KOH by heating with a 60 ^{o}C water bath for 14 hours. An aliquot of this solution was used to measure protein, RNA, and DNA content. RNA and DNA was separated by adding 1 ml of 60% perchloric acid to 2 ml of KOH protein solution. Bovine serum albumin, yeast RNA type XI, and calf thymus DNA (sigma) were used to prepare the standard curves for protein, RNA, and DNA, respectively.

Hydroxyproline Assays: Hydroxyproline was measured in one ml aliquot of lung homogenate that was used for measuring DNA, RNA, and protein according to the procedure described by Woessner, 1961. Briefly, the protein was precipitated by adding 0.25 ml of 50% trichloroacetic acid to one milliliter of homogenate. The pellet was hydrolyzed with 6 N HCl at 110 ^{o}C overnight. Radiolabeled ^{3}H-hydroxyproline was used as internal standard that could be evaluated by liquid scintillation counting. The supernatant was decolorized and filtered; an aliquot of hydroxyproline was measured spectrophotometrically at 557 nm.

These measurements were made since they are sensitive indicators of adverse lung responses to inhaled pollutants and are indicative of inflammatory and potentially injurious cellular level changes. Hydroxyproline levels are indicative of early changes in metabolism that tends to lead to pulmonary fibrosis. Fibrosis in the lung is a common disease entity in people living in urban areas and may be related to the inhalation of air pollutants.

Pathological Methods.

The purpose of the gross and microscopic techniques that were used in this study was to utilize quantifiable measurements of structural and cellular changes in the respiratory parenchyma that are representative of the most significant degenerative diseases of the pulmonary interstitium in man: interstitial fibrosis, chronic bronchiolitis, and emphysema. Since these diseases are generally accepted to have inflammatory changes as their pathologic basis, measurements concentrated on the morphologic correlate of acute inflammation, i.e., inflammatory cell infiltration and edema formation, in the acute studies, and on the consequences of persistent inflammation

(fibrosis, airway wall thickening, and structural damage to the interstitium) in the subchronic studies.

In the 3-day studies, small-airway inflammation was quantified by counting the numbers of inflammatory cells within alveolar ducts. Cell counts were limited to alveolar ducts because these ducts are a well-defined anatomic structure that can be identified in light-microscopic sections. They are also likely to be at the site of significant particle deposition and are the most sensitive region of the rat pulmonary parenchyma to ambient oxidant exposure (Barr et al., 1988). Along with the terminal bronchioles, the alveolar ducts are part of the most important site of obstruction due to narrowing in the small airway disease associated with chronic obstructive pulmonary disease (COPD) in man (Spencer, 1985).

In the 30-day study, responses included number of free cells characteristic of inflammation, a subjective but semiquantitative evaluation of pulmonary fibrosis, and measurements of airway wall thickness, airway lumen diameter, and alveolar diameter. These parameters reflect the changes present in the broad category of human chronic obstructive pulmonary disease (COPD). These changes include narrowing of small airways due to inflammation and destruction of connective tissue in the interstitium, resulting in the dilation of alveoli associated with emphysema.

Preliminary Elastase Pretreatment (Impaired Rats) Experiments

The experimental production of emphysema by the intratracheal injection of elastase has several variables that must be considered when designing experiments using this model (Busch, et al., 1984). Not all species are susceptible to similar doses. Not all elastase preparations are of the same potency, despite apparent equivalency in biochemical activity. The immediate pulmonary reaction to elastase instillation is alveolar hemorrhage and if this response is severe, death rapidly ensues. Therefore, the margin between effective dose and lethal dose is rather slim. Post-instillation infectious pneumonia is a reported problem, as are complications from anesthesia. For these reasons, we spent considerable time and a modest amount of resources

determining an appropriate dose range for anesthetics, appropriate instillation techniques and volumes and appropriate, effective, nonlethal doses of elastase. Once determined, a single lot-number of elastase was used throughout. We found that an anesthetic "cocktail" of ketamine (100 mg/ml), acepromazine (5 mg/ml), and atropine (0.02 mg/ml), administered as a 0.1 ml/100 g intramuscular injection, was an effective and safe anesthetic. Intratracheal instillation was facilitated by use of a local anesthetic (Cetacaine tm) applied to the larynx. The elastase (Sigma Chemical Corp., Lot # 94F-80501) dose determined to be effective but generally non-lethal was 25 U/100gm. An instillation volume of 0.5 ml was the maximum that was routinely tolerated. Average death loss rate was less than 10% with this routine. Post-instillation pneumonia was not a problem in our experiments. We attribute this to the use of disease- and contamination-free rats (verified by extensive serologic screening for respiratory pathogens).

The pretreatment with elastase was nevertheless subject to considerable individual variation. Thus, each set of elastase-pretreated rats differed somewhat from the others with respect to the absolute degree of impairment. In comparison, aerosol and gas exposure conditions were much more precise.

Necropsy, Lung Fixation and Gross Pathology

Six animals from each exposure group of 12 were randomly assigned to the pathology group. Two of these animals from each group in the acute exposures and all 6 animals per group in the sub-chronic study were bled by heart puncture for hematology and clinical chemistry evaluation. The lungs of each animals were removed at necropsy, and weighed after the heart and esophagus had been removed. The lungs were then fixed by intratracheal infusion of neutral buffered formalin at 20 cm water pressure. After 24-48 hours of fixation, lung volumes were determined by volume displacement.

Tissue Processing

The lungs were cut into 16 transverse sections, approximately 2 mm thick, with 8 sections being obtained from the left and 8 from the right lobes. The odd numbered sections were used for light microscopy, while the even numbered sections were used for scanning electron microscopy. Lungs from exposure CL-1 had all 8 sections embedded for light microscopy using standard techniques for paraffin embedding. Analysis of variance in exposure series CL-1 demonstrated that one section per lung provided adequate sampling for statistical purposes (Appendix C). In all subsequent studies, paraffin embedding was done on one randomly selected, odd numbered section per animal.

Lung block number 4 (in exposure CL-1) or one randomly selected block (in subsequent exposures) was processed for scanning electron microscopy by dehydration in a graded series of ethanols and amyl acetate, followed by critical point drying. Dried sections were stub mounted and sputtercoated with carbon.

Inflammatory Cell Counts: Light Microscopy

A count of the number and type of inflammatory cells present in proximal respiratory ducts was made on all paraffin embedded blocks. Proximal alveolar ducts were defined as the segment of alveolar duct delimited by the first five consecutive alveolar lumina distal to the junction of the terminal bronchiole (cuboidal epithelium) with the alveolar duct (squamous epithelium). Alveolar sacs that opened into the lumen of the alveolar duct were included in the counts as were profiles of alveolar sacs that were contiguous with the alveolar duct wall but for which no opening to the duct was present. All identifiable terminal bronchiole-alveolar duct junctions in each slide were evaluated separately. We had intended to differentiate cell types in these counts, but, in practice, found only macrophages, so results were expressed as macrophages/alveolus. In order to determine the minimum number of sections and regions needed for meaningful statistical analysis, counts were made on all eight sections from each of the healthy (non-elastase treated) animals from the first exposure. A nested analysis of variance was performed on these counts

(Appendix C) to determine what proportion of the variance in the inflammatory cell numbers could be ascribed to variation within each level, viz., treatment group, individual animal, lung cross-section, or alveolar duct region. It showed that the primary source of variation was between individual alveolar duct regions but that significant variation existed between experimental groups while the differences between sections in an animal contributed little to the overall variance. This result allowed us to evaluate confidently only one section per animal to determine differences between groups thus cutting sectioning and evaluation time significantly in the remaining exposures. The reason for the marked variation between individual alveolar ducts is unclear. We have seen this phenomenon in other ozone exposures and have hypothesized that it may be due to ventilation perfusion differences, differences in the number of airway generations preceding the alveolar duct, or simply the limitations of counting cells in a three dimensional structure in a two dimensional field.

Inflammatory cell counts - Scanning Electron Microscopy

Inflammatory cell counts using scanning electron microscopy (SEM) were done in a manner similar to that used for light microscopy. Terminal bronchiole-alveolar duct junctions were located using differences in epithelial morphology. The region outlined by the first contiguous five alveolar sacs was included in the counts. Roughly spherical 10-15 micron structures with loose attachments to the alveolar wall were counted as inflammatory cells and, based on light microscopic results, were classified as macrophages.

Quantimet Image analysis

Alterations in parenchymal structure induced by elastase pretreatment (impaired rats) are difficult to document due to their irregular distribution within the lung and their tendency, because of the intratracheal administration, to affect limited regions of parenchyma. This is particularly true in alveolar ducts (Busch, et al., 1984). To overcome this problem in documenting the effectiveness of the elastase pretreatment, we used the Quantimet image analysis system to summarize surface to volume relationships of

large regions of lung. A low magnification field (approximately 40X) of the dorso-lateral quarter of each paraffin section was digitized. All non-parenchymal structures (vessels, conducting airways etc.) were eliminated from analysis. The digitized parenchyma was then analyzed to give an average alveolar size and an overall surface-to-volume ratio for alveolar septae versus alveolar lumens.

Morphometric Analysis of Parenchymal Remodeling

The relative proportion of the lung composed by various airway and parenchymal structures was determined by point and intercept counting, employing a stratified random sampling technique. In this approach, randomly selected blocks of lung tissue were divided into parenchyma and non-parenchyma at low magnification, followed by more detailed sampling of parenchymal structures of interest at successively higher magnifications (Elias and Hyde, 1983). All counts were done with a Lietz microscope fitted with an American Optical projection screen. Entire slides were examined at a magnification of 16X. Transparencies of morphometric grids (Weibel, 1979) were overlaid on the projected image on the screen and points of intercepts of the grids overlying structures of interest were counted. In the counts, points overlying parenchyma (all alveolarized structures including alveolar ducts and respiratory bronchioles) were distinguised from those overlying non-parenchyma (all conducting airways to the terminal bronchiole/alveolar duct junction as well as large vascular structures).

At 40X, regions of parenchyma, selected by random movements (using a random number generator) of the calibrated microscope stage, were counted. Stage movements began at the dorsal border of the lobe and continued until the ventral border of the section was encountered, yielding a count of five to eight fields per section. At this magnification, the parenchyma was subdivided into terminal bronchioles, respiratory bronchioles, alveolar ducts, or alveolar sacs. Criteria for identification of structures have been previously described (Barr, 1983). Most important in this procedure are the criteria for inclusion in the categories of terminal bronchiole and respiratory bronchiole. Terminal bronchioles were defined as the segment of airway between the junction of

squamous and cuboidal epithelium at the first alveolar sac out to the narrowest cross section of the terminal conducting airway. Respiratory bronchioles were defined as those segments of conducting airway which had wall partly composed of alveolar structures but had intervening walls lined by cuboidal epithelium. These bronchioles were composed of a complex mixture of extracellular matrix (collagen and elastin) and occasional mesenchymal cells (smooth muscle and/or fibroblasts). Alveolar ducts were linear tubes formed of contiguous alveolar sacs whose lumens opened into the airway lumen.

To better characterize the site of the primary ozone-induced lesion, all terminal bronchiole junctions were further evaluated at 100X. Structures in this region were subcategorized as (1) terminal bronchiole wall and lumen versus (2) respiratory bronchiole wall and lumen. At this same magnification, the number of intercepts of grid lines with terminal bronchiole walls was recorded to determine the arithmetic mean of thickness.

Relative volume of each structure as part of the total lung volume was determined by the following formula:

$$\frac{V_i}{V_l} = \frac{P_i}{P_t} \times \frac{P_{ti}}{P_l}$$

where V_i is the volume of the structure in question, V_l is the volume of the lung, P_i is the number of points overlying the structure, P_t is total points in that region, and (P_{ti}/P_l) provides a correction factor for the relative volume of that region in the lung as a whole as determined at the lower magnification. This allows all structures to be expressed as a percentage of the total lung volume. Mean arithmetic thickness of the terminal bronchiole wall was calculated as:

$$T = \frac{d \times P_t}{4 \times P_i}$$

where T is the mean arithmetic thickness, d is the (magnification corrected) length of the intersecting grid line, P_t the points overlying the wall and P_i the intercepts of the grid line with the wall.

Subjective Evaluation of Light Microscopic Pathology

Subjective evaluation of histopathology was made using hematoxylin- and eosin-stained paraffin sections of all pathology group rats in the study. In addition, in the sub-chronic study, sections stained with Masson's trichrome were evaluated for location and extent of collagen fiber deposition. Separate scores (graded 1-4) were assigned for the severity of emphysematous change, fibrosis, pigment deposition terminal airway wall changes (edema and epithelial hyperplasia) and overall lesion severity. A score of 0 implied no difference from normal, 1 was an equivocal change and 4 was most severe. Slides were randomized and evaluated without knowledge of the treatment group.

Statistical Analysis of Results

Data on lung morphometric parameters, inflammatory cell counts, and biochemical measurements that showed treatment-related changes were analyzed as a 2x2x2 factorial analysis of variance. In this analytical model there were three factors, each at two levels. The factors were: lung impairment by elastase; aerosol (either the California-type or the London-type); and gas (either ozone or SO_2). The two levels of each factor were: present or absent. Each of the eight cells contained six rats, 48 in all. Each rat received 0,1,2 or 3 of the factors. One cell had none (healthy controls); three cells had one (impairment, aerosol or gas); three cells had two (impairment + aerosol, impairment + gas, aerosol + gas); and one had three (impairment + aerosol + gas).

There were thus three main effects: differences due to impairment, or to aerosol, or to gas. The factor means were based on 24 rats, each taken over the four cells in which the factor was present or was absent. In addition, there were three two-way interactions available for testing: [impairment x aerosol]; [impairment x gas]; and [aerosol x gas], and one three-way interaction: [impairment x aerosol x gas]. The interactions test the hypotheses that the factors are independent, i.e., that each factor makes an additive contribution to the measured parameter. A statistically significant

interaction is indicative of an exacerbation or of an inhibition by one factor in the presence or absence of another factor. Clearly, the ascertainment of the presence of interactions has important biological significance with respect to synergism, potentiation, or amelioration of responses.

In the fixed-effects model of the analysis of variance, the effects (or factors) are assumed to have been chosen in advance (hence fixed) and not selected randomly. Each of the main effects and interactions was tested for significance against the error term. With 48 rats in the experiment there are 40 degrees of freedom for testing significance.

For example, Table 4 provides typical results of the analysis of variance for data on inflammatory cells per alveolus. Means and standard errors (SE) of these values for the six rats in each cell are given. The results from the four cells in which, e.g., ozone was absent (-) or present (+) are also shown. The test for the significance of the effect produced by ozone compared the two means: 0.0286 vs. 0.1394. This difference was found to be very highly significant. Similarly, the aerosol effect was significant (0.0581 vs. 0.1100). The effect of impairment, however, was not significant (0.0731 vs. 0.0945).

There were two significant interactions: [ozone x aerosol] and [ozone x aerosol x impaired]. The mean for the 12 rats that received neither aerosol nor ozone was 0.0236. Rats that got ozone only had a mean of 0.0924. Rats that got aerosol alone had a mean of 0.0336. Those that received both ozone and aerosol had a mean of 0.1863. The addition of ozone alone produced an increase of 0.0688 macrophages/alveolus (0.0924-0.0236), while the addition of ozone to rats given aerosol produced an increase of 0.1527 (0.1863-0.0336). Thus the effect of ozone in the presence of aerosol was non-additive, and could be said to be exacerbative, synergistic, potentiative, etc, and the significant ozone x aerosol interaction observed is explained.

The analyses were performed by a general linear model analysis of variance computer program (Numbercruncher Statistical Analysis System version 5.0, 2/87). Lesion scores in subjective evaluations were analyzed by a

non-parametric equivalent of analysis of variance, the Kruskal-Wallis test. A probability level of less than 0.05 was considered to describe a significant difference. A nearly significant probability level less than 0.1 was considered to represent a strong tendency.

Lung Clearance and Permeability.

Two sensitive indicators of lung impairment are marked increase in lung clearance half-time for insoluble particles and marked reduction in clearance half-time for soluble aerosols. Each of these possibilities was studied by permitting exposed rats to briefly inhale ^{99m}Tc-labeled aerosols of somewhat soluble DTPA or insoluble iron oxide. After these separate inhalations, the rats were restrained in special tubes placed in contact with a gamma camera radiation detector. This camera provided images of the radioactive ^{99m}Tc label and allow the observation and quantification of the clearance of the test particles from the lungs of the rats. In this way it was possible to measure systemic clearance of the somewhat soluble DTPA particles indicative of lung permeability and also tracheobronchial clearance rates of the insoluble iron oxide particles.

The epithelial surface of the respiratory airways of the lung is a naturally protective barrier to the entry of foreign substances into the systemic circulation. When there is damage to this surface (such as separation of cell junctions) the lung becomes leaky. Human lung physiologists have recently begun studying this phenomenon in man utilizing inhaled ^{99m}Tc radiolabeled DTPA and modern nuclear medicine techniques. We modified these methods to test the lungs of the rats under study in this project. Three rats from each exposure atmosphere were permitted to inhale ultrafine aerosols of ^{99m}Tc DTPA generated by nebulization. Lung burdens were followed using a gamma camera as described below. Measurements were made the day that exposures ended in each case.

Technetium-99m (^{99m}Tc) labeled DTPA for nebulization was prepared by the elution of ^{99m}Tc as sodium pertechnetate from a generator containing the radioactive parent molybdenum-99, and this was bound to DTPA by use of a

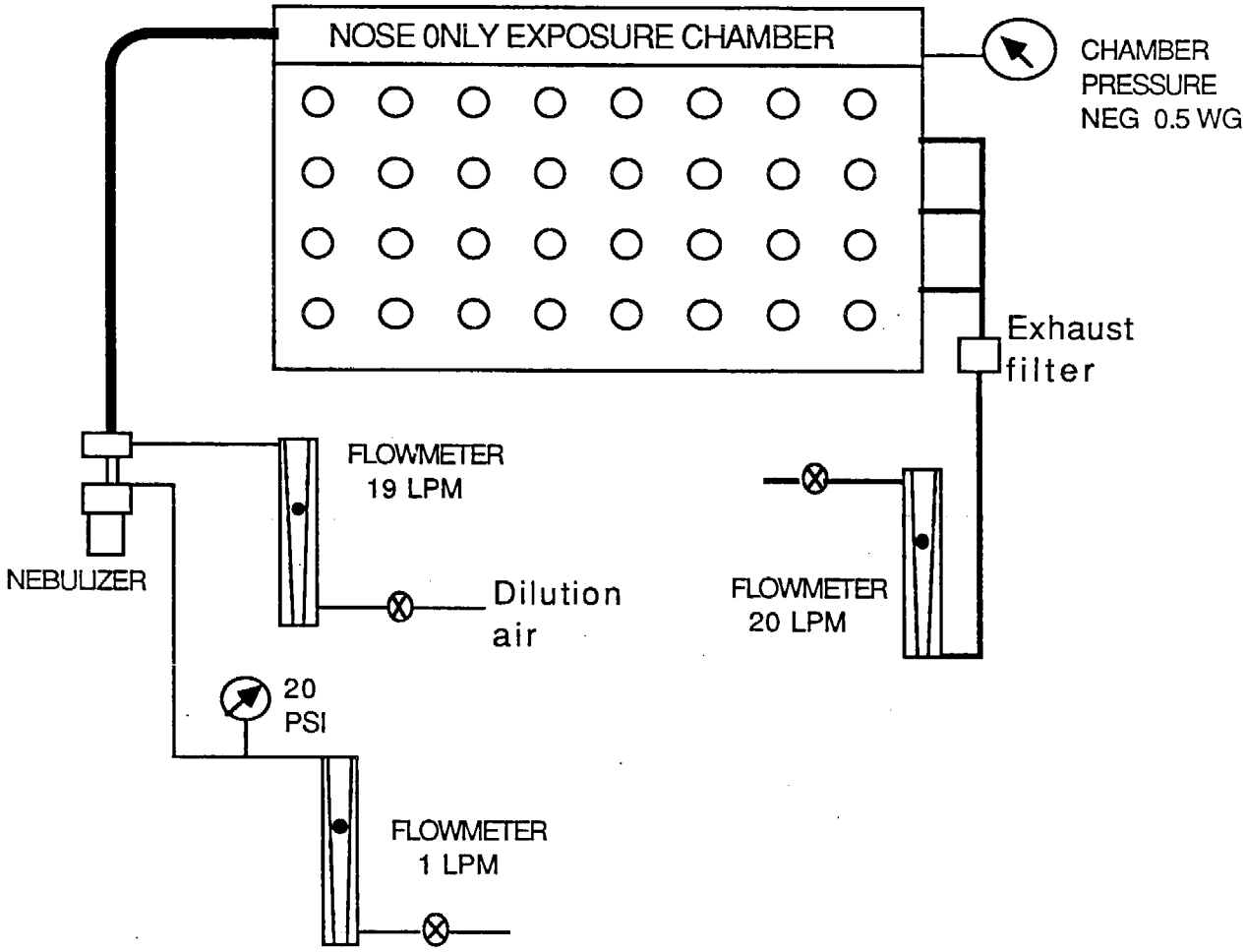
standard radiopharmaceutical kit (Medi-Physics). After the technetium-99m was chelated to the DTPA, it was loaded into a Lovelace generator (In-Tox products, Albuquerque, NM.) and nebulized using compressed air at 20 psig. Air was added to the exhaust of the nebulizer to make up a combined flow of twenty liters per minute of air to dry the aerosol and deliver it to the exposure chamber. The aerosol generation and exposure system is shown in Figure 19. Studies of the particle size, using a Mercer-type, one liter per minute cascade impactor, indicated the aerosol to be 0.46 micrometer mass median aerodynamic diameter with a GSD=1.70. The rats were loaded into nose-only exposure tubes and their positions were adjusted with the plunger to assure that their noses were in a position which provided optimal exposure to the aerosol. The exposure chamber was designed to hold a maximum of forty-eight rats. The groups of rats assigned for the tests were exposed for five minutes to the aerosol, and then clean air was allowed to flow through the chamber for an additional minute before the rats were removed.

When the clearance of insoluble particles slows (with corresponding increase in clearance half-time), the lung is less capable of clearing itself of potentially toxic or injurious particles. These are normally cleared via the tracheo-bronchial tree on a cilia-propelled mucus escalator. Viable aerosols such as bacteria, that land in the airways, have more time to multiply and cause injury if the clearance rate is slowed. Hence, an increase in clearance half-time can be detrimental for either animals or man. For this reason we quantified the clearance half-time for insoluble particles using modern nuclear medicine techniques. Three rats from each exposure atmosphere were permitted to inhale respirable aerosols of ^{99m}Tc radiolabeled insoluble iron oxide aerosols, and the activity in the lung was followed for one day with a gamma camera. These rats were then transferred to lung biochemistry.

Figure 19: Technetium-99m aerosol delivery system used to briefly expose rats to ^{99m}Tc-labeled DTPA for lung clearance studies.

TECHNETIUM AEROSOL DELIVERY

DTPA AEROSOL



Technetium-99m labeled iron oxide for aerosolization was prepared by adding the technetium elution to approximately two to four milliliters of a solution of colloidal iron which was made by adding 150 g of ferric chloride to 300 ml of boiling distilled water with 100 ml of hydrochloric acid. The solution was dialyzed for several days by a constant flow (10 ml/min) of distilled water from a glass container into a graduated cylinder in which the dialysis bags were suspended. The clear solution turned a burgundy color when dialysis was complete. The eluted ^{99m}Tc was added to the iron colloid solution and placed in a Lovelace nebulizer just prior to use. The total added activity varied from 150 millicuries (mCi) to 382 mCi. The aerosol generation and exposure system is shown in Figure 20. The aerosol was carried with air through a quartz tube placed in a tube furnace regulated to 400^{o}C which oxidized the iron and created a spherical aerosol. Tests of the aerosol with a Mercer cascade impactor indicate an aerosol mass median aerodynamic diameter of 1.46um with a GSD=1.74. After the aerosol was oxidized in the furnace, it was mixed with nineteen liters of air per minute to cool and carry the aerosol at the proper flow rate required for operation of the exposure chamber. The rats were exposed for five minutes and then clean air was allowed to flow through the chamber before the animals were removed.

Four male Fischer-344 SPF rats were used in two preliminary studies to establish protocols. Two were exposed to aerosols ^{99m}Tc-labeled DTPA and two were exposed to the aerosols of ^{99m}Tc-labeled iron oxide. The rats were restrained in nose-only exposure tubes for exposure to the aerosols and subsequent imaging with the space adjustment plunger holding the rats in secure positions (Fig. 20). The rats were placed in right lateral recumbency on a horizontally positioned gamma camera and returned to the same position if they moved. It became obvious that an alternate method of restraint was necessary, since a radiograph of a restrained rat revealed that too much pressure on the space adjuster resulted in marked compression of the thorax. This compression was unacceptable because it interfered with visualization of the lungs in the scintigraphic images. Relaxation of the space adjusters, allowed too much movement. An improved method of restraint was introduced for exposure CL-3 utilizing rectangular plastic rat boxes (Fig. 21). The boxes minimized rotational movement, and rear movement was prevented with a wooden peg mounted

just behind the rear legs. Multiple peg holes were drilled in the boxes to accommodate rats of different sizes. These boxes were separated by lead shields to prevent interferences in gamma counting.

Gamma counts for imaging were collected in all studies in a spatial matrix form on a Searle Large Field of View Gamma Camera with a parallel-hole collimator. During the preliminary and first studies, the collimator and gamma camera were placed face up (in a horizontal position) with the rats in right lateral recumbency within their respective tubes. A plexiglas cover, divided into sections with lead bars, positioned the gamma camera during all imaging to prevent radiation interferences as well as hold the rats next to the camera during radiation counting. Each section was labeled and each rat was assigned to only one section during all of its imaging studies (Fig. 21). One position contained a receptacle for a ^{99m}Tc standard. For CL-2, the gamma camera was placed in a vertical position, but the rats were restrained in round tubes until CL-3. From then on they were restrained in rectangular boxes.

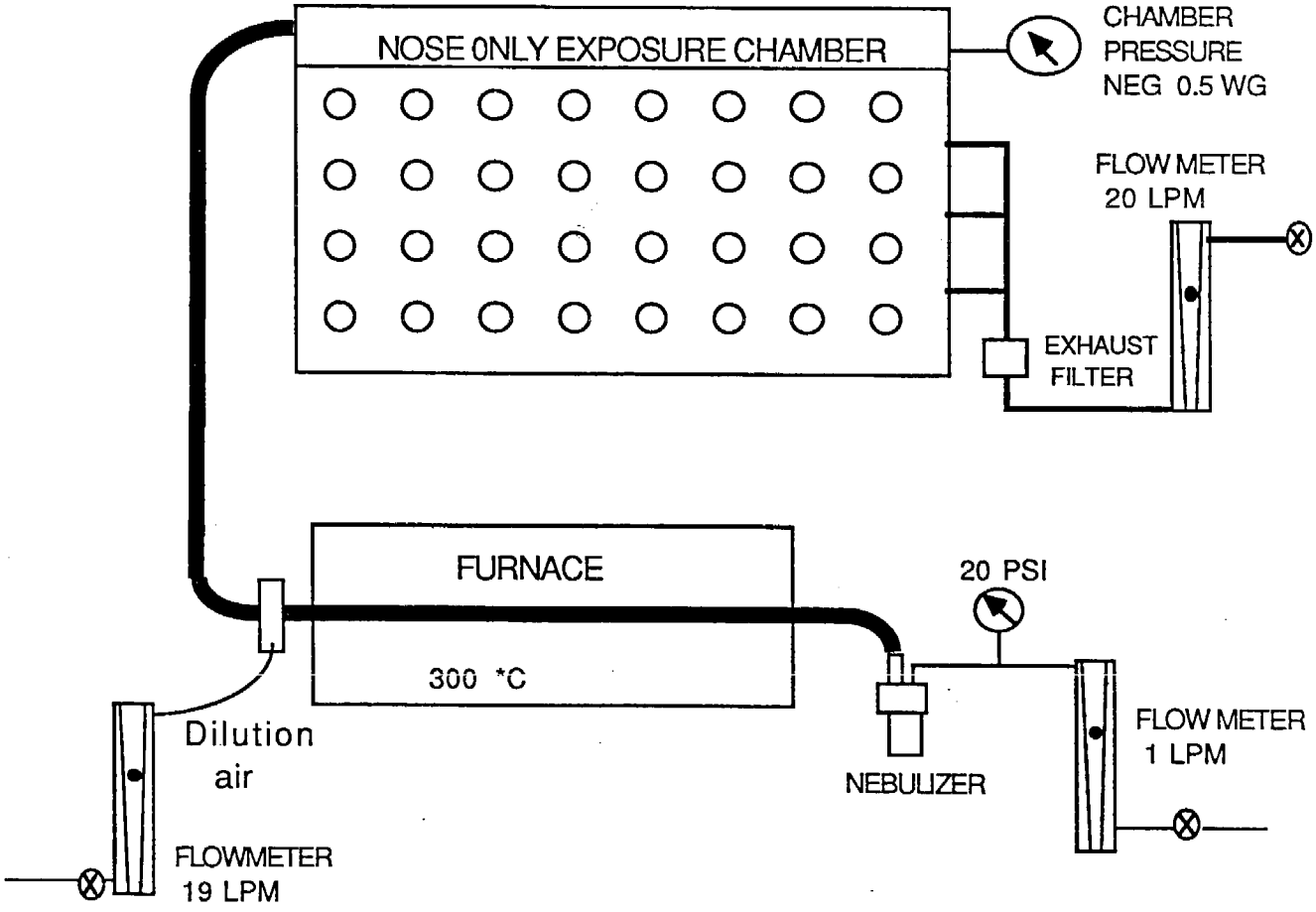
Images were initially acquired in 64x64 detector pixel matrix format on the gamma camera. After the first preliminary study and for all subsequent exposures a 128x128 detector pixel matrix was used to provide for optimal resolution. In all studies a ^{99m}Tc standard was placed in the field of view and used to correct all measurements for radioactive decay or changes in camera sensitivity. During the preliminary studies, two rats exposed to DTPA were counted to provide sixty consecutive one-minute images. Two rats exposed to iron oxide were counted to provide 120 consecutive one-minute images.

Image acquisition for exposures CL-1 through CL-7 involved simultaneous evaluation of eight rats in a counting group. The groups were consecutively exposed and counted according to a double-blind random assignment from each treatment group. Images were accumulated for sixty one-minute periods for the DTPA exposed rats and for five consecutive one-minute periods for the iron-oxide exposed rats. Additional counts were then made at 4 to 8 hour intervals for up to 30 hours. The acquisition duration, data, and time were recorded automatically by the computer and stored with the image.

Figure 20: Technetium-99m aerosol delivery system used for brief exposure of rats to ^{99m}Tc-labeled iron oxide aerosol for lung clearance studies.

TECHNETIUM AEROSOL DELIVERY

IRON OXIDE AEROSOL



nose-only cylindrical exposure tube

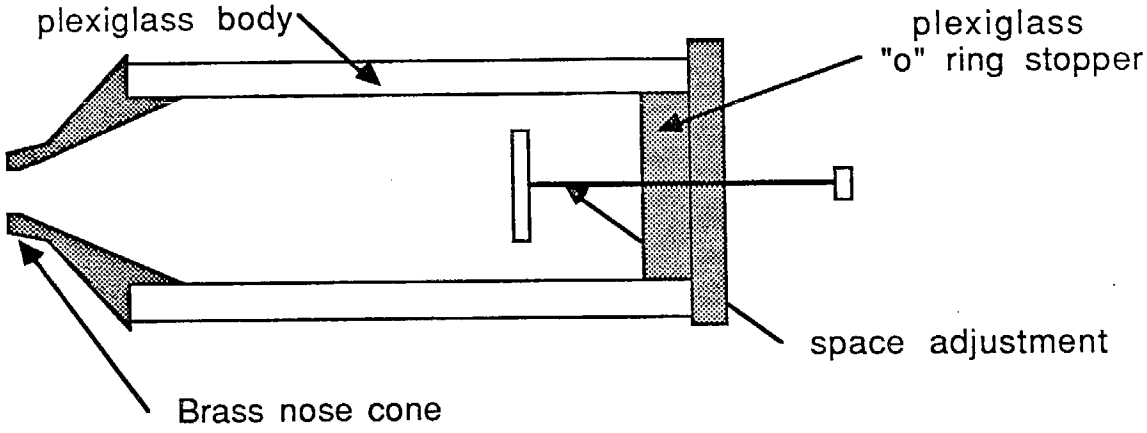
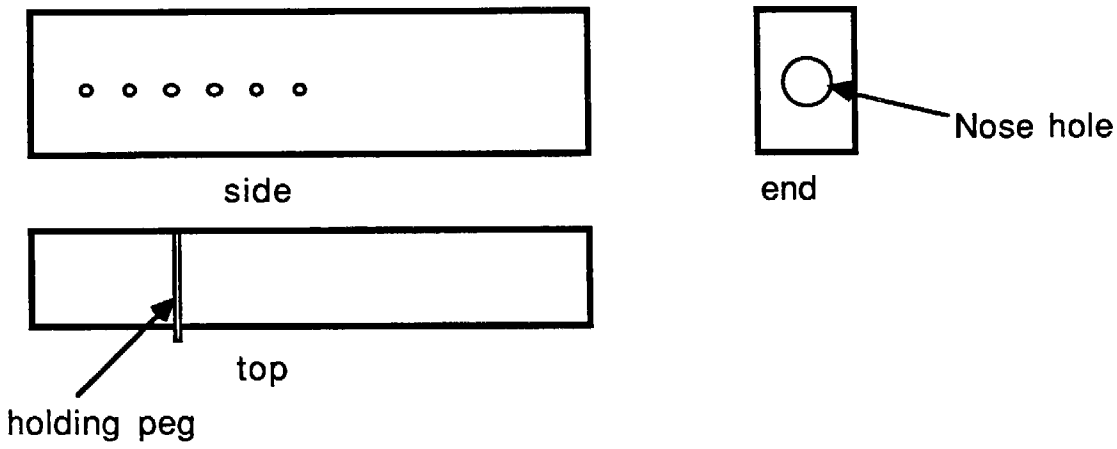


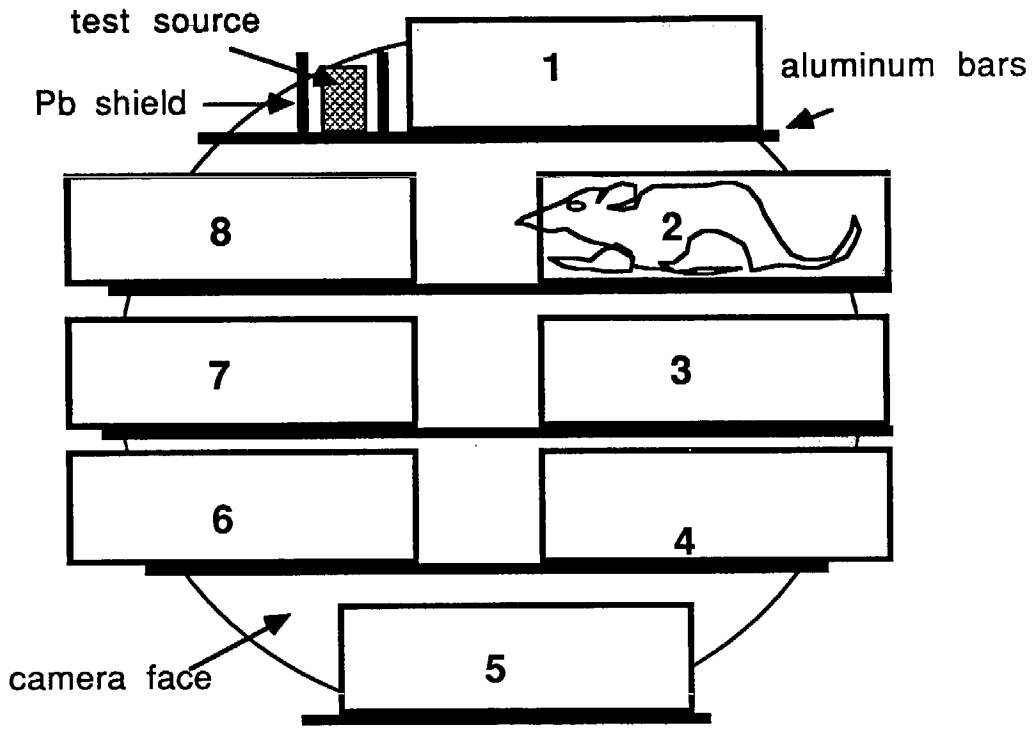
Figure 21: Technetium imaging system used in the gamma camera lung clearance studies of inhaled ^{99m}Tc-labeled DTPA and iron oxide aerosols. Rectangular plastic holders (upper) with wooden dowel holding peg used in this experiment for the restraint and proper placement of rats on the face of the imaging gamma ray camera (lower).

TECHNETIUM IMAGING

RAT HOLDER for IMAGING CAMERA



imaging camera rat holder configuration



Because a low signal-to-noise ratio was observed on the second day of imaging the iron oxide exposured rats, it was necessary to correct for the varying radiation background. Ten-minute background images were acquired with empty rat boxes in normal counting locations. The average counts per minute per pixel ranged from two to four, and these were subtracted from each pixel in the time-related images of the rats.

All images were acquired on disk and stored on magnetic tape. A processing procedure packed the 128x128 matrix into a 4x64 matrix. A 32x32 portion of the image was converted to a 64x64 matrix and displayed on the full screen as a smoothed image. Each region of interest (ROI) outlining the lung was manually selected from this improved image to match the perceived contour of each rat's lungs. Care was taken to exclude the nose and the viscera from the ROI.

Data on activity versus time were constructed for each rat lung ROI. The stability of the gamma camera was assessed by analyzing each standard data set. If an exponential curve fit yielded a half-time of 6 ± 0.5 hours corresponding to the known decay rate of ^{99m}Tc, the clearance curves were automatically corrected for the ^{99m}Tc decay. If the data were more variable, they were normalized using the reference standard count rates. The data were transferred by magnetic tape to a Data General MV/8000 computer, and clearance functions (one or two component exponentials) were fit by weighted least-squares.

The experimental protocol for exposures CL-3 through CL-7 was identical to that in the previous exposures with the exception of activity aerosolized, time of aerosolization, and sequence of aerosolization and imaging. The amount of activity aerosolized for the DTPA rats of exposure CL-3 exceeded amounts used in the previous studies. This level of activity proved to be detrimental because the nose and abdomen became even more difficult to separate from the lung regions. A total of 300-400 millicuries was found to be an optimal amount of activity for the aerosolization of the test particles, and was used for CL-6 and CL-7.

Time of ^{99m}Tc aerosol exposure was increased to ten minutes for the two iron oxide exposures in CL-6 and CL-7 because of the low counts that were observed after a five minute aerosolization. The sequence of acquisition and imaging of DTPA and iron oxide groups was changed. During exposures CL-1, CL-2, and CL-4 all iron oxide groups were exposed to the aerosol and imaged once before any DTPA rats were exposed and imaged. We began to be concerned with the amount of time that elapsed before imaging the DTPA rats and decided on exposures CL-3, CL-5, CL-6 and CL-7 to expose and image all DTPA rats before exposing the iron oxide rats to the aerosol.

ANIMAL CARE

The animal experimentation and care in this project were conducted in accordance with the principles of the American Association for Accreditation of Laboratory Animal Care (AAALAC) in fully accredited facilities and following the Guiding Principles in the Care and Use of Laboratory Animals of the U.S. Department of Health, Education, and Welfare (DHEW) and the Animal Welfare Act. Protocols were reviewed and approved by the University of California, Davis, campus Veterinarian and the Animal Research Use Committee to assure conformance with high standards of care and humane treatment of the animals at all times. These regulations were enforced by regular inspections by AAALAC, the U.S. Department of Agriculture, and the Office of the Campus Veterinarian.

Male specific pathogen-free (SPF) Fischer-344 70-day old rats were used in these studies. The animals were purchased from Bantin and Kingman, Inc., Fremont California. They transported by van in filtered containers. They were subjected to microbiological testing during the quarantine period after arrival in Davis to insure that the animals were delivered free from infection. Two rats from each shipment (one shipment per exposure) were used as health screens. In addition, one rat from each exposure chamber was also screened at the end of the 30-day subchronic studies. Serum from these rats was assayed for pneumonia virus of mice, Retrovirus-type 3, Encephalomyelitis virus (GDVII), Sendai Virus, Kilham rat virus, Toolan's H-1 virus (all by hemagglutination inhibition tests), Mouse adenovirus Lymphocytic choriomeningitis virus (by complement fixation), Mycoplasma pulmonis, Rat coronavirus, and sialodacryoadenitis virus (by ELISA test). Tests were done by Microbiological Associates Inc., Bethesda, MD. No evidence of infection by any of the respiratory pathogens listed was present in any of the animals.

Rats were housed singly in wire cages. The cages were kept within the filtered air exposure chambers at the UCD Air Pollution Exposure Facility at the Primate Research Center (CPRC). Access by animal care personnel was under strict conditions of hygiene to prevent infections. Attendants wore sterile garb, surgical masks, and sterile gloves when handling animal cages or animals.

RESULTS

Exposure Conditions, Aerosols and Gases.

The chemical constituents of the aerosols were verified with filter samples collected during the exposures. The measured constituents of the synthetic California-type aerosol and the synthetic London-type aerosol are summarized in Table 2 along with some representative test data where the wet (nebulizer) and dry (Wright dust feed, WDF) generators were operated separately. The concentrations of the aerosols and gases in all of the exposures are summarized in Table 3. Size distributions are described by mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). Data for each of the seven exposures are summarized in Appendix A.

Hematology and Clinical Chemistry

Hematology and clinical blood chemistry was performed on blood from two rats in each exposure group in the short term exposures. The six rats in the pathology group were all bled for hematology and serum chemistry in the 30-day exposure. Summaries of these clinical tests are presented in Appendix B. All values were within normal ranges for clinical hematology of rats and no treatment group had values that were outside of these normal ranges.

Gross Pathology (lung weights, volumes)

Averages of lung weight and volume, body weight, and lung weight and volume to body weight ratios are found in Appendix C. A small but consistent increase in lung weight was seen in all impaired elastase-pretreated animals regardless of other treatment assignments. Similarly, an increase in lung volume was evident in all elastase treated groups in all seven exposures. These relationships were consistent when expressed as uncorrected weights or normalized to body weight. The impaired rat elastase pretreatment-associated trends in lung volume and weight were also evident in the 30 day study (CL-7).

Table 2: Constituents of Synthetic London and California Aerosols

CALIFORNIA-TYPE AEROSOL	Measured nominal values in exposures and tests						
date Impactor sample	1	2	3	4	5	6	7
6-13 Nebulizer only	0	0.21	0.17	0.22	0.82	1.14	1.31
6-19 WDF only	0.05	0.08	0.17	0.54	1.48	1.05	0.54

TEST: GENERATOR	MMAD (um)	GSD	Test Concentration
Nebulizer	0.83	1.88	0.40 mg/m^3
WDF	1.14	1.83	3.22 mg/m^3
Combined	1.17	1.80	

Overall: CL-1 to CL-3		CL-7	
Relative Humidity	78±3%	Relative Humidity	77±3%
$NH_4HSO_4/(NH_4)_2SO_4$	29.7%	$NH_4HSO_4/(NH_4)_2SO_4$	30.1%
NH_4NO_3	25.8%	NH_4NO_3	26.2%
Carbon	17.6%	Carbon	18.0%
$VOSO_4$	0.011%	$VOSO_4$	0.011%
$NiSO_4$	0.013%	$NiSO_4$	0.013%
$PbSO_4$	0.035%	$PbSO_4$	0.035%
$MnSO_4$	0.032%	$MnSO_4$	0.032%
Natural clay	26.4%	Natural Clay	27.0%

LONDON-TYPE AEROSOL	Measured nominal values in exposures and tests						
Date Imp. sample	1	2	3	4	5	6	7
7-28 Nebulizer	0.11	0.03	0.05	0.18	0.87	3.00	3.17
7-28 WDF	0.01	0.03	0.17	0.50	0.98	0.80	2.30

TEST: GENERATOR	MMAD (um)	GSD	Test Concentration
Nebulizer	0.705	2.56	0.553 mg/m^3
WDF	0.716	1.53	0.446 mg/m^3
Combined	0.85	1.70	

Overall: CL-4 to CL-6		CL-7	
Relative Humidity	78±3%	Relative Humidity	77±3%
$NH_4HSO_4/(NH_4)_2SO_4$	35.7%	$NH_4HSO_4/(NH_4)_2SO_4$	40.1%
Coal Fly ash	33.2%	Coal Fly Ash	29.9%
Carbon	33.2%	Carbon	29.9%

Table 3: Exposure Data Summary

Exp#	Ch#	MMAD (um)/GSD	Aer.conc-SD (mg/m^3)/SD	GAS ppm	humidity %RH	solution pH	Temp. ^oC
		CALIFORNIA TYPE AEROSOL		O_3			
C1-1	13	1.48/2.21	0.53/0.05		78	1.87	22.8
	14	1.48/2.21	0.58/0.06	0.410	77	1.87	22.8
	15			0.400	78		22.8
C1-2	13	1.44/2.22	1.42/0.10		78	1.89	25.5
	14	1.44/2.22	1.47/0.15	0.39	78	1.87	24.4
	15			0.400	78		24.1
C1-3	13	1.17/1.72	2.99/0.75		81	1.90	22.2
	14	1.17/1.72	2.96/0.96	0.409	82	1.90	22.5
	15			0.400	80		23.0
		LONDON TYPE AEROSOL		SO_2			
C1-4	13	0.801/1.78	0.83/0.11		81	1.95	23.3
	14	0.801/1.78	0.99/0.96	1.07	80	1.95	22.7
	15			1.07	80		23.1
C1-5	13	0.801/1.78	4.68/0.79		76	2.0	22.2
	14	0.801/1.78	5.02/0.78	21.89	77	2.0	23.0
	15			20.98	76		22.3
				O_3			
CL-6	13	0.801/1.78	3.21/1.26		78	2.0	23.2
	14	0.801/1.78	2.64/0.86	0.39	79	2.0	23.7
	15			0.39	79		23.0
		CALIFORNIA TYPE AEROSOL		O_3			
CL-7	14	1.17/1.97	1.05/0.58		76	2.0	23.2
	15	1.11/2.35	1.02/0.48	0.37	77	2.0	22.7
	16			0.38	79		23.0
		LONDON TYPE AEROSOL		SO_2			
	15	0.88/1.68	1.10/0.36	0.96	75	2.0	23.4

There was an increase in both responses in the ozone+aerosol+impaired group as compared with the ozone exposed impaired animals, but these differences were not evident when the data were normalized with respect to body weight.

Body weights were remarkably consistent within the short term exposures. Means for no groups differed by more than 20 g and standard error within each group was generally under 10 g. No treatment-related difference in body weight was apparent. Animals in exposure CL-1 were, on average, slightly larger than those in CL-3 to CL-6 while those in exposure CL-2 were slightly smaller (avg 204 vs 235 g). While it was expected that there would be some effect of elastase pretreatment to decrease body weight in the 30-day exposure, this was not a consistent finding. It was also expected that the ozone or sulfur dioxide treatment would decrease body weight, primarily due to the decreased appetite resulting from nasal injury. There was a small decrease (approximately 15 g) in the average weight in the ozone and ozone + impaired groups, but this was not duplicated in the groups treated with ozone in combination with aerosol.

Quantimet Analysis of Elastase Induced Emphysema

Results of quantimet analysis of surface to volume ratios and mean alveolar size are given for exposures CL-1, CL-3 and CL-7 are found in Appendix C. Elastase pretreatment used to impair rats consistently increased the average alveolar cross sectional size and the surface area to alveolar perimeter ratio (an estimate of volume to surface area) in the acute studies. This was not a repeatable finding in the subchronic study. We attribute the differences in this last study to the random location of samples used for this analysis as compared to the use of consistently located sections for each animal evaluated in the first studies. The differences between exposure CL-1 and CL-3 are related to improved consistency of inflation at fixation when the problems associated with dissecting the apical lobes were eliminated.

Light Microscopic Inflammatory Cell Counts

Results of light microscopic cell counts in the proximal alveolar duct are found in Appendix C; the 3-way analysis of variance are provided in Tables 4-9.

Significant increases in the numbers of inflammatory cells were present in all ozone treated animals. As stated in the methods, these cells all had the morphologic features of alveolar macrophages. It was generally found that animals pre-treated with elastase alone had increased numbers of inflammatory cells. These differences were not statistically significant in the first two exposures (CL-1 and CL-2) but were significant in all subsequent exposures. Analysis of variance showed that the elastase effect to increase airway inflammation was independent of gas treatment (O_3 or SO_2) and was the only treatment effect detected in exposures CL-4 and CL-5 (SO_2 exposures).

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. California aerosol (CL-1 and CL-2) inhalation resulted in increased inflammatory cell numbers but exposure to London-type aerosol did not (CL-6). Significant interaction (exacerbation of this response) between ozone and aerosol treatment was evident in exposures CL-1 CL-2, with a similar but non-statistically verifiable tendency in CL-3. London-type aerosol also exacerbated the ozone inflammatory effect in CL-6. A significant interaction between ozone, aerosol and impairment was evident in exposure CL-1 and CL- 6 and similar but non-significant tendencies were evident in exposure CL-2 but not in exposure CL-3. No increases in the number of proximal alveolar duct inflammatory cells were evident in any of the London-type aerosol/SO_2 exposures. Only slight increases in the extent of inflammatory cell accumulation occurred in the ozone and aerosol treated animals as the concentration of aerosol was increased in exposures 1 through 3. These increases were well within the range of variability, were not statistically significant and were not interpreted to represent a realistic dose response effect.

Table 4: Exposure CL-1 Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory Cells/alveolus
Gas: Ozone at 0.4 ppm
Aerosol: California-type at 0.55 mg/m^3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	0.0188	0.0047
Impaired (Unexposed)	6	0.0285	0.0087
CA Aerosol	6	0.0216	0.0046
CA Aerosol + Impaired	6	0.0457	0.0085
Ozone	6	0.1084	0.0188
Ozone + Impaired	6	0.0765	0.0150
Ozone + CA Aerosol	6	0.1436	0.0254
Ozone + CA Aerosol + Impaired	6	0.2290	0.0474

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
Ozone	-	24	0.0286	0.0039	
	+	24	0.1394	0.0182	0.0001
CA Aerosol	-	24	0.0581	0.0097	
	+	24	0.1100	0.0214	0.0014
Impaired	-	24	0.0731	0.0136	
	+	24	0.0945	0.0204	ns

Significant Interactions:
Ozone x CA Aerosol $p = 0.0085$
Ozone x CA Aerosol x Impaired $p = 0.0356$

Table 5: Exposure CL-2 Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory Cells/alveolus
Gas: Ozone at 0.4 ppm
Aerosol: California-type at 1.45 mg/m^3

Treatment	N	Mean	SE
None (Healthy unexposed	6	0.0170	0.0034
Impaired (Unexposed)	6	0.0210	0.0052
CA Aerosol	6	0.0130	0.0072
CA Aerosol + Impaired	6	0.0214	0.0071
Ozone	6	0.0882	0.0113
Ozone + Impaired	6	0.0965	0.0341
Ozone + CA Aerosol	6	0.1540	0.0381
Ozone + CA Aerosol + Impaired	6	0.2167	0.0306

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
Ozone	-	24	0.0182	0.0029	
	+	24	0.1389	0.0178	0.0001
CA Aerosol	-	24	0.0557	0.0115	
	+	24	0.1013	0.0215	0.0053
Impaired	-	24	0.0681	0.0153	
	+	24	0.0889	0.0199	ns

Significant Interactions:
Ozone x CA Aerosol $p = 0.0039$

Table 6: Exposure CL-3 Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory Cells/alveolus
Gas: Ozone at 0.4 ppm
Aerosol: California-type at 2.98 mg/m^3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	0.0096	0.0038
Impaired (Unexposed)	6	0.0455	0.0132
CA Aerosol	6	0.0023	0.0012
CA Aerosol + Impaired	6	0.0534	0.0107
Ozone	6	0.1328	0.0253
Ozone + Impaired	6	0.0800	0.0148
Ozone + CA Aerosol	6	0.1621	0.0221
Ozone + CA Aerosol + Impaired	6	0.1208	0.0219

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
Ozone	-	24	0.0277	0.0061	
	+	24	0.1240	0.0117	0.0001
CA Aerosol	-	24	0.0670	0.0121	
	+	24	0.0847	0.0149	ns
Impaired	-	24	0.0767	0.0169	
	+	24	0.0749	0.0096	0.0003

Significant Interactions:
none

Table 7: Exposure CL-4 Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory Cells/alveolus
Gas: SO_2 at 1.07 ppm
Aerosol: London-type at 0.91 mg/m^3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	0.0199	0.0058
Impaired (Unexposed)	6	0.0280	0.0072
LT Aerosol	6	0.0130	0.0035
LT Aerosol + Impaired	6	0.0569	0.0167
Sulfur Dioxide	6	0.0157	0.0074
Sulfur Dioxide + Impaired	6	0.0381	0.0096
SO_2 + LT Aerosol	6	0.0218	0.0047
SO_2 + LT Aerosol + Impaired	6	0.0447	0.0110

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
SO_2	-	24	0.0295	0.0057	
	+	24	0.0300	0.0047	ns
LT Aerosol	-	24	0.0254	0.0040	
	+	24	0.0341	0.0061	ns
Impaired	-	24	0.0176	0.0027	
	+	24	0.0419	0.0058	0.0005

Significant Interactions:
none

Table 8: Exposure CL-5 Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory Cells/alveolus
Gas: SO_2 at 21 ppm
Aerosol: London-type at 5.0 mg/m^3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	0.0162	0.0047
Impaired (Unexposed)	6	0.0181	0.0070
LT Aerosol	6	0.0109	0.0025
LT Aerosol + Impaired	6	0.0213	0.0067
Sulfur Dioxide	6	0.0008	0.0026
Sulfur Dioxide + Impaired	6	0.0623	0.0236
SO_2 + LT Aerosol	6	0.0044	0.0016
SO_2 + LT Aerosol + Impaired	6	0.0043	0.0163

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
SO_2	-	24	0.0241	0.0040	
	+	24	0.0294	0.0084	ns
LT Aerosol	-	24	0.0336	0.0075	
	+	24	0.0200	0.0052	ns
Impaired	-	24	0.0098	0.0017	
	+	24	0.0438	0.0077	0.0001

Significant Interactions:
none

Table 9: Exposure CL-6 Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory Cells/alveolus
Gas: Ozone at 0.4 ppm
Aerosol: London-type at 2.93 mg/m^3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	0.0219	0.0052
Impaired (Unexposed)	6	0.0543	0.0136
LT Aerosol	6	0.0118	0.0022
LT Aerosol + Impaired	6	0.0162	0.0057
Ozone	6	0.0917	0.0251
Ozone + Impaired	6	0.0082	0.0118
Ozone + LT Aerosol	6	0.1070	0.0123
Ozone + LT Aerosol + Impaired	6	0.1655	0.0303

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
Ozone	-	24	0.0260	0.0050	
	+	24	0.1116	0.0121	0.0000
LT Aerosol	-	24	0.0626	0.0092	
	+	24	0.0751	0.0155	ns
Impaired	-	24	0.0581	0.0110	
	+	24	0.0796	0.0142	ns

Significant Interactions:
Ozone x LT Aerosol p = 0.0026
Ozone x LT Aerosol x Impaired p = 0.0420

Subjective LM analysis

A summary of results of light microscopic evaluation for the short term exposures is given in Appendix C. The primary criterion of interest in these sections was the extent of mural thickening in the terminal bronchiole-alveolar duct region. This thickening most likely represents local edema formation, cell swelling and early reactive hyperplasia of epithelial cells and is representative of the well documented changes associated with ozone exposure. There was a clear distinction between control and ozone treatment groups with the typical small airway lesion being readily detectable in all ozone treated animals. No histopathologic changes were detectable in either the parenchyma or the intrapulmonary airways in any of the SO_2 treated animals. No differences in lesion intensity was apparent in the four exposure groups treated with ozone alone. Elastase pretreatment appeared to diminish the alveolar duct wall thickening slightly. Aerosol treatment did not have an effect on the light microscopic appearance of the ozone lesion in the small airways despite increasing concentrations of aerosol in exposures CL-1 through CL-3. The combination treatment of ozone and aerosol in impaired rats resulted in much more variable intensity of small airway lesions from region to region within animals and between animals in a group. The highest dose of aerosol (CL-3) appeared to have the mildest lesion of the California aerosol groups while rats in the ozone + London aerosol exposure (CL-6) had an even lesser response.

A more detailed analysis of several criteria was done for the 30 day exposure CL-7. Separate scores were given to the extent of fibrosis evident in paraffin sections stained with Masson's trichrome, the wall thickening, the amount of aerosol pigment retained, the extent of emphysema evident and an overall score based on lesion severity in small airways. The data are summarized in Table 10 with the Mann Whitney analysis of variance. Significant responses included increased lung pigmentation associated with both California-type aerosol exposures and ozone exposures. Impaired rats showed a significant level of pulmonary emphysema, while exposure to ozone was responsible for a significant level of lung lesions, mural changes, and observations of pulmonary fibrosis. A significant interaction was associated with the combination of ozone and California-type aerosol, indicating that the aerosol significantly exacerbated the fibrosis initiated by the ozone.

Table 10: Subjective Analysis of Exposure 7
30 Day Exposure to Indicated Atmospheres
(Nominal averages for six rats per group less unusables)

Treatment	Lesion	Mural Changes	Fibrosis	Pigmentation	Emphysema
None	0.0	0.0	0.0	0.0	0.8
Impaired	0.0	0.0	0.4	0.3	2.0
Ozone	1.8	1.7	0.9	0.6	0.8
Ozone + Impaired	2.3	2.2	1.4	1.7	1.0
CA Aerosol	0.2	0.1	0.0	0.8	0.4
CA Aerosol + Impaired	0.1	0.1	0.1	0.8	1.6
CA Aerosol + Ozone	2.4	2.4	1.3	2.1	0.9
CA Aerosol + Ozone + Impaired	2.4	2.3	1.9	2.9	2.1
LT Aerosol + SO_2	0.1	0.1	0.1	1.2	0.3
LT Aerosol + SO_2 + Impaired	0.3	0.3	0.3	1.5	1.6

0 = no lesion or response.
1 = equivocal lesion or response
2 = moderate but definite lesion or response.
3 = moderately severe.
4 = severe.

MANN-WHITNEY ANALYSIS OF VARIANCE FOR SUBJECTIVE OBSERVATIONS IN EXPOSURE 7

Treatment	Lesion		Mural Changes		Fibrosis		Pigmentation		Emphysema	
CA Aerosol	-	+	-	+	-	+	-	+	-	+
N	22	33	22	33	22	33	22	33	22	33
Median	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	1.0
P	ns		ns		ns		0.0005		ns	
Ozone	-	+	-	+	-	+	-	+	-	+
N	33	22	33	22	33	22	33	22	33	22
Median	0.0	2.0	0.0	2.0	0.0	1.0	0.0	1.5	0.0	1.0
P	<0.00005		<0.00005		<0.00005		0.03		ns	
Impaired	-	+	-	+	-	+	-	+	-	+
N	30	25	30	25	30	25	30	25	30	25
Median	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	2.0
P	ns		ns		ns		ns		0.0005	

Significant Interactions:

CA Aerosol x Ozone (Fibrosis) p = 0.0183

Figure 22: Light microscopic appearance of terminal bronchiole and proximal alveolar duct in the 30-day study.

a) Inhaled clean air for 30 days. Rats exposed to SO_2 had a similar appearance.

b) Effect of elastase pretreatment in animals exposed to clean air for 30 days. Focal regions of simplified alveolar structures with enlarged lumens and dilated alveolar ducts are evident.

c) Effect of 30 days exposure to 0.4 ppm ozone. Note the accumulation of inflammatory cells in alveoli and the separation of alveolar ducts in conducting airways by segments of thickened wall lined by cuboidal epithelium (respiratory bronchioles).

d) Terminal bronchiole form a rat exposed for 30 days to 1 mg/m^3 California-type aerosol. No differences from clean air group are apparent.

e) Remodeled respiratory bronchiole from an animal treated for 30 days with ozone at 0.4 ppm and California-type aerosol at 1 mg/m^3. Note pigment accumulation in inflammatory cells.

f) Respiratory bronchiole from an elastase pre-treated rat exposed for 30 days to 0.4 ppm ozone and 1 mg/m^3 California-type aerosol. Note the increased number of inflammatory cells.

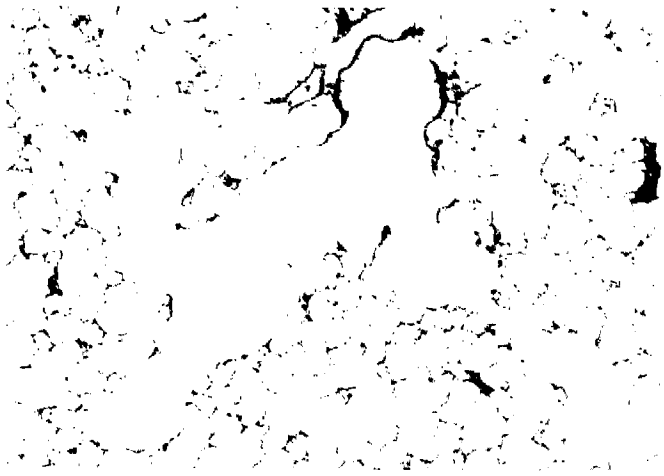


Fig. 22a

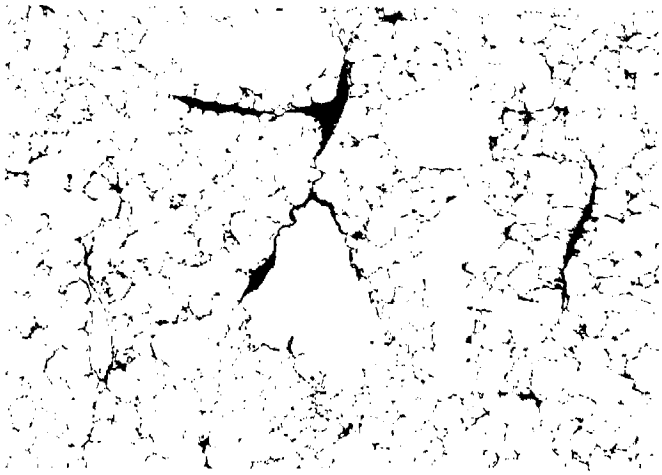


Fig. 22b

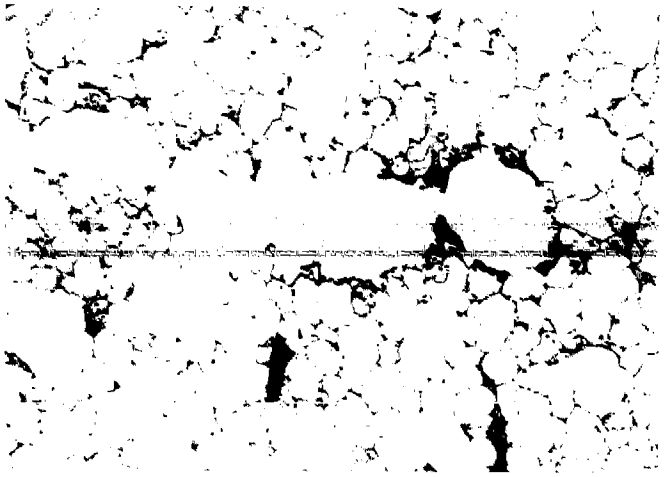


Fig. 22c

Fig. 22d

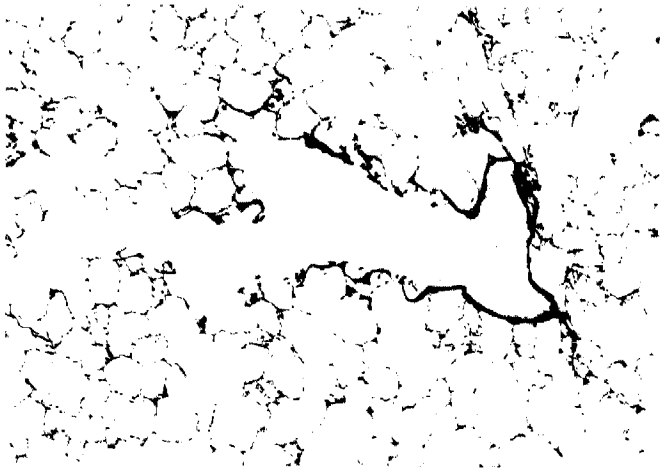


Fig. 22e

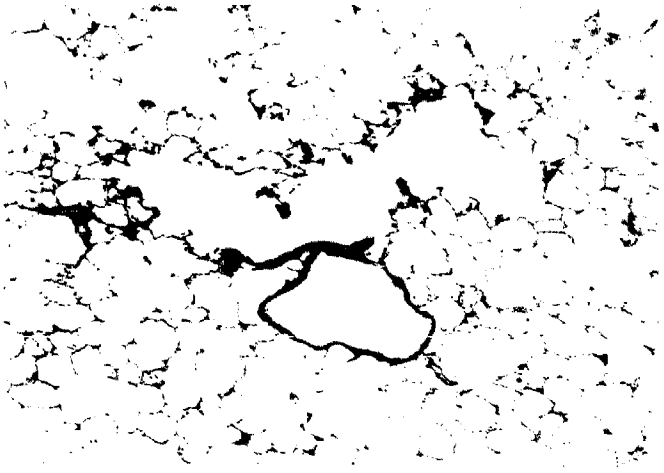


Fig. 22f

Pigmentation was observed for both ozone and aerosol exposures in the 30-day study (CL-7). Two types of pigment were observed in the histologic sections. A light tan-brown pigment was present in macrophages in the alveoli of lung impaired elastase pretreated animals. This pigment resembled hemosiderin and probably arose as a consequence of the initial hemorrhagic reaction to elastase instillation. While the presence of this pigment caused a slight increase in the subjective scoring of pigment accumulation, it was generally easily distinguished from the pigment associated with the retention of aerosol particles in the lung. The latter was black because of the carbon content and tended to be in more regular sized, larger granules than did hemosiderin. Significant black aerosol pigment was evident only in the aerosol exposed animals from the 30-day study. In aerosol plus ozone exposed animals, the aerosol pigment was found in the form of granules in macrophages which accumulated in the alveolar ducts. The aerosol pigment in impaired rats that were aerosol plus ozone exposed was not only more dense in alveolar duct macrophages but appeared to fill the cytoplasm with black debris rather than to be present as discrete granules. Aerosol pigment deposition in animals exposed to aerosol alone was randomly distributed in the alveolar parenchyma in the form of individual black granules that were either extracellular or in individual alveolar macrophages. Lung impairment by elastase pre-treatment was without effect on the random distribution of aerosol pigment in animals not exposed to ozone.

A subtle but significant increase in the amount of collagen was detected in the interstitium of the newly formed respiratory bronchioles in the ozone + aerosol + impaired group. No changes in the location or amount (subjectively evaluated) of London-type aerosol were detected in the groups exposed to London-type aerosol + SO_2.

Morphometric Analysis of Exposure CL-7

Morphometric analysis allowed quantification of the relative volumes of the parenchymal components of the lung to be expressed as a percent of total lung volume as determined from data found in Appendix C, Table C-23. These results are presented for the subchronic 30-day exposure set, CL-7, in Table 11A and Table 11B.

Table 11A: Exposure CL-7 small-airway structures as percent of lung volume for terminal bronchiole wall (TBw), terminal bronchiole lumen (TBlu). respiratory bronchiole wall (RBw), and respiratory bronchiole lumen (RBlu) with the mean arithmetic thickness of the terminal bronchiole wall (Mean T, mm) and the number of macrophages per alveolus in the proximal alveolar duct (Mac/Alv).

Treatment		TBw	TBlu	RBw	RBlu	Mean T	Mac/Alv
None (Healthy)	Mean	0.203	1.320	0.005	0.007	0.026	0.010
	SE	0.047	0.208	0.004	0.003	0.005	0.006
Impaired (Unexposed)	Mean	0.344	2.137	0	0	0.032	0.058
	SE	0.047	0.403	0	0	0.004	0.010
Ozone	Mean	0.401	2.555	0.028	0.072	0.044	0.449
	SE	0.062	0.448	0.011	0.028	0.003	0.100
Ozone + Impaired	Mean	0.381	2.368	0.009	0.037	0.046	0.650
	SE	0.098	0.690	0.006	0.018	0.010	0.135
CA Aerosol	Mean	0.343	2.150	0.008	0.031	0.042	0
	SE	0.060	0.419	0.004	0.021	0.015	0
CA Aerosol + Impaired	Mean	0.172	0.846	0	0	0.028	0.087
	SE	0.129	0.508	0	0	0.005	0.036
O_3 + CA Aerosol	Mean	0.365	2.631	0.031	0.056	0.025	0.389
	SE	0.040	0.638	0.029	0.038	0.005	0.036
O_3 + Aerosol + Impair	Mean	0.204	1.299	0.039	0.137	0.048	1.044
	SE	0.068	0.386	0.014	0.044	0.014	0.274
SO_2 + LT Aerosol	Mean	0.333	2.377	0.003	0.014	0.030	0.017
	SE	0.113	0.717	0.003	0.014	0.004	0.005
SO_2 + Aerosol + Impair	Mean	0.169	1.392	0	0	0.032	0.007
	SE	0.058	0.339	0	0	0.007	0.005

Table 11B: Analysis of Variance Including Probability Values for the Data on Exposure CL-7 Lung Small-airway Changes.

Treatment	TBw	TBlu	RBw	RBlu	Mean T	Cells/Alv
Ozone	ns	ns	0.0097	0.0008	ns	0.0000
Aerosol	ns	ns	ns	ns	ns	ns
Impaired	ns	ns	ns	ns	ns	0.0199
Interactions:						
CA Aerosol x Ozone	ns	ns	ns	ns	ns	ns
CA Aerosol x Impair	ns	ns	0.029	0.0145	ns	ns
Ozone x Impaired	ns	ns	ns	0.0507	ns	0.0943
Ozone x CA x Impair	ns	ns	0.043	0.0069	ns	ns
SO_2 x LT Aerosol	ns	ns	ns	ns	ns	ns
SO_2 x LT x Impair	ns	ns	ns	ns	ns	ns

As in the subjective analysis and in the short term exposures, no changes due to SO_2 exposure were evident. Ozone exposure resulted in a significant increase of the relative volume of remodeled quasi-respiratory bronchiole in the terminal airways. Also evident in the O_3 treated animals was a significant increase in inflammatory cell infiltrate. A apparent tendency to terminal bronchiole wall thickening in ozone treated animals was not statistically significant. Lung impairment by elastase pretreatment significantly increased the inflammatory infiltrate in the proximal alveolar duct in both the ozone exposed impaired group and in the aerosol with ozone exposed impaired group. Aerosol treatment alone had no effect in any of the parameters evaluated and no ozone x aerosol interaction was apparent. The combined treatment of ozone and aerosol in the impaired elastase pretreated animals resulted in a marked increase in quasi-respiratory bronchiole formation.

SEM Cell Counts

Scanning electron microscopy (SEM) counts of inflammatory cells (pulmonary alveolar macrophages) in proximal alveolar ducts were done on exposures CL-1 and CL-2. These are presented in Tables 12-13. The results of initial exposure counts showed the SEM counts gave little additional value to the counts obtained by light microscopy and were more variable and operator dependent. For these reasons, as well as the lack of significant light microscopic changes in the SO_2 exposures, successful SEM counts were limited to exposures CL-1 and CL-2. SEM does provide a dramatic visualization of the small airway changes associated with remodeling of the terminal airways and representative photographs are shown in Figure 23.

Table 12: Exposure CL-1 SEM Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory cells/alveolus by SEM
Gas: Ozone at 0.4 ppm
Aerosol: California at 0.55 mg/m3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	0.0381	0.0050
Impaired (Unexposed)	5	0.0705	0.0107
CA Aerosol	4	0.0491	0.0132
CA Aerosol + Impaired	4	0.1096	0.0239
Ozone	6	0.131	0.0183
Ozone + Impaired	4	0.0835	0.0277
Ozone + CA Aerosol	6	0.1557	0.0223
Ozone + CA Aerosol + Impair	6	0.2119	0.0533

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
Ozone	-	19	0.0639	0.0094	
	-	22	0.1256	0.0182	0.0001
CA Aerosol	-	21	0.0808	0.0135	
	+	20	0.1315	0.0138	0.0147
Impaired	-	22	0.0935	0.0132	
	+	19	0.1189	0.0142	ns

Significant Interactions:
none

Table 13: Exposure CL-2 SEM Small Airway Inflammation Analysis of Variance

Parameter: Inflammatory cells/alveolus by SEM
Gas: Ozone at 0.4 ppm
Aerosol: California at 1.45 mg/m^3

Treatment	N	Mean	SE
None (Healthy Unexposed)	2	0.0422	0.0842
Impaired (Unexposed)	2	0.0118	0.0012
CA Aerosol	2	0.0538	0.0368
CA Aerosol + Impaired	2	0.0939	0.0336
Ozone	5	0.0733	0.0094
Ozone + Impaired	1	0.1403	0.1404
Ozone + CA Aerosol	5	0.1209	0.0191
Ozone + CA Aerosol + Impair	3	0.0580	0.0142

ANALYSIS of VARIANCE

Treatment		N	MEAN	SE	P
Ozone	-	8	0.0751	0.0101	
	+	14	0.0918	0.0107	ns
CA Aerosol	-	10	0.0733	0.0101	
	+	12	0.0816	0.0132	ns
Impaired	-	14	0.0725	0.0111	
	+	8	0.0802	0.0131	ns

Significant Interactions:
none

Figure 23: Scanning electron microscopic appearance of terminal bronchioles and alveolar ducts from healthy and impaired rats exposed for 30 days to clean air, ozone with and without California-type aerosol, SO_2 with London-type aerosol, in the subchronic exposures.

a) Normal terminal airway representative of control animals (clean air) or animals exposed to SO_2 with London-type aerosol.

b) Effect of elastase pretreatment in clean air exposed animal. Alveolar ducts are lined by enlarged, flattened alveolar structures.

c) Terminal airway of rat exposed for 30 days to 1 mg/m^3 California type aerosol: no difference from control appearance.

d) Effect of 30 days exposure to 0.4 ppm ozone. Note the accumulation of inflammatory cells in alveoli and the separation of alveolar ducts in conducting airways by segments of thickened wall lined by cuboidal epithelium (respiratory bronchioles).

e) Effect of 30 days exposure to 0.4 ppm Ozone with 1 mg/m3 California type aerosol. Note apparently greater inflammatory response and more dramatic appearance of respiratory bronchiole formation compared with ozone alone (not statistically verified).

f) Effect of ozone, aerosol and elastase pre-treatment. There is marked inflammatory cell accumulation, and a broad, flattened and dilated respiratory bronchiole surface.

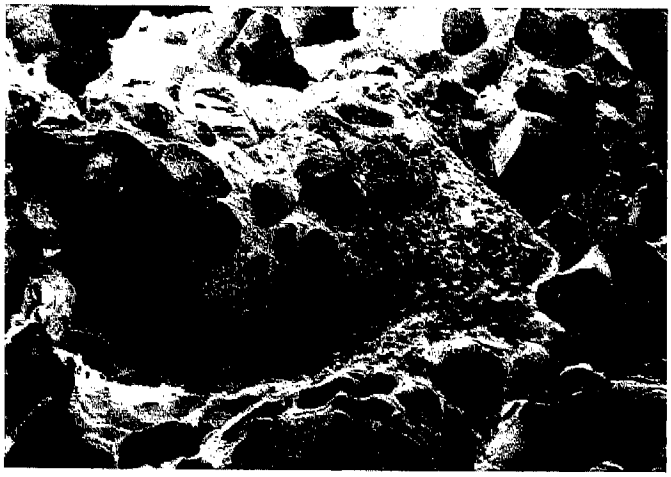


Fig. 23a

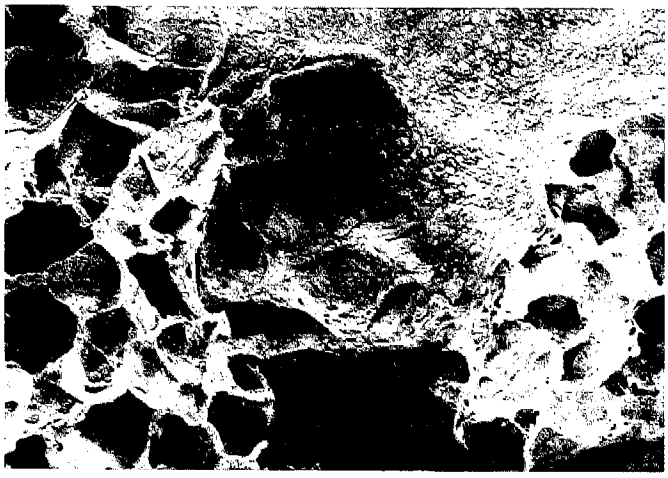


Fig. 23b

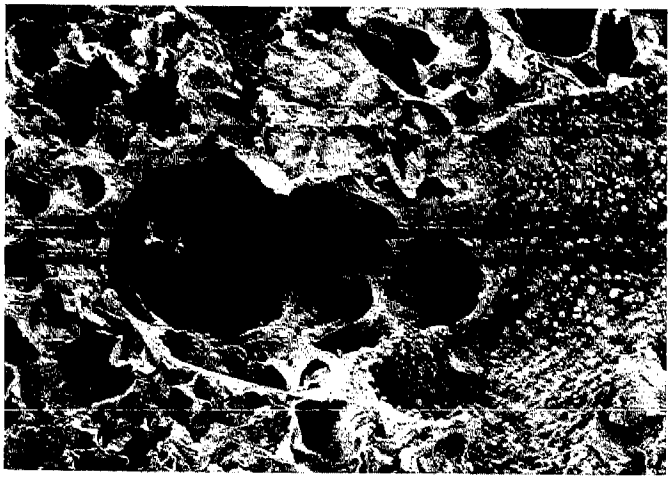


Fig. 23c

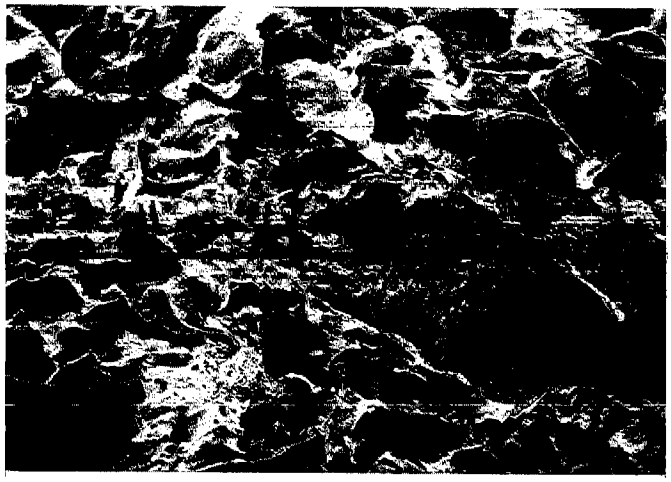


Fig. 23d

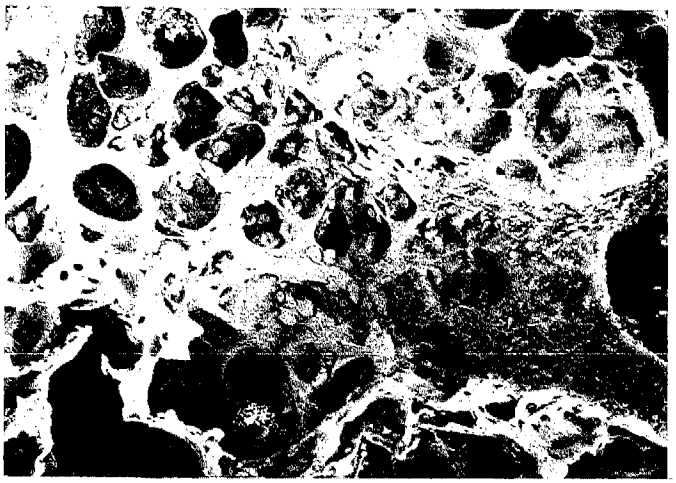


Fig. 23e

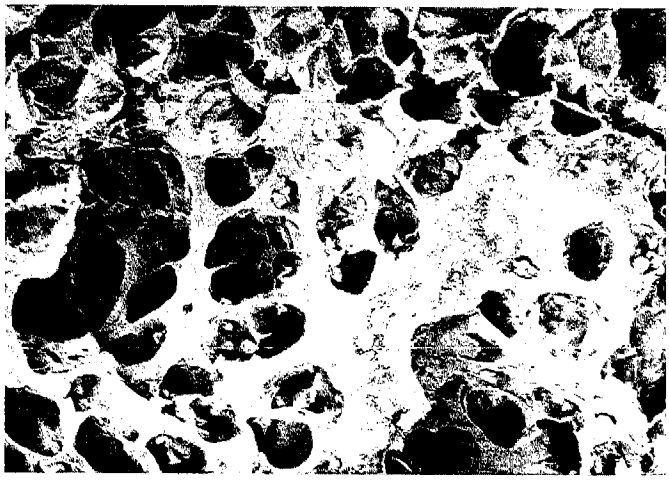


Fig. 23f

Results, Lung Biochemistry

Determinations of DNA, RNA, total protein in lung tissue were performed as described in the methods section. Protein and hydroxyproline assays were done on the right apical lobe from animals in exposure sets CL-1 and CL-4, but there were no statistically significant findings (Appendix D). Lung DNA, RNA, and protein measurements were done on the left lobe of animals from exposure sets CL-2 and CL-5. DNA, RNA, protein and hydroxyproline assays were performed on the left lobe of all animals in the 30-day subchronic exposure (CL-7). Results of these assays are presented in Appendix D and summarized in Tables 14-23.

The results of the 3-days studies (Tables 14-19) include: (1) significant increase in total lung DNA, RNA, and/or protein in rats exposed to synthetic California-type aerosol (1.45 mg/m^3), the synthetic London-type aerosol (in impaired rats) with and without SO_2, and ozone (0.4 ppm), but aerosol alone made little change in healthy rat values; (2) the lungs of impaired rats were more sensitive to the exposure of ozone or London-type aerosol with or without SO_2 than those of the healthy rats; (3) the protein content of the lungs of impaired rats exposed to SO_2 (21 ppm) was decreased significantly. The increase in the level of DNA, RNA, and/or protein content in the lung of the exposed animals may be explained by: (a) increased number of inflammatory cells (macrophage), (b) primary cellular response, and/or (c) pulmonary edema (source of protein). These biochemical changes should be considered as potentially detrimental to the health of the exposed individuals.

The results of the subchronic (30-day) study (Tables 20-23) showed significant decreases in total lung DNA and RNA, and significant increases in total lung hydroxyproline in rats exposed to California-type aerosol, but little change in healthy rats exposed only to aerosols. In addition, ozone exposure tended to increase and elastase treated impaired rats had significant increases in lung hydroxyproline. London-type aerosol with SO_2 exacerbated this increase in impaired rats. Increase in the lung level of hydroxyproline suggests that the lung may become fibrotic. Lung impairment from elastase pretreatment treatment also increased the sensitivity of lung to aerosol and gas exposure as observed in the acute exposure.

Table 14: Exposure CL-2 Biochemical Analysis of Lung DNA Content

Parameter: Lung DNA content (mg)
Gas: Ozone at 0.4 ppm
Aerosol: California Type at 1.45 mg/m3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	3.18	0.28
Impaired (Unexposed)	6	4.08	0.33
CA Aerosol	6	3.61	0.58
CA Aerosol + Impaired	6	5.43	0.81
Ozone	6	4.23	0.30
Ozone + Impaired	6	4.34	0.30
Ozone + CA Aerosol	6	4.63	0.30
Ozone + CA Aerosol + Impaired	6	5.57	0.43

Analysis of Variance

Treatment		N	Mean	SE	P
Ozone	-	24	4.08	0.25	
	+	24	4.69	0.22	0.062
CA Aerosol	-	24	3.96	0.14	
	+	24	4.81	0.26	0.011
Impaired	-	24	3.91	0.18	
	+	24	4.86	0.24	0.005

Significant Interactions:

None

Table 15: Exposure CL-2 Biochemical Analysis of Lung RNA Content

Parameter:	Lung RNA content (mg)
Gas:	Ozone at 0.4 ppm
Aerosol:	California Type at 1.45 mg/m3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	17.4	1.9
Impaired (Unexposed)	6	21.5	1.6
CA Aerosol	6	17.0	2.3
CA Aerosol + Impaired	6	23.7	3.3
Ozone	6	22.2	1.3
Ozone + Impaired	6	23.3	1.5
Ozone + CA Aerosol	6	21.5	1.8
Ozone + CA Aerosol + Impaired	6	25.0	2.5

Analysis of Variance

Treatment		N	Mean	SE	P
Ozone	-	24	19.9	1.1	
	+	24	23.0	0.9	0.043
CA Aerosol	-	24	21.1	0.7	
	+	24	21.8	1.2	ns
Impaired	-	24	19.5	0.9	
	+	24	23.4	1.1	0.014

Significant Interactions:

None

Table 16: Exposure CL-2 Biochemical Analysis of Lung Protein Content

Parameter:	Lung protein content (mg)
Gas:	Ozone at 0.4 ppm
Aerosol:	California Type at 1.45 mg/m3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	143	15
Impaired (Unexposed)	6	192	9
CA Aerosol	6	158	15
CA Aerosol + Impaired	6	250	28
Ozone	6	196	15
Ozone + Impaired	6	198	12
Ozone + CA Aerosol	6	229	13
Ozone + CA Aerosol + Impaired	6	221	18

Analysis of Variance

Treatment		N	Mean	SE	P
Ozone	-	24	186	8	
	+	24	211	7	0.039
CA Aerosol	-	24	182	6	
	+	24	215	9	0.009
Impaired	-	24	182	7	
	+	24	215	9	0.006

Significant Interactions:

Ozone x Impaired P = 0.003

Table 17: Exposure CL-5 Biochemical Analysis of Lung DNA Content

Parameter:	Lung DNA content (mg)
Gas:	Sulfur dioxide at 21 ppm
Aerosol:	London Type at 5.0 mg/m3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	5.10	0.46
Impaired (Unexposed)	6	4.93	0.26
LT Aerosol	6	4.24	0.31
LT Aerosol + Impaired	6	6.21	0.42
Sulfur dioxide	6	4.74	0.15
Sulfur dioxide + Impaired	6	5.34	0.24
Sulfur dioxide + LT Aerosol	6	4.04	0.24
SO_2 + LT Aerosol + Impaired	6	5.81	0.44

Analysis of Variance

Treatment		N	Mean	SE	P
SO_2	-	24	5.12	0.17	
	+	24	4.98	0.13	ns
LT Aerosol	-	24	5.03	0.14	
	+	24	5.08	0.17	ns
Impaired	-	24	4.53	0.15	
	+	24	5.57	0.16	0.001

Significant Interactions:

Impaired x London type aerosol	P = 0.002
Impaired x SO_2 x London aerosol	P = 0.006

Table 18: Exposure CL-5 Biochemical Analysis of Lung RNA Content

Parameter:	Lung RNA content (mg)
Gas:	Sulfur Dioxide at 21 ppm
Aerosol:	London Type at 5.0 mg/m3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	19.3	1.3
Impaired (Unexposed)	6	18.6	1.6
LT Aerosol	6	18.7	1.3
LT Aerosol + Impaired	6	21.3	1.8
Sulfur dioxide	6	17.0	0.4
Sulfur dioxide + Impaired	6	17.4	0.4
Sulfur dioxide + LT Aerosol	6	16.7	1.2
SO_2 + LT Aerosol + Impaired	6	21.4	1.0

Analysis of Variance

Treatment		N	Mean	SE	P
SO_2	-	24	19.5	0.7	
	+	24	18.1	0.4	ns
LT Aerosol	-	24	18.1	0.5	
	+	24	19.5	0.6	ns
Impaired	-	24	17.9	0.5	
	+	24	19.7	0.6	0.041

Significant Interactions:

Impaired x London type aerosol	P = 0.048
Impaired x SO_2 x London aerosol	P = 0.048

Table 19: Exposure CL-5 Biochemical Analysis of Lung Protein Content

Parameter:	Lung protein content (mg)
Gas:	Sulfur dioxide at 21 ppm
Aerosol:	London Type at 5.0 mg/m3

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	232	18
Impaired (Unexposed)	6	208	18
LT Aerosol	6	271	16
LT Aerosol + Impaired	6	242	28
Sulfur dioxide	6	220	10
Sulfur dioxide + Impaired	6	218	8
Sulfur dioxide + LT Aerosol	6	188	17
SO_2 + LT Aerosol + Impaired	6	244	10

Analysis of Variance

Treatment		N	Mean	SE	P
SO_2	-	24	238	10	
	+	24	218	6	ns
LT Aerosol	-	24	220	7	
	+	24	236	9	ns
Impaired	-	24	228	7	
	+	24	228	8	ns

Significant Interactions:

Impaired x SO_2 P = 0.032

Table 20: Exposure CL-7 Biochemical Analysis of Lung DNA Content

Parameter: Lung DNA content (mg)
Gas: Ozone at 0.4 ppm
Aerosol: California Type at 1.0 mg/m3
(Additional Treatment: Sulfur dioxide at 1 ppm with London type aerosol at 1.1 mg/m3)

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	3.98	0.27
Impaired (Unexposed)	6	4.61	0.31
California Aerosol	6	4.17	0.14
California Aerosol + Impaired	6	4.12	0.19
Ozone	6	4.29	0.40
Ozone + Impaired	6	4.69	0.24
Ozone + California Aerosol	6	3.65	0.12
Ozone + CA Aerosol + Impaired	6	4.08	0.20
London Aerosol with SO_2	6	3.98	0.22
London Aerosol/SO_2 + Impaired	6	3.88	0.33

Analysis of Variance

Treatment		N	Mean	SE	P
Ozone	-	24	4.22	0.11	
	+	24	4.18	0.12	ns
(CA) Aerosol	-	24	4.39	0.14	
	+	24	4.01	0.08	0.034 (decrease)
Impaired	-	24	4.02	0.12	
	+	24	4.38	0.11	0.054

Significant Interactions:

None

Table 21: Exposure CL-7 Biochemical Analysis of Lung RNA Content

Parameter: Lung RNA content (mg)
Gas: Ozone at 0.4 ppm
Aerosol: California Type at 1.0 mg/m3
(Additional Treatment: Sulfur dioxide at 1 ppm with London type aerosol at 1.1 mg/m3)

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	20.5	1.2
Impaired (Unexposed)	6	20.3	0.5
California Aerosol	6	20.5	0.8
California Aerosol + Impaired	6	19.0	1.1
Ozone	6	20.1	0.9
Ozone + Impaired	6	22.6	1.2
Ozone + California Aerosol	6	19.3	0.3
Ozone + CA Aerosol + Impaired	6	20.1	0.8
London Aerosol with SO_2	6	20.4	0.7
London Aerosol/SO_2 + Impaired	6	21.1	0.5

Analysis of Variance

Treatment		N	Mean	SE	P
Ozone	-	24	20.1	0.4	
	+	24	20.5	0.4	ns
(CA) Aerosol	-	24	20.9	0.5	
	+	24	19.3	0.4	0.079 (decrease)
Impaired	-	24	20.1	0.4	
	+	24	20.5	0.4	ns

Significant Interactions:
Ozone x Impaired P = 0.054

Table 22: Exposure CL-7 Biochemical Analysis of Lung Protein Content

Parameter: Lung protein content (mg)
Gas: Ozone at 0.4 ppm
Aerosol: California Type at 1.0 mg/m3
(Additional Treatment: Sulfur dioxide at 1 ppm with London type aerosol at 1.1 mg/m3)

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	250	19
Impaired (Unexposed)	6	259	11
California Aerosol	6	262	12
California Aerosol + Impaired	6	247	21
Ozone	6	257	21
Ozone + Impaired	6	290	33
Ozone + California Aerosol	6	262	7
Ozone + CA Aerosol + Impaired	6	257	21
London Aerosol with SO_2	6	253	12
London Aerosol/SO_2 + Impaired	6	247	8

Analysis of Variance

Treatment		N	Mean	SE	P
Ozone	-	24	255	8	
	+	24	267	10	ns
CA Aerosol	-	24	264	10	
	+	24	257	8	ns
Impaired	-	24	258	7	
	+	24	263	11	ns

Significant Interactions:

None

Table 23: Exposure CL-7 Biochemical Analysis of Lung Hydroxyproline Content

Parameter: Lung Hydroxyproline Content (mg)
Gas: Ozone at 0.4 ppm
Aerosol: California Type at 1.0 mg/m3
(Additional Treatment: Sulfur dioxide at 1 ppm with London type aerosol at 1.1 mg/m3)

Treatment	N	Mean	SE
None (Healthy Unexposed)	6	1.60	0.17
Impaired (Unexposed)	6	1.98	0.13
California Aerosol	6	1.83	0.19
California Aerosol + Impaired	6	1.77	0.14
Ozone	6	1.64	0.09
Ozone + Impaired	6	1.88	0.22
Ozone + California Aerosol	6	1.89	0.10
Ozone + CA Aerosol x Impaired	6	2.53	0.19
London Aerosol with SO_2	6	1.75	0.16
London Aerosol/SO_2 + Impaired	6	2.14	0.15

Analysis of Variance

Treatment		N	Mean	SE	P
Ozone	-	24	1.80	0.07	
	+	24	1.99	0.07	0.097
CA Aerosol	-	24	1.78	0.07	
	+	24	2.01	0.07	0.048
Impaired	-	24	1.74	0.07	
	+	24	2.04	0.08	0.009

Significant Interactions:
London Aerosol with SO_2 x Impaired P = 0.038

Results of Lung Clearance Studies with ^{99m}Tc-labeled Aerosols

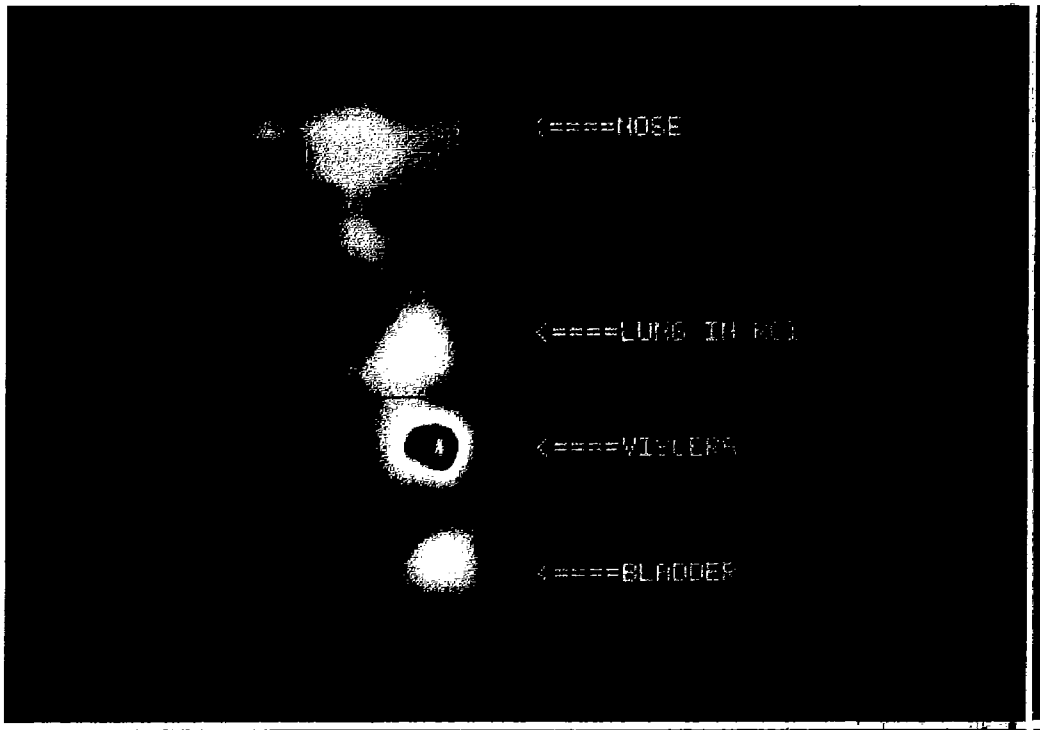
For each exposure CL-1 to CL-6, forty-eight unanesthetized Fischer-344 SPF male rats were imaged eight at a time on the Searle large field of view gamma scintillation camera with parallel-hole collimator as described in the methods section. Twenty-four rats were exposed to a technetium-labeled aerosol and the other twenty-four were exposed to a technetium labeled iron oxide aerosol. An additional 12 rats (6 for each aerosol) were studied in exposure CL-7 because there was one additional exposure chamber (Figure 3). Examples of the region-of-interest (ROI) image analysis for determination of lung burdens in rats of ^{99m}Tc-labeled particles for these studies are shown in Fig. 24.

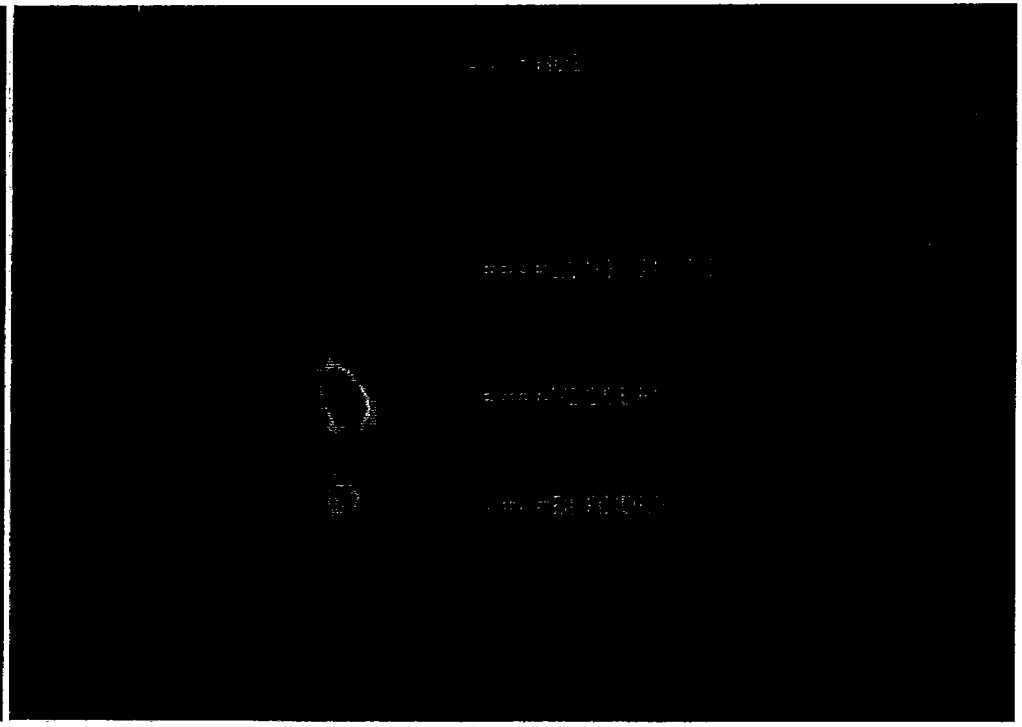
The measured average clearance half-times and their statistical distribution are given in Appendix E for exposures CL-2 to CL-6. No usable data on lung clearance were obtained from CL-1 because of technical problems that were later resolved. The results for these three-day acute exposures show no responses that are statistically significant because of fairly large variability between measurements and small changes, if any.

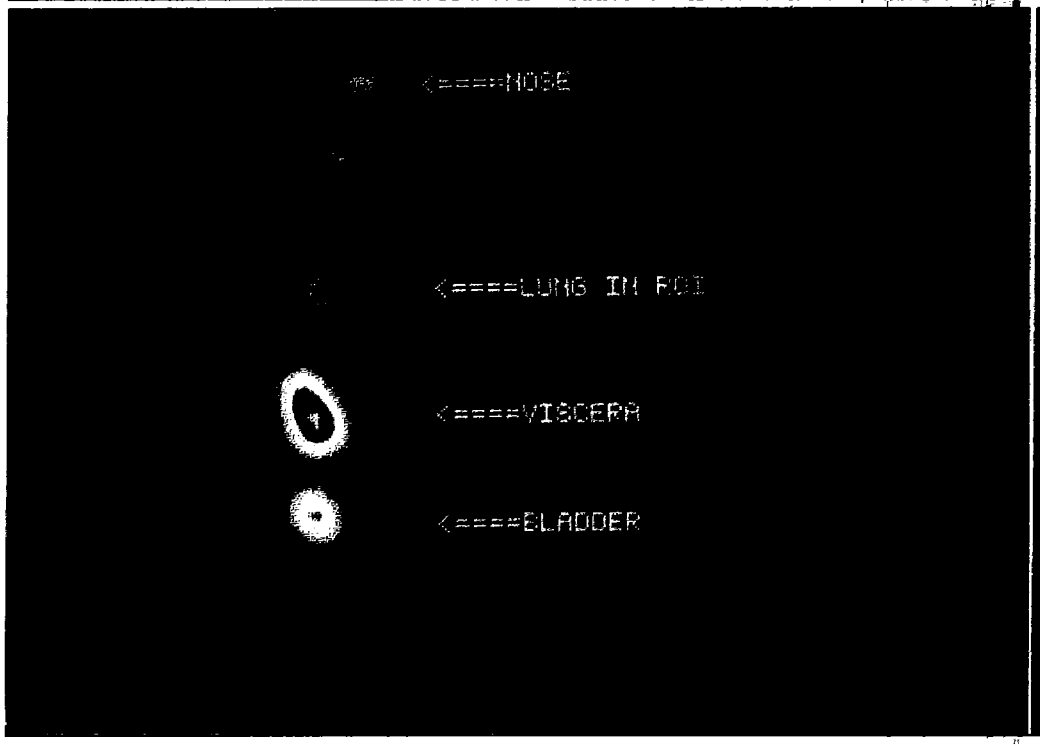
The measured average clearance half-times and their statistical distribution for exposure CL-7 are also given in Table 24. Although there was no apparent change in DTPA clearance, there were several marked tendencies and some significant differences among the iron oxide particle tracheobronchial clearance rates observed in CL-7. There was no evidence that lung impairment evoked changes in lung permeability or in tracheobronchial clearance, and impaired rats yielded responses very much like the unimpaired rats with respect to effects of inhalation exposure, and were grouped together for improved statistical power.

Analysis of the tracheobronchial clearance rates for of individual rats showed a distribution that was decidedly non-Gaussian. For this reason the Mann-Whitney nonparametric test of variance was used to evaluate these data. The aerosol-exposed rats consisted of two types: (1) those that responded and had much longer clearance half-times (slower clearance) than unexposed rats, and (2) those that had no response or slightly shorter clearance half-times.

Figure 24: Examples of the region of interest (ROI) image analysis of lung burdens burdens of ^{99m}Tc-labeled particles observed with a Searle large-field gamma scintillation camera.


<====NOSE
<====LUNG IN ROI
<====VISCERA
<====BLADDER




<====NOSE
<====LUNG IN ROI
<====VISCERA
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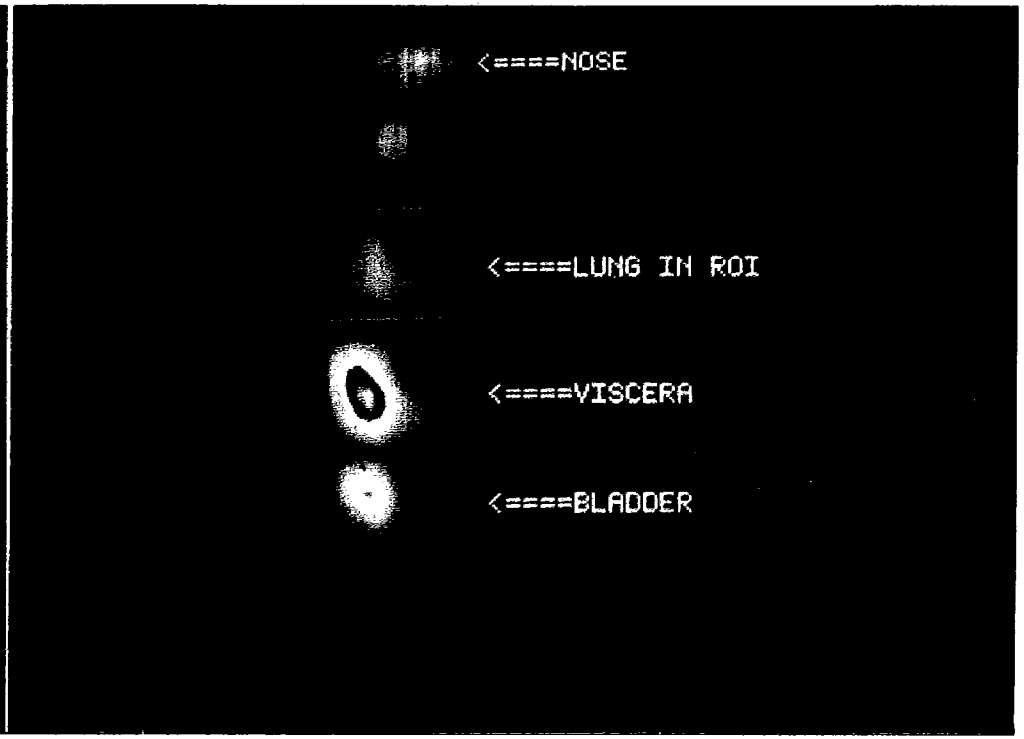
<====NOSE
<====LUNG IN ROI
<====VISCERA
<====BLADDER

Table 24: Exposure CL-7 (30 day) DTPA and Iron Oxide Clearance Half Times (MEAN ± SE, three rats per group)

*TREATMENT	DTPA (minutes)	IRON OXIDE (**) LONG (hours)	IRON OXIDE (**) SHORT
NONE (H)	45.3±4.2	90.2±27.5	0.44±0.03
IMPAIRED (I)	48.2±5.0	97.0±18.8	0.56±0.38
OZONE (H)	51.4±15.5	64.1±24.8	0.30±0.20
OZONE (I)	68.0±6.9	40.6±4.9	0.37±0.09
CA AEROSOL (H)	53.3±8.3	126 ±47	0.70±0.25
CA AEROSOL (I)	59.5±13.0	274 ±165	1.03±0.27
OZONE & CA AEROSOL (H)	45.5±1.6	48.0±9.1	0.36±0.15
OZONE & CA AEROSOL (I)	53.4±6.4	58.2±4.5	0.47±0.15
SO2 & LT AEROSOL (H)	53.6±4.3	126 ±50	0.90±0.40
SO2 & LT AEROSOL (I)	53.2±3.5	118 ±51	0.50±0.40

* (H) : HEALTHY, (I): IMPAIRED

**LONG: LONG RETENTION PART OF FITTED DOUBLE-EXPONENTIAL FUNCTION.

SHORT: SHORT RETENTION PART OF FITTED DOUBLE-EXPONENTIAL FUNCTION.

NON PARAMETRIC MANN WHITNEY ANALYSIS OF VARIANCE
FOR THE LONG COMPONENT HALF TIME FOR INSOLUBLE IRON OXIDE PARTICLES

Treatment		N	MEDIAN	P
Ozone	-	18	113.6	
	+	12	47.8	0.0056

In fact, there was a gap in tracheobronchial clearance rates between these responders versus non-responders with respect to the longer clearance half times for some California-type and London-type aerosol exposed rats. Based upon the Mann-Whitney nonparametric test, there was a significant increase in clearance rate (P=0.0056) in ozone exposed rats compared to other rats. Likewise, there was a significant difference (P=0.017) between the aerosol (without ozone) exposed rats and those that were exposed to ozone (with or without aerosol) in that ozone speeded tracheobronchial clearance (shortened the half-time; P=0.0056), while the aerosol exposure (combining California-type and London-type exposures) tended to slowed clearance (P=0.096).

Summary

The overall results are summarized in Tables 25 and 26. Table 25 provides summary information for the main findings in the 3-day studies. Table 26 provides summary information for the main findings in the 30-day study.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. In the 3-day studies, there was a significant increase in lung DNA and protein in rats exposed to California-type aerosol (1.45 mg/m^3); exposure to London-type aerosol (5 mg/m^3), sulfur dioxide (21 ppm), and London-type aerosol in combination with sulfur dioxide yielded no significant effects. In addition, there was a significant increase in lung inflammatory cells in rats exposed to California-type aerosol, but this was not observed for London-type aerosol. Impaired rats breathing only clean air or exposed to various pollutant atmospheres had a statistically significant increase in lung DNA, RNA and/or protein. Ozone (0.4 ppm) caused small airway inflammation in both healthy and impaired rats, and the combination of either California-type aerosol (as low as 0.55 mg/m^3) or London type aerosol (2.9 mg/m^3) significantly exacerbated this response. This augmentation of inflammation was not a quantifiable dose-response function of aerosol concentration, but occurred at the lowest concentration (0.55 mg/m^3) with the California-type aerosol.

In the 30-day subchronic study, there were a significant decrease in lung DNA and an increase in lung hydroxyproline in rats California-type aerosol (1 mg/m^3) as well as a tendency toward reduction in the rate of tracheobronchial clearance in both healthy and impaired rats. London-type aerosol (1.1 mg/m^3) with sulfur dioxide (1 ppm) had a tendency to decrease the rate of tracheobronchial clearance and caused some lung pigmentation, but elicited no other observed responses. Hence, California-type aerosol was more effective in yielding observable biochemical changes in these subchronic exposures. Ozone (0.4 ppm) caused lung small-airway lesions and fibrosis, and increases in rate of tracheobronchial clearance. Increased rate of tracheobronchial clearance also occurred when ozone and California-type aerosol were combined. Impaired rats had small airway lesions associated with emphysema and increased lung DNA and hydroxyproline, even those exposed to clean air. The combination of California-type aerosol and ozone yielded a significant exacerbation of pulmonary fibrosis and lung pigmentation (associated with particle deposits). The combination of California-type aerosol with or without ozone in impaired rats led to a synergistic exacerbation of lung small airway lesions, and enhanced particle accumulations in the lung. London-type aerosol (1.1 mg/m^3) with sulfur dioxide (1 ppm) significantly increased the lung content of hydroxyproline in impaired rats.

The acute and subchronic studies were similar in showing a lesser effectiveness for London-type aerosol than California-type aerosol in yielding significant changes in exposed rats as summarized in Tables 25 and 26. Both showed that the combination of California-type aerosol and ozone tends to exacerbate the lung injury caused by ozone. The subchronic study showed significant effects of ozone (increase) and both aerosols (decrease) on tracheobronchial clearance, while there were no significant changes in tracheobronchial clearance in the acute studies.

Table 25: Summary of significant ($p<0.05$) responses and interactions in rats to 3-Day exposures to California-type (CA) aerosol (0.55 to 3 mg/m^3), ozone (0.4 ppm), London-type (LT) aerosol (0.8 to 5 mg/m^3) and sulfur dioxide (up to 21 ppm). [ns = not significant response, if any]

EXPOSURES CL-1 THROUGH CL-3

TREATMENT/ EXPOSURE	SMALL AIRWAY INFLAMMATION	ALTERED TRACHEOBRONCHIAL CLEARANCE	ENHANCED LUNG PERMEABILITY	LUNG BIOCHEMISTRY
Impaired	ns	ns	ns	Increased DNA, RNA & protein
CA Aerosol	yes	ns	ns	Increased DNA & protein
Ozone	yes	ns	ns	Increased RNA & protein
SIGNIFICANT INTERACTIONS:				
Ozone x Impaired	----	----	----	Increased protein
CA Aerosol x Ozone x Impaired	yes	----	----	----
CA Aerosol x Ozone	yes	----	----	----

EXPOSURES CL-4 THROUGH CL-6

TREATMENT/ EXPOSURE	SMALL AIRWAY INFLAMMATION	ALTERED TRACHEOBRONCHIAL CLEARANCE	ENHANCED LUNG PERMEABILITY	LUNG BIOCHEMISTRY
Impaired	yes	ns	ns	Increased DNA & RNA
LT Aerosol or SO_2	ns	ns	ns	ns
SIGNIFICANT INTERACTIONS:				
LT Aerosol x Impaired	----	----	----	Increased DNA & RNA
SO_2 x Impaired	----	----	----	Decreased protein
LT Aerosol x SO_2 x Impaired	----	----	----	Increased DNA & RNA
LT Aerosol x Ozone	yes	----	----	Increased DNA & RNA
LT Aerosol X Ozone x Impaired	yes	----	----	----

Table 26: Summary of significant ($p<0.05$) responses and interactions in rats to 30-day subchronic exposure (CL-7) to California-type aerosol (CA) (1 mg/cubic-meter), ozone (0.4 ppm), and London-type aerosol (LT) (1.1 mg/cubic-meter) with sulfur dioxide (1 ppm). [ns = not significant response, if any]

TREATMENT/EXPOSURE	SMALL AIRWAY INFLAMMATION	SMALL AIRWAY LESIONS	LUNG BIOCHEMISTRY (NUCLEIC ACIDS)	INCREASED LUNG HYDROXYPROLINE	ENHANCED LUNG TISSUE PIGMENTATION	ALTERED TRACHEOBRONCHIAL CLEARANCE RATE	SMALL AIRWAY FIBROSIS
Impaired	yes	ns	Increased DNA	yes	ns	ns	ns
CA Aerosol	ns	ns	Decreased RNA	yes	yes	[decrease $p<0.1$]	ns
LT Aerosol + Sulfur Dioxide	ns	ns	ns	ns	yes	[decrease $p<0.1$]	ns
Ozone	ns	yes	ns	[yes, $p<0.1$]	yes	increased	yes
SIGNIFICANT INTERACTIONS:							
CA Aerosol x Impaired	----	yes	----	----	----	----	---
LT Aerosol x Impaired	----	----	----	yes	----	----	---
CA Aerosol x Ozone	----	----	----	----	yes	[increase $p<0.1$]	yes
Ozone x Impaired	[yes $p<0.1$]	yes	----	----	----	----	---
CA Aerosol x Ozone x Impaired	----	yes	----	----	yes	----	---

DISCUSSION

The main objectives of the project discussed with respect to the forgoing results are as follows:

(1) Evaluation of acute and subchronic responses of the lung to inhaled London-type aerosol (LT) or California-type aerosol (CA) alone and in combination with episodal levels of ozone (California) or sulfur dioxide (London).

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. An important observed difference in pulmonary response was that there were small but statistically significant changes in lung biochemical values in rats exposed to California-type aerosol in both the acute (increased lung DNA and protein) and subchronic studies (increased lung hydroxyproline and decreased RNA) that were not observed with the London-type aerosol. In other respects, the two aerosols tended to elicit similar responses. Both aerosols tended to decrease, to a similar extent, tracheobronchial clearance in the subchronic studies. Both aerosols exacerbated, to a similar extent, the small airway inflammation caused by ozone exposure as observed in the 3-day studies. London-type aerosol interacted with elastase pretreatment (in impaired rats) to increase lung DNA and RNA content in the acute studies. Sulfur dioxide exposure was without effect alone or in combination with either aerosol except for small statistically significant lung biochemical changes in combination with elastase pretreatment (in impaired rats) and/or London-type aerosol.

(2) Quantification of specific responses that relate to health effects in human populations, including epithelial damage (measured as lung permeability), lung clearance impairment, biochemical responses (indicative of potential fibrosis and/or inflammation), inflammatory cell accumulation in the lung, and cellular-level abnormalities in the lung.

There was evidence of lung injury in some of the acute exposure experiments. This was seen in data on small-airway inflammation and lung biochemical changes in DNA, RNA and lung protein. No treatment-associated differences were detected in tracheobronchial clearance or lung permeability. For the 30-day subchronic exposure studies the main responses included treatment-associated changes in particle accumulation, small-airway lesions, fibrosis, changes in tracheobronchial clearance, hydroxyproline synthesis and lung nucleic acid content. No changes in lung permeability were detected in the subchronic studies.

(3) Comparison of responses between London-type aerosol (LT) with and without sulfur dioxide with California-type aerosol (CA) with and without ozone for the purpose of relating the basis (London episodes) of current air pollution standards to appropriate standards in California.

Neither aerosol was effective by itself in causing significant responses in healthy rats, but some significant aerosol effects were observed in association with ozone exposure or lung impairment. The California-type aerosol yielded lung biochemical changes and exacerbation of effects associated with ozone or lung impairment. These responses were either not observed at all with the London-type aerosol, with or without SO_2, or occurred at much higher concentrations or only in impaired rats. The exacerbation of ozone inflammatory response was observed at 0.55 mg/m^3 for California-type aerosol but at 3 mg/m^3 for the London-type. The combination of California-type aerosol and ozone was much more effective in causing a variety of significant detrimental pulmonary alterations which did not exist or were much less severe than with the London-type aerosol and SO_2. Hence, there is a clear indication that the California-type aerosol should be considered potentially more hazardous than the London-type aerosol.

(4) Comparison of responses in impaired individuals (simulated emphysematous lung disease in elastase pretreated rats) to healthy individuals.

One of the most common interactions that was regularly significant in all parts of these studies was the exacerbation of injury in impaired animals by

exposure to particulate matter and gaseous pollutants. In the acute studies, lung biochemical changes were caused by lung impairment from elastase pre-treatment, and these changes were exacerbated by ozone and London-type aerosol exposures, together and separately. A similar exacerbation was observed with respect to small-airway inflammation. In the subchronic study, significant levels of small airway lesions were observed in impaired rats exposed to California-type aerosol, while no significant response was observed for unexposed impaired rats or for aerosol exposed healthy rats.

(5) Comparison of effects elicited by acute and subchronic exposures.

The overall responses associated with the 3-day and 30-day exposures are contrasted in Tables 25 and 26. An important response in the acute studies was acute small-airway inflammation in the lungs of rats exposed to California-type aerosol and ozone. Small-airway inflammation was not an important response in the subchronic study. On the other hand, the subchronic exposures led to changes in tracheobronchial clearance that were not detectable in the acute studies and increased lung hydroxyproline, quantifiable small airway lesions, observable pulmonary fibrosis, and apparent accumulations of particles associated with lung pigmentation. Both study conditions showed aerosol-ozone interactions with synergism or exacerbation of responses associated with this combination.

General Discussion

Results of the pathologic analysis of the 3-day exposure studies demonstrate that the well documented small-airway inflammation caused by ozone (Last, et al., 1986) does not occur with exposures to California-type aerosol or London-type aerosol alone, with or without SO_2, in concentrations within the potential range of environmental exposure. A fairly consistent result of lung impairment from elastase pretreatment was to increase the amount of inflammatory infiltrate in proximal alveolar ducts of ozone exposed animals compared to ozone-exposed healthy animals. A tendency for interaction of aerosol and ozone to increase the inflammatory infiltrate in proximal alveolar ducts occurred in all of the acute studies involving ozone exposures, and was

significant in two of the 3-day ozone exposures including impaired rats. There appeared to be little detectable difference in the ability of the London-type aerosol versus the California-type aerosol to promote and exacerbate adverse macrophage response from ozone exposures.

The consequences of persistent inflammation in the terminal airways could include destruction of airway walls, fibrosis and airway remodeling. Of these, fibrosis of small airway walls and structural remodeling of the proximal alveolar duct were documented in association with ozone exposure in this study. Compared with ozone exposures alone, there was a quantifiable and significant increase in the extent of airway remodeling as a consequence of the ozone x California-type aerosol interaction when influenced by impairment from elastase pretreatment. This augmented remodeling was associated with visibly detectable increases in the amount of pigment retention in ozone-exposed animals and with increases of stainable collagen in small-airway structures of these same rats. The extent of observed fibrosis was small. This study is the first to document that the interaction of particulate aerosols with ozone causes a quantifiable, structural change in the small airways of the lung. Since this change includes collagen deposition, it is most likely irreversible. The decreased compliance of small-airway walls that this implies would probably cause functional alterations in small-airway resistance and perhaps in alveolar clearance as well.

Given the vagaries of dose and effectiveness that other investigators have experienced in using the elastase model of emphysema (Busch, et al., 1984), we felt that it was important to document that the dose used in our experiments was effective in producing significant parenchymal alterations. We found that the intercept method was time consuming and not sufficiently accurate to detect the changes which occurred in our animals. These changes were apparent histologically (as demonstrated in the subjective light-microscopic analysis) and were quantifiable using the image analysis system. Contrary to the experience of others, death losses in our animals occurred at the time of dosing due to pulmonary hemorrhage. Post-instillation infections were not a problem in our experiments.

In the 30-day study, the augmented inflammation that was apparent in impaired rats as a consequence of added exposure to ozone can be explained by several alternate mechanisms. The elastase treatment itself is not without some inflammatory response and, while this response was gone by the time of the exposures, it is possible that this prior inflammatory process had somehow stimulated pulmonary inflammation to create a greater response to a second stimulus, in this case, ozone exposure. It is also possible that elastase treatment somehow alters macrophage function causing diminished phagocytosis or lysosomal breakdown of phagocytized material. Other possible explanations relate to the architectural alterations induced in the parenchyma. Dilatation in the alveolar duct and loss of elastic recoil should theoretically slow parenchymal clearance. If parenchymal clearance were diminished, the increased numbers of inflammatory cells could then represent decreased migration out of the inflammatory site rather than increased influx. It has been reported that, in hamsters, elastase treatment causes goblet cell metaplasia in large airways (Snider, et al., 1984). While this response could also alter mucociliary clearance, this change was not evident in the elastase treated rats in this study. Another effect of decreased alveolar clearance due to elastase treatment would be to allow the accumulation of secretory products, inflammatory mediators or cell debris that could serve as chemotactants. The consequence of elastase pre-treatment was to increase retention of the particulate in the 30-day study. This change was visually apparent in the histologic sections in that the elastase treated ozone-exposed animals had dark accumulations of pigment in macrophages in the terminal bronchiole-alveolar duct regions while much lighter accumulations were seen in the ozone + aerosol exposed animals not treated with elastase. Again it is uncertain whether this represents a clearance failure or increased deposition of aerosol. Unfortunately, the evidence of increased aerosol retention is limited to a subjective observation and this area deserves further study.

Other model systems have demonstrated synergistic effects of particulate aerosols to augment ozone-induced inflammation (Last, et al., 1986). In these studies, a single-component aerosol of ammonium sulfate stimulated the inflammatory response to ozone by an as yet undetermined mechanism. It was proposed that the acidity of the aerosol and local alterations in pH induced by

the ammonium sulfate were important in this process. Our aerosols were also moderately acidic and had acidic ammonium bisulfate. It would seem that the effect of aerosol pH on inflammatory response to ozone exposure deserves further investigation. This study is apparently the first to demonstrate that these synergistic effects occur with aerosols and atmospheres of composition and concentration similar to those that occur in the environment.

The major consequence of persistent injury and inflammation in the 30-day study primarily as a result of ozone exposures was the formation of structures resembling respiratory bronchioles in a species (rat) in which they are generally considered to be very attenuated or absent. This change has recently been documented in rats exposed to 0.96 ppm ozone for 60 days (Barr, 1988). It apparently represents structural remodeling of the proximal alveolar duct as a consequence of persistent injury. It appears to have an interstitial component of fibrosis and an epithelial component of hyperplasia and metaplasia of the normal type I epithelium of the alveolar duct to a cuboidal epithelium resembling that in the terminal bronchiole. The relevance of this change to species with more complex small airways such as man is uncertain except that this change seems likely to result in a significant loss of small airway compliance and a similar interstitial process in man would be expected to similarly affect small airway flexibility and reduce lumen size. The other important conclusion regarding this change is that, unlike the inflammatory infiltration, these changes are likely to be irreversible consequences of pollutant exposure. This study documents that this change can occur earlier, as seen in the 3-day studies, and at a lower exposure concentration than previously described and further illustrates that the interaction both of aerosol, and lung impairment by elastase pre-treatment combined with aerosol exposure, exacerbates this ozone induced lesion.

The observed differences in tracheobronchial clearance associated with the 30-day CL-7 exposures indicates the potential importance of the inhalation of pollutant airborne particulate matter in adversely altering tracheobronchial clearance. This slowed clearance may be the forerunner of bronchitis and congestive lung impairment. The increased tracheobronchial clearance associated with ozone exposures may counteract the aerosol effect, but the

pathological results show that aerosol and ozone together tend to reduce the deep lung alveolar clearance of inhaled particles, and may also tend to cause certain forms of congestive lung impairment. This should be more extensively investigated in future studies.

Future studies should consider further the altered tracheobronchial clearance and the implications of the inhalation of various toxic or infectious agents in combination with particulate air pollutants. In addition, pulmonary clearance (half-time about 80 days in rats) was not studied in this project, and may be significantly altered by inhaled particulate materials.

When sulfur dioxide is inhaled by man, a major portion reaches the lung because of the common practice by people of inhaling through the mouth. Less than 1% reaches the lung for inhalation via the nose (Speizer and Frank, 1966). Rats and other small laboratory animals are compulsive nose breathers with complex nasal passages. In rodents more than 95% of inhaled sulfur dioxide is absorbed in the nasal passages and never reaches the lungs (Strandberg, 1964; Dalhamm & Strandberg, 1964). In addition, levels of 400 ppm are required for six weeks to demonstrate experimental bronchitis in rats (Reid, 1963). On the other hand, the rat has been shown to be a satisfactory model for deposition of respirable particles (Raabe et al., 1977).

These results indicate that only a very few percent of inhaled SO_2 reaches the lungs of rats. This fact may explain the minimal response in the biochemical, clearance, and pathological tests performed in this study. The mild response of rats to SO_2 at levels up to 21 ppm in the London smog acute exposures indicates that rats are not a good model for one-to-one modeling of the exposure of people to sulfur dioxide. If no more than 5% of sulfur dioxide is expected to pass the nose and enter the lung, there would, therefore, have been an effective concentration of 1 ppm when it reached the lung. Oral breathing people may respond to 1 ppm. In addition, some people may be particularly sensitive to SO_2 gas, and some asthmatics have violent respiratory bronchiolar constriction upon exposure to even low concentrations of sulfur dioxide (Incaudo and Gershwin, 1986). Rats do not similarly respond. Future work should take into consideration these phenomena.

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APPENDIX A

Exposure Chamber Gas Analysis and Aerosol Data

Table A-1: TEST OF AEROSOL NEBULIZER USED IN CHAMBER ATMOSPHERE

Babington Type Nebulizers (Solosphere and Hydrosphere)

Solosphere output characteristics with inlet closed
Flow rate in liters per minute (LPM) corresponding to a gage pressure in pounds per square inch (PSIG). Using this data 20 PSIG was chosen for exposures.

Pressure (PSIG)	10	16	20	26	30
Flow (LPM)	4.23	6.17	7.14	8.9	9.8

At pressures above 30 psi nebulizer sputters releasing large droplets

Droplets	Mass Median Aerodynamic Diameter (MMAD) = 9.03 um
	Geometric Standard Deviation (GSD) = 2.207
Dry Particles	Mass Median Aerodynamic Diameter (MMAD) = 1.05 um
	Geometric Standard Deviation (GSD) = 2.119
Volume Output	Inlet closed using pressure of 20 psi = 0.507 ml/min
	Inlet open using pressure of 20 psi = 1.554 ml/min

		NEBULIZER SOLUTION		
Chemical	Molecular Weight	Nominal Percent	Calculated Percent	Actual Percent
NH_4HSO_4	115.11	50	53.4	53.1
NH_4NO_3	80.5	45	46.4	46.7
$VOSO_4*2H_2O$	162/199	1	.02	.02
$NiSO_4*6H_2O$	154/262	1	.02	.02
$PbSO_4$	303.2	1	.06	.06
$MnSO_4$	150.9	1	.06	.06

CHAMBER CONCENTRATION CALCULATION

Calculation is for total aerosol chamber concentration of 1.6 milligrams per cubic meter. Amount of total chemical in nebulizer solution (c) = 3.3 milligrams per liter (mg/l). Flow through chamber (Q) = 2.1 Cubic meters per minute (m^3/min); 50% of aerosol was from nebulizer 2.1 m^3/min X 0.8 mg/m^3 = 1.68 mg/min / 0.507 ml/min = 3.314 mg/ml.

EFFICIENCY OF GENERATED MATERIAL IN CHAMBER

Efficiency is calculated by comparing the concentration, based on the material generated versus the amount of material collected on filters from the chamber during the actual exposures. Exposures listed below are for exposures number 1 through 7. These are designated CL-1 to CL-7.

California type aerosol		London type aerosol	
Exp #	Efficiency	Exp#	Efficiency
CL-1	85%	CL-4	71%
CL-2	57%	CL-5	71%
Cl-3	76%	CL-6	64%
Cl-7	48%	Cl-7	59%

Table A-2: Exposure Solution Analysis

(Analysis was performed using the 1.65 mg salts/ml solution)

micrograms of salt per milliter of water = ug/ml

Chemical Form	CL-1	CL-2
NO_3	781 ug/ml	744 ug/ml
SO_4	887	857
Mn	0.97	0.92
Pb	1.04	0.97
Ni	0.38	0.31
V	0.31	--

Nebulizer Solution Analysis

Starting versus ending concentration of nebulizer solutions.

Analysis was performed using the nebulizer solution from the second exposure.

Chemical Form	Starting Stock	Exposure Reservoir	Ratio %
NO_3	744 ug/ml	1230 ug/ml	165
SO_4	857	1540	180
Mn	0.92	2.42	263
Pb	0.97	1.61	166

Table A-3: Chemical Analysis

(Analysis of original 3.3 milligram per milliter (mg/ml) stock solution.)

Chemical	Calculated (ug/ml)	Found (ug/ml)	% Recovery
SO_4	1774	1974	111.3
NO_3	1542	1571	101.9
Mn	1.93	1.90	98.4
Pb	2.07	2.03	98.1
Ni	0.75	0.75	100.0

ANALYSIS OF CL-1 EXPOSURE SOLUTION 1.65 mg/ml (pH = 2.11)
(Reservoir #13 and #14 refer to nebulizer solutions used on those chambers)

Chemical	Calculated (ug/ml)	Reservoir #13 (ug/ml)	Ratio %	Reservoir #14 (ug/ml)	Ratio %
SO_4	887	1162	131	1048	118
NO_3	781	949	122	928	118
Mn	0.97	1.10	113	1.05	108
Pb	1.04	1.50	144	1.34	129
Ni	0.38	0.94	247	0.49	129

MASS BALANCE OF FILTER SAMPLES
(Analysis of chemical form are from filter samples taken from chamber during exposure number one and dissolved in water to extract the salts and analyzed)

FILTER	NO_3/NH_4NO_3	$SO_4/(NH_4)_2SO_4$	TOTAL	FILTER WT	SAMPLE %
36	2.82/3.77 mg	2.62/3.34 mg	7.10 mg	16.1 mg	44
36A	2.19/2.92	2.47/3.13	6.06	12.8	47
41	2.63/3.51	2.05/2.61	6.13	12.5	49
37	2.94/3.92	2.19/2.79	6.70	15.9	42
37A	1.42/1.89	2.53/3.21	5.11	13.9	37
43	1.58/2.11	2.41/3.06	5.17	12.4	42
38	1.96/2.61	1.94/2.47	5.08	11.4	45
38A	2.71/3.62	1.79/2.28	5.89	13.1	45
40	2.31/3.08	2.29/2.91	5.99	12.3	49
39	1.86/2.48	2.43/3.09	5.57	14.8	38
39A	3.01/3.31	1.87/2.37	5.67	12.1	47

FILTER	NO_3/NH_4NO_3	$SO_4/(NH_4)_2SO_4$	TOTAL (mg)	FILTER WT	SAMPLE %
S1	not found mg	0.07/0.09 mg	0.09 mg	0.13 mg	68
S2	0.17/0.22	0.15/0.19	0.41	0.52	79
S3	0.55/0.74	1.22/1.55	2.29	5.83	39
S4	0.89/1.19	2.45/3.12	4.31	10.15	42
S5	0.89/1.19	1.58/2.01	3.20	8.94	36
S6	0.62/0.83	0.83/1.06	1.89	4.86	39
S7	2.63/3.51	0.82/1.04	4.55	8.02	57

Table A-4: Mass Balance of Filter Samples for CL-2 in mg

Separate Nebulization Test
(Analysis was from filters sampling from the chamber for this test only)

Filter#	NO_3/NH_4NO_3	$SO_4/(NH_4)2SO_4$	Total Wt.	% salts
2	1.63 mg	2.06 mg	3.35 mg	79.0
3	0.63	3.32	2.09	68.1
4	1.48	2.48	3.67	79.3
5	0.60	3.87	2.32	71.0
7	0.71	3.07	2.18	72.8
Sierra Impactor Stages				
1	0.11 mg	1.82 mg	0.20 mg	73.7
2	0.13	2.38	0.31	31.1
3	1.36	1.52	2.07	35.6
4	4.82	1.50	7.25	50.9
5	4.58	1.44	6.60	57.5
6	3.29	1.30	4.26	63.6
7	5.29	0.89	4.70	58.5
Pooled Filter Samples				
13A	7.62	1.54	11.80	51.1
13B	5.32	1.64	8.75	42.1
14A	5.55	1.68	9.32	50.2
14B	6.34	1.80	11.44	50.2

Table A-5: Aerosol Source Materials

California Type Aerosol

CLAY = 60 percent by weight of California Type Aerosol

Vendor	Material
Southern Clay Products P.O. Box 44 Gonzales, Texas 78629 (512) 672-2891	Mineral Colloid sample# G85082703 Na-Montmorillonite - 40215 98% < 240 mesh cat # 100=120

CARBON = 40% of dust by weight of California Type Aerosol

Asbury Graphite Mills, Inc. Asbury, Warren County, N.J. 08802-0144	M-150 Med. grind Graphite

Example of calculation of amount needed
3 day exposure - run 23 hrs/day = t (minutes)
2 chambers - Q (flow rate) = 2.1 m^3/min for one chamber

Chamber concentration wanted = 0.4 mg/m^3 Qt x efficiency of Wright Dust Feed (WDF) / amount of material.
The WDF aerosol is 50% by weight of total concentration in chamber.

Assuming 13 % WDF efficiency.
amt = flow rate (2.1 m^3/min) X time (4140 min) X 2 chambers X concentration

	calculated			actual collection		
exp #	conc. (mg/m^3)	amt (mg)	total wt(g)	conc(mg/m^3)	amt(mg)	total (g)
1	0.2	3478	26.7	0.24	4173	32.1
2	0.4	6955	53.2	0.48	8346	54.2
3	0.8	13901	107.0	0.96	16692	128.4

	40%	60%
total used = 214.7	graphite=85.8g	clay=128.8g

30 day exposure used a concentration of 0.5 mg/m^3 calculated use at 13 % efficiency = 802 grams.

Actual use for the total exposure was 478 grams.

LONDON TYPE AEROSOL

Lamp Black; 50% of dust was lamp black material.

Vendor	Material
Cabot Corp.	Monarch 800-cs-9719
125 Hight St.	S/D G-5266
Boston, Mass	

Coal flyash; 50% of dust was coal flyash.

On hand at LEHR	Western fly ash
	size classified <5uM
	Collected from power plant
	described Raabe, et al.,
	1979.

London Type: 60% of aerosol is from WDF.

Assuming the same efficiency of generation by the Wright Dust Feed of 13 %:

Calculated amt of material for Cl- 4 of about 1.0 mg/m^3:
lamp black=33.2 g flyash=33.2 g total = 66.5 g

Actual amount used was 33.6 g with the efficiency of generation = 24% for the 30 day subchronic experiment 665 g was calculated and actual use was 335 g.

Separate studies were conducted of the efficiency of aerosolization of both the nebulized salt aerosol and the dispersed dry dust. These results are summarized in Table A-6. These efficiencies were used to plan the various exposures, each requiring a different average concentration of both materials but in the same proportion in all cases.

Table A-6:Generation Efficiency

Column headings for table A-6.

Exp # = Exposure number from CL-1 (first exposure) to last (CL-7).
Ch # = Chamber samples were taken from (chambers 13 through 15 were used).
mat used = Material used, either dust for Wright Dust Feed or solution.
time = Time elapsed in minutes.
rate = The usage rate in milligram per minute as average for the exposure.
Q sys = Flow rate through the chamber for the time of exposure.
calc conc = Concentration calculated using the actual amount of material used.
act conc = Concentration calculated by using material collected on filters.
eff = Efficiency of generation calculated by dividing calc conc by act conc.

Exp #	Ch#	mat used	time	rate	Q sys	calc conc	act conc	eff
dust		(g)	(min)	(mg/min)	(m^3/min)	(mg/m^3)	(mg/m^3)	%
CL-1	13	17.7	4140	4.28	2.1	2.04	0.27	13
	14	18.2		4.40		2.09	0.29	14
nebulizer.		(1) (g/1)						
CL-1	13	1.86x1.65=3.07		0.74	2.1	0.35	0.27	77
	14	1.65x1.65=2.72		0.66		0.31	0.29	93
aerosol = dust + nebulizer								
CL-1	13			5.02	2.1	2.39	0.53	22
	14			5.06		2.41	0.58	24
dust								
CL-2	13	32	4140	7.73	2.1	3.86	0.71	18
	14	46		11.11		5.29	0.74	14
nebulizer		(1) (g/1)						
CL-2	all	13.4x1.65=22.11		5.34		2.54	1.45	57
aerosol								
CL-2	13			10.40	2.1	4.95	1.42	29
	14			13.78		6.56	1.47	22
dust								
Cl-3	13	110.6	4140	26.7	2.1	12.74	1.5	12
	14	97.0		23.4		11.16	1.48	13
nebulizer.								
CL-3	all	20.0x1.65=33.0		7.97	2.1	3.80	2.90	76
aerosol								
CL-3	13		4140	30.2	2.1	14.38	2.99	21
	14			27.42		13.05	2.96	23
dust								
CL-4	13&14	33.64	4140	8.13	2.1	3.86	0.91	24
nebulizer								
CL-4	all	6.8x1.65=11.2		2.71	2.1	1.29	0.92	71
aerosol								
CL-4	13			5.85	2.1	2.79	0.83	30
	14					0.99		35

Exp #	Ch#	mat used	time	rate	Q sys	calc conc	act conc	eff
dust								
CL-5	13&14	110.2	4140	26.62	2.1	12.68	4.85	38
nebulizer.								
CL-5	all	14.1x3.33=46.95		11.34	2.1	5.4	4.85	90
aerosol								
CL-5	13		4140	18.98	2.1	9.04	4.68	52
	14						5.02	56
dust								
CL-6	13&14	97.4	4140	23.54	2.1	11.21	2.93	26
nebulizer								
CL-6	all	12.0x3.33=39.96		9.65	2.1	4.6	2.93	64
aerosol								
CL-6	13			16.65	2.1	7.93	3.21	40
	14					2.64		33
dust								
CL-7	13&14	478	41400	11.56	2.1	5.50	1.03	19
	15	335	41400	8.10	2.1	3.86	0.55	14
nebulizer								
CL-7	13 & 14	55.8x3.33=185		4.49	2.1	2.14	1.03	48
	15	24.2x3.33=80.4		1.94	2.1	0.93	0.55	59
aerosol								
CL-7	13			8.03	2.1	3.82	1.05	27
	14			8.03			1.02	27
	15			10.04		4.78	1.10	23

Table A-7: Data Summary for Exposure CL-1: April 4-6, 1986

Column headings for tables A-7 through A13.
fil # = Filter number, assigned in sequence for record keeping.
holder = Filter holder used for taking samples from chamber.
chamber = Exposure chamber number associated with the sample and aerosol.
collected = Amount of material that was measured on the filter by weight.
conc. = Concentration calculated in the chamber for given period.
Vol = Volume of air sampled by the filter for given period.
dT = Elapsed time for a given sample period.

Exposure summary: 0900 April 4 to 0800 April 7 for 23hrs/day = 4,140 min.

material	device	used	Chamber #	conc. averages
clay + graphite	WDF-13	17.8 g	13	0.53 +/- 0.05 mg/m^3
	WDF-14	18.2	14	0.58 +/- 0.06
1.65 mg/L sol.	Neb-13	1.86	13	0.45
	Neb-14	1.65	14	0.40

Mass Median Aerodynamic Diameter (MMAD) = 1.479 um GSD = 2.21
pH of stock sol 3.3 mg/ml = 1.87, 1.65 mg/ml = 2.11, after exp(#14) = 2.10

filter samples filter type Versapore 800 47mm

	filter #	filter	chamber	collected	conc(mg/m^3)	Vol(m^3)	dT(min)
4th	36	4	14	16.14	0.65	24.8	1380
	37	12	14	15.9	0.64	24.8	1380
	38	7	13	11.4	0.46	24.8	1380
	39	8	13	14.8	0.59	24.8	1380
5th	36a	4	14	13.9	0.57	24.3	1350
	37a	12	14	14.9	0.61	24.3	1350
	38a	7	13	12.8	0.52	24.3	1350
	39a	8	13	13.1	0.57	25.3	1350
6th	40	7	13	12.34	0.52	23.8	1320
	41	4	14	12.5	0.53	23.8	1320
	42	8	13	12.0	0.51	23.8	1320
	43	12	14	12.3	0.52	23.8	1320

Sierra impactor: stage#	1	2	3	4	5	6	7
mg collected	0.13	0.52	5.83	10.15	8.94	4.86	8.02

pH measurements: Measurements are taken on five filters put together and allowed to sit in double distilled water for several hours. Double distilled water has a pH of 6.4 to 7.0

Versapore blank (lot # 66401)= 4.272
Nuclepore (lot # 83D7A96) = 7.053
Gelman type A/E (lot #8292) = 8.377

pH from a series of filters taken from chamber (designated 36-43 and SI# 1-7)

#36 = 6.994
#37 = 6.952
#37a = 6.971
#38 = 6.826
#38a = 6.346
#39 = 6.856
#39a = 6.161
#40 = 6.592
#41 = 6.994
#42 = 6.585
#43 = 6.855
SI# 3 = 7.104
SI# 4 = 7.095
SI# 5 = 7.319
SI# 6 = 7.413
SI# 7 = 7.578

Table A-8: Data Summary for Exposure CL-2: June 3, 1986

Exposure summary: 0900 May 30 to 0800 June 2 for 23hrs/day = 4,140 min

material	device	amt	ch#	conc. averages
clay + graphite	WDF-13	32g	13	1.42 +/- 0.10 mg/m^3
	WDF-14	46g	14	1.47 +/- 0.15 mg/m^3
1.65 mg/L sol.		13.4 L used		3.1ml/min
MMAD = 1.44		GSD= 2.22		

filter samples filter type Versapore 800 47mm

	filter No.	holder	chamber	collected	conc (mg/m^3)	Vol(m^3)	dT(min)
30th	1	4	13	5.84	1.35	4.32	240
	2	20	13	5.69	1.42	4.32	240
	3	8	14	5.84	1.35	5.69	240
	4	12	14	6.14	1.42	4.32	240
	5	4	13	4.21	1.29	3.24	180
	6	8	14	4.63	1.43	3.24	180
31st	7	4	13	6.32	1.41	4.46	875
	8	20	13	6.22	1.39	4.46	865
	9	8	14	4.75	1.26	3.76	865
	10	12	14	5.16	1.36	3.79	865
	11	4	13	6.38	1.48	4.28	830
	12	20	13	7.04	1.64	4.28	830
	13	8	14	5.66	1.57	3.61	830
	14	12	14	5.34	1.47	3.64	830
1st	15	4	13	4.12	1.44	2.86	555
	16	20	13	4.10	1.44	2.86	555
	17	8	14	3.78	1.58	2.40	551
	18	12	14	3.98	1.65	2.41	551
	19	4	13	9.64	1.43	6.75	1422
	20	20	13	10.36	1.47	7.05	1500
	21	8	14	8.59	1.39	6.19	1422
	22	12	14	9.03	1.37	6.57	1500
2nd	23	4	13	1.52	1.27	1.19	78
	24	8	14	2.13	1.78	1.19	78

Sierra impactor stage #	1	2	3	4	5	6	7
mg collected	0.42	1.41	9.64	23.71	19.45	11.84	17

Total weight collected from chamber #13 = 2.11mg *
Total weitht collected from chamber #14 =1.73mg

*dust= carbon + clay from Wright dust feed

Table A-9: Data Summary for Exposure CL-3: JULY 15, 1986

Exposure summary: 0900 July 11 to 0800 July 14 for 23hrs/day = 4,140 min
conc. totals
chamber 13 aerosol = 2.99 mg/m^3 MMAD = 1.17 GSD = 1.72
chamber 14 aerosol = 2.96 mg/m^3
Ozone = 0.409 ppm SD=0.026
chamber 15 Ozone = 0.422 ppm SD = 0.01

filter samples ------collected on --Versapore 800, 47 mm

filter	No.	time	dT	holder	chamber	collected	conc (mg/m^3)
11th	1	9.15	13.17	4	13	9.78	2.39
	2	9.15	13.17	12	14	9.35	2.29
	3	9.15	21.0	7	13	16.8	2.59
	4	9.15	21.0	20	14	15.6	2.42
	5	22.25	7.83	4	13	7.13	2.95
	6	22.25	7.83	12	14	6.29	2.59
	7	7.47	6.62	7	13	6.24	3.04
	8	7.47	6.62	20	14	6.65	3.25
	9	14.24	9.52	4	13	4.81	2.31
	10	14.24	9.52	12	14	7.41	3.56
12th	11	23.55	6.72	7	13	5.83	2.80
	12	23.55	6.72	20	14	2.03	0.98
	13	7.00	6.60	7	13	4.32	2.12
	14	7.00	6.60	20	14	4.25	2.08
	15	13.36	7.57	4	13	4.84	2.08
	16	13.36	7.54	12	14	5.09	2.18
	17	21.10	2.75	7	13	3.87	4.65
	18	21.10	2.75	20	14	3.61	4.25
13th	19	1.00	7.0	4	13	6.77	3.12
	20	1.00	7.0	12	14	8.00	3.70

Sierra impactor stage#	1	2	3	4	5	6	7
mg collected	0.14	1.05	5.48	16.14	17.79	22.22	10.59

Table A-10: Data Summary for Exoposure CL-4: August 12, 1986

Exposure summary: 0900 Aug. 8 to 0800 Aug. 11 for 23hrs/day = 4,140 min.

Aerosol concentration totals

chamber 13 aerosol = 0.83 mg/m3 SD=0.11 MMAD = 0.795

chamber 14 aerosol = 0.99 mg/m3 SExposure GSD = 1.838

SO_2 = 1.0 ppm

chamber 15 SO_2 = 1.0 ppm

filter samples ------collected on --Versapore 800,47mm

filter No.	time	dT	holder	chamber	collected	conc(mg/M3)
9th 1	9.15	7.47	4	13	3.26	0.74
2	9.15	21.25	7	13	9.08	0.68
3	9.15	7.47	8	14	4.61	1.05
4	9.15	21.25	12	14	11.13	0.87
5	16.43	20.75	4	13	9.8	0.80
6	16.43	20.75	8	14	11.9	0.92
10th 7	6.30	24.33	7	13	12.59	0.88
8	6.30	24.33	2	14	12.57	0.816
9	13.28	42.62	4	13	23.83	0.95
10	13.28	42.62	8	14	28.55	1.07
11th 11	6.40	25.42	7	13	14.27	0.95
12	6.40	25.42	12	14	17.68	1.16

Sierra impactor stage#	1	2	3	4	5	6	7
mg collected	0.11	0.15	0.59	2.06	5.15	10.21	11.27

Table A-11: Data Summary for Exposure CL-5: September 16, 1986

Exposure summary: 0900 Sep. 12 to 0800 Sep. 15 for 23hrs/day = 4,140 min.

Aerosol concentration totals
chamber 13 aerosol = 4.68 mg/m^3 SD=0.79 MMAD = 0.894
chamber 14 aerosol = 5.02 mg/m^3 SD=0.78 GSD = 1.997
SO_2 =~ 4.0 ppm
chamber 15 SO_2 =~ 4.0 ppm

filter samples ------collected on --Versapore 800,47mm

filter	No.	Vol(m^3)	dT	holder	chamber	collected	conc(mg/m^3)
12th	1	2.47	480	4	13	13.30	5.39
	2	5.82	1126	7	13	23.21	3.98
	3	2.47	480	8	14	14.50	5.88
	4	5.82	1126	12	14	32.13	5.52
	5	6.36	1234	4	13	23.04	3.61
	6	6.36	1234	8	14	32.06	5.04
13th	7	5.57	1081	7	13	24.65	4.41
	8	5.57	1081	12	14	31.82	5.71
	9	5.10	990	4	13	22.05	4.31
	10	5.10	990	8	14	27.65	5.42
	11	6.13	1189	7	13	25.94	4.22
	12	6.13	1189	12	14	24.88	4.06
14th	13	4.64	900	4	13	22.15	4.77
	14	4.64	900	8	14	16.30	3.52
	15	4.44	862	7	13	25.58	5.76
	16	4.44	862	12	14	23.11	5.20
	17	3.09	600	4	13	17.77	5.74
	18	3.09	600	8	14	14.95	4.83

Sierra impactor stage#	1	2	3	4	5	6	7
mg collected	0.24	0.24	0.65	2.68	5.61	7.00	8.36

Table A-12: Data Summary for Exposure CL-6: October 14, 1986

Exposure summary: 0900 Oct. 10 to 0800 Oct. 13 for 23 hrs/day = 4,140 min.

Conc. totals

Chamber 13 aerosol = 3.21 mg/m^3 SD=1.26 MMAD = 0.932 um

Chamber 14 aerosol = 2.64 mg/m^3 SD=0.86 GSD = 1.873

O3 = 0.4 ppm

Chamber 15 O3 =~ 0.4 ppm

filter samples ------collected on --Versapore 800,47mm

filter	No.	Vol(m3)	dT	holder	chamber	collected	conc(mg/m^3)
12th	1	2.06	420	4	13	12.13	5.89
	2	3.86	805	7	13	19.15	4.96
	3	2.26	420	8	14	3.43	1.51
	4	4.35	805	12	14	9.70	2.23
	5	4.77	975	4	13	16.87	3.53
	6	5.26	975	8	14	16.84	3.26
13th	7	6.15	1139	7	13	17.07	2.77
	8	6.15	1139	12	14	16.56	2.70
	9	6.99	1428	4	13	23.75	3.39
	10	6.99	1428	8	14	22.79	3.25
	11	8.19	1546	7	13	21.36	2.61
	12	7.58	1546	12	14	20.70	2.73
14th	13	6.45	1317	4	13	15.76	2.44
	14	6.38	1317	8	14	13.81	2.16
	15	3.18	650	7	13	6.72	2.13
	16	3.18	650	12	14	7.20	2.26

Sierra impactor stage #	1	2	3	4	5	6	7
mg collected	0.0	0.30	0.47	4.46	9.94	9.49	11.12

Table A-13: Data Summary for Exposure CL7: November 15- December 14, 1986

Filter collection from 9:00 Nov 15 to 08:00 Dec 14, 1986
collected 23 hours per day = 41400 min.

Chamber #13 aerosol (CA) = 1.05 mg/m^3 SD = 0.58 mg/m^3
Chamber #14 aerosol (CA) = 1.02 mg/m^3 SD = 0.48 mg/m^3 0.37 ppm O_3
Chamber #15 aerosol (LT) = 1.10 mg/m^3 SD = 0.36 mg/m^3 with 0.96 ppm SO_2
Chamber #16 ozone = 0.38 ppm

Date	fil #	Holder	Ch#	Vol (m^3)	dT (min)	collected	conc.(mg/m^3)
11-15	1	4	13	2.8	572	1.61	0.57
	2	12	14	2.9	572	2.54	0.87
	3	7	15	2.9	572	3.48	1.19
	4	4	13	3.7	768	2.42	0.65
	5	12	14	3.9	768	2.54	0.65
	6	7	15	3.9	768	6.82	1.74
11-16	7	4	13	7.4	1517	4.76	0.64
	8	12	14	7.8	1517	5.35	0.68
	9	7	15	7.7	1517	14.93	1.93
11-17	10	4	13	6.7	1369	8.49	1.26
	11	12	14	6.9	1369	5.53	0.80
	12	7	15	6.9	1369	11.40	1.65
11-18	13	4	13	1.3	275	2.62	2.01
	14	12	14	1.4	275	1.91	1.36
	15	7	15	1.4	275	2.33	1.66
	16	4	13	5.5	1140	7.61	1.38
	17	12	14	5.8	1140	4.39	0.75
	18	7	15	5.8	1140	7.54	1.30
11-19	19	4	13	7.7	1580	13.96	1.81
	20	12	14	8.0	1580	1.90	0.23
	21	7	15	8.0	1580	11.85	1.48
11-20	22	4	13	6.2	1278	3.41	0.55
	23	12	14	6.5	1278	4.03	0.62
	24	7	15	6.5	1278	5.55	0.85
11-21	25	4	13	7.6	1562	3.72	0.48
	26	12	14	7.9	1562	4.40	0.55
	27	7	15	7.9	1562	5.76	0.72
11-22	28	4	13	7.6	1560	7.06	0.92
	29	12	14	7.9	1560	3.81	0.48
	30	7	15	7.9	1560	11.10	1.40
11-23	31	4	13	6.6	1360	1.65	0.25
	32	12	14	6.8	1360	1.02	0.15
	33	7	15	6.8	1360	6.27	0.92
11-24	34	4	13	8.6	1775	9.99	1.16
	35	12	14	8.8	1775	10.74	1.22
	36	7	15	8.8	1775	15.36	1.74
11-26	37	4	13	7.2	1475	4.30	0.59
	38	12	14	7.3	1475	6.52	0.89
	39	7	15	7.3	1475	9.04	1.23

Date	fil #	Holder	Ch#	Vol (m^3)	dT (min)	collected	conc.(mg/m^3)
11-27	40	4	13	7.0	1430	5.16	0.73
	41	12	14	7.1	1430	6.50	0.91
	42	7	15	7.1	1430	6.64	0.93
11-28	43	4	13	14.3	2920	10.47	0.73
	44	12	14	14.6	2920	12.20	0.83
	45	7	15	14.6	2920	13.67	0.93
11-30	46	4	13	6.7	1375	6.04	0.90
	47	12	14	6.8	1375	12.46	1.83
	48	7	15	6.8	1375	2.16	0.31
12-1	49	4	13	6.7	1370	1.42	0.21
	50	12	14	6.8	1370	3.74	0.55
	51	7	15	608	1370	5.47	0.80
12-2	52	4	13	6.6	1365	3.93	0.59
	53	12	14	6.8	1365	6.53	0.96
	54	7	15	6.8	1365	7.13	1.04
12-3	55	4	13	6.7	1385	8.97	1.33
	56	12	14	6.9	1385	10.21	1.47
	57	7	15	6.9	1385	6.33	0.91
12-4	58	4	13	7.0	1430	7.97	1.13
	59	12	14	7.1	1430	10.00	1.40
	60	7	15	7.1	1430	7.21	1.01
12-5	61	4	13	6.8	1395	7.53	1.10
	62	12	14	6.9	1395	11.59	1.67
	63	7	15	6.9	1395	6.72	0.97
12-6	64	4	13	6.9	1410	6.44	0.93
	65	12	14	7.0	1410	12.74	1.82
	66	7	15	7.0	1410	6.21	0.88
12-7	67	4	13	6.7	1380	3.37	0.50
	68	12	14	6.9	1380	15.11	2.18
	69	7	15	6.9	1380	5.47	0.79
12-8	70	4	13	6.8	1400	12.05	1.77
	71	12	14	7.0	1400	5.99	0.85
	72	7	15	7.0	1400	6.11	0.87
12-9	73	4	13	6.9	1410	11.19	1.62
	74	12	14	7.0	1410	7.87	1.12
	75	7	15	7.0	1410	5.78	0.82
12-10	76	4	13	6.7	1380	8.51	1.27
	77	12	14	6.9	1380	6.28	0.91
	78	7	15	6.9	1380	8.71	1.26
12-11	79	4	13	6.9	1420	8.90	1.28
	80	12	14	7.1	1420	10.31	1.45
	81	7	15	7.1	1420	8.06	1.13
12-12	82	4	13	6.9	1420	8.75	1.26
	83	12	14	7.1	1420	11.32	1.59
	84	7	15	7.1	1420	6.96	0.98
12-13	85	4	13	6.9	1410	13.27	1.92
	86	12	14	7.0	1410	7.65	1.09
	87	7	15	7.0	1410	6.83	0.97
12-14	88	4	13	6.7	1372	19.93	2.97
	89	12	14	6.8	1372	8.33	1.22
	90	7	15	6.8	1372	9.58	1.40
12-14	86	4	13				

Table A-14: Sierra Impactor Data Summary for Exposure CL-7

Sierra impactor data: Q= 21 LPM

Sierra impactor effective size(um)			10	5.3	2.5	1.4	0.91	0.48	<0.48
Date	MMAD/GSD	Ch#	1	2	3	4	5	6	7
11/20	1.03/2.05	13	.34	.28	.45	1.92	4.60	2.78	4.0
11/21	0.945/1.89	14	0	0	.12	1.35	4.05	2.13	3.48
11/24	0.98/1.83	15	0	0	.18	.83	2.15	2.28	2.04
12/2	0.88/1.68	13	0	0	1.0	5.55	13.51	17.64	16.53
12/4	1.14/2.12	14	.7	.77	1.96	5.48	8.15	8.17	7.58
12/6	0.96/2.28	15	0	.23	0.84	2.42	3.67	3.72	5.15
12/8	1.17/1.96	13	.03	.44	2.71	11.19	18.13	12.21	13.12
12/9	1.00/2.14	14	0	.13	1.37	3.86	6.58	5.55	7.86
12/10	1.11/2.35	15	0	.22	.66	2.23	2.71	1.74	3.10

Average for chamber #13 = 1.02 um
Average for chamber #14 = 1.03 um
Average for chamber #15 = 1.02 um

Concentration mean for entire exposure, chamber #13 =1.05 ug/m^3 SD =0.58
Concentration mean for entire exposure, chamber #14 =1.02 ug/m^3 SD =0.48
Concentration mean for entire exposure, chamber #15 =1.10 ug/m^3 SD =0.36

EXPOSURE REPORT ON CALIFORNIA-LONDON #1 (Ch 4.2-15)

OZONE ONLY GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
04-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #1 (Ch 4.2-15)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN ON 2/26/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE FOR THREE DAYS AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT.

(NOTE: THE UV PHOTOMETRIC STANDARD WAS USED FOR THIS EXPOSURE.)

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #5
4. EXPOSURE CHAMBER: 4.2-15
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	04/04/86	09:00	04/07/86	08:00	2	23	0	422	0.39	0.01	0.27	0.41

EXPOSURE REPORT #1 (CHAMBER 4.2-14)

OZONE PLUS "CALIFORNIA" AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
02-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #1 (CHAMBER 4.2-14)

EXPOSURE PROTOCOL

BANTIN & KINGMAN F344 MALE RATS BORN ON 02/02/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE PLUS "CALIFORNIA" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF 80 PERCENT FOR THREE DAYS.

(NOTE: THE UV PHOTOMETRIC STANDARD WAS USED FOR THIS EXPOSURE.)

EXPOSURE DETAILS

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #5
4. EXPOSURE CHAMBER: 4.2-14
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP

	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
GRP	START DATE	START TIME	STOP DATE	STOP TIME	DAYS	HRS	MIN	SAMP SIZE	MEAN PPM	STD DEV	MIN PPM	MAX PPM
1	04/04/86	09:00	04/07/86	08:00	2	23	0	421	0.39	0.01	0.33	0.41

EXPOSURE REPORT #2 (CHAMBER 4.2-14)

OZONE (0.4 PPM) PLUS "CALIFORNIA"
AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
02-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #2 (CHAMBER 4.2-14)

EXPOSURE PROTOCOL

BANTIN & KINGMAN F344 MALE RATS BORN 03/23/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE PLUS "CALIFORNIA" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. CHAMBER: 4.2-14
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	05/30/86	09:00	06/02/86	08:00	2	23	0	415	0.39	0.01	0.27	0.43

EXPOSURE REPORT ON CALIFORNIA-LONDON #2 (CHAMBER 4.2-15)

OZONE (0.4 PPM) GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
02-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #2 (CHAMBER 4.2-15)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN 03/23/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. CHAMBER: 4.2-15
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	05/30/86	09:00	06/02/86	08:00	2	23	0	415	0.40	0.02	0.23	0.42

EXPOSURE REPORT #2 (CHAMBER 4.2-14)

OZONE (0.4 PPM) PLUS "CALIFORNIA"
AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
02-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #2 (CHAMBER 4.2-14)

EXPOSURE PROTOCOL

BANTIN & KINGMAN F344 MALE RATS BORN 03/23/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE PLUS "CALIFORNIA" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. CHAMBER: 4.2-14
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP

	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
G R P	START DATE	START TIME	STOP DATE	STOP TIME	DAYS	HRS	MIN	SAMP SIZE	MEAN PPM	STD DEV	MIN PPM	MAX PPM
1	05/30/86	09:00	06/02/86	08:00	2	23	0	415	0.39	0.01	0.27	0.43

EXPOSURE REPORT ON CALIFORNIA-LONDON #3 (CHAMBER 4.2-15)

OZONE (0.4 PPM) GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
03-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #3 (CHAMBER 4.2-15)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN 05/03/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. CHAMBER: 4.2-15
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	07/11/86	09:00	07/14/86	08:00	2	23	0	411	0.40	0.02	0.19	0.43

EXPOSURE REPORT ON ~~CALIFORNIA-LONDON~~ #3 (CHAMBER 4.2-14)

OZONE (0.4 PPM) PLUS "CALIFORNIA"
AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
03-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #3 (CHAMBER 4.2-14)

EXPOSURE PROTOCOL

BANTIN & KINGMAN F344 MALE RATS BORN 05/03/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE PLUS "CALIFORNIA" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. CHAMBER: 4.2-14
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN
7. STEVE TEAGUE, L.E.H.R. HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP

	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
G R P	START DATE	START TIME	STOP DATE	STOP TIME	DAYS	HRS	MIN	SAMP SIZE	MEAN PPM	STD DEV	MIN PPM	MAX PPM
1	07/11/86	09:00	07/14/86	08:00	2	23	0	413	0.39	0.02	0.32	0.44

EXPOSURE REPORT ON CALIFORNIA-LONDON #4 (Ch 4.2-15)

SULFUR DIOXIDE (1.0 PPM) ONLY GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
04-SEP-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #4 (Ch 4.2-15)

EXPOSURE PROTOCOL

BANTIN & KINGMAN F344 MALE RATS BORN ON 06/01/86 WERE CONTINUOUSLY EXPOSED TO 1.0 PPM SULFUR DIOXIDE AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: ONE HOUR AVERAGE OUT OF EVERY 2 HOURS.
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: MELOY SULFUR ANALYZER #5
4. EXPOSURE CHAMBER: 4.2-15
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN

EXPOSURE STATISTICS BY GROUP

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	08/08/86	09:00	08/11/86	08:00	2	23	0	33	1.07	0.03	1.02	1.12

EXPOSURE REPORT ON ~~CALIFORNIA-LONDON~~ #4 (Ch 4.2-14)

SULFUR DIOXIDE (1.0 PPM) PLUS "LONDON"
AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
04-SEP-86

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN ON 06/01/86 WERE CONTINUOUSLY EXPOSED TO 1.0 PPM SULFUR DIOXIDE PLUS "LONDON" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: ONE HOUR AVERAGE OUT OF EVERY 2 HOURS.
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: MELOY SULFUR ANALYZER #5
4. EXPOSURE CHAMBER: 4.2-14
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP
****

GRP	EXPOSURE PERIOD START DATE	START TIME	STOP DATE	STOP TIME	ELAPSED TIME DAYS	HRS	MIN	POLLUTANT CONCENTRATIONS SAMP SIZE	MEAN PPM	STD DEV	MIN PPM	MAX PPM
1	08/08/86	09:00	08/11/86	08:00	2	23	0	34	1.07	0.07	0.95	1.24

EXPOSURE REPORT ON CALIFORNIA-LONDON #5 (CHAMBER 4.2-14)

SULFUR DIOXIDE (10 PPM) PLUS "LONDON"
AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
06-OCT-86

EXPOSURE REPORT ON CALIFORNIA-LONDON #5 (CHAMBER 4.2-14)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN 07/05/86 WERE CONTINUOUSLY EXPOSED TO 10 PPM SULFUR DIOXIDE (SEE NOTE 8 BELOW) PLUS "LONDON" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THREE DAYS.

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: ONE HOUR AVERAGE OUT OF EVERY TWO HOURS
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. CHAMBER: 4.2-14
5. EXPOSURE TECHNICIAN: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: TIM DUVALL, MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.
8. THE ANIMALS WERE EXPOSED TO A HIGHER CONCENTRATION OF SULFUR DIOXIDE THAN REQUESTED BECAUSE A RANGE SWITCH WAS INADVERTENTLY TRIPPED TO THE WRONG POSITION WHEN THE ANALYZER WAS MOVED FROM THE CALIBRATION AREA TO THE EXPOSURE CHAMBERS. NO TESTING TIME WAS AVAILABLE TO DIAGNOSE THE PROBLEM BEFORE THE EXPOSURE BEGAN. WE NEED MORE ADVANCED NOTICE TO CHANGE EXPOSURE PARAMETERS.

EXPOSURE STATISTICS BY GROUP
****

G R P	EXPOSURE PERIOD START DATE	START TIME	STOP DATE	STOP TIME	ELAPSED TIME DAYS	HRS	MIN	POLLUTANT CONCENTRATIONS SAMP SIZE	MEAN PPM	STD DEV	MIN PPM	MAX PPM
1	09/12/86	09:00	09/15/86	08:00	2	23	0	31	21.89	6.23	15.36	30.15

EXPOSURE REPORT ON CALIFORNIA-LONDON #6 (Ch 4.2-15)

OZONE GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS

EXPOSURE REPORT ON CALIFORNIA-LONDON #6 (Ch 4.2-15)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN ON 07/26/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE FOR THREE DAYS AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT.

(NOTE: THE UV PHOTOMETRIC STANDARD WAS USED FOR THIS EXPOSURE.)

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. EXPOSURE CHAMBER: 4.2-15
5. EXPOSURE TECHNICIANS: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	10/10/86	09:00	10/13/86	08:00	2	23	0	416	0.39	0.02	0.24	0.43

EXPOSURE REPORT ON CALIFORNIA-LONDON #6 (CHAMBER 4.2-14)

OZONE PLUS "LONDON" AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS

EXPOSURE REPORT ON CALIFORNIA-LONDON #6 (CHAMBER 4.2-14)

CPRC EXPOSURE FACILITY

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN ON 07/26/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE PLUS "LONDON" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF 80 PERCENT FOR THREE DAYS.

(NOTE: THE UV PHOTOMETRIC STANDARD WAS USED FOR THIS EXPOSURE.)

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #2
4. EXPOSURE CHAMBER: 4.2-14
5. EXPOSURE TECHNICIANS: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	10/10/86	09:00	10/13/86	08:00	2	23	0	419	0.38	0.02	0.27	0.49

EXPOSURE REPORT ON CALIFORNIA-LONDON #7 (CHAMBER 4.2-16)

OZONE GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
20-JAN-87

EXPOSURE REPORT ON CALIFORNIA-LONDON #7 (CHAMBER 4.2-16)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN ON 09/06/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE AT AN ELEVATED RELATIVE HUMIDITY OF 80 PERCENT FOR THIRTY DAYS.

(NOTE: THE UV PHOTOMETRIC STANDARD WAS USED FOR THIS EXPOSURE.)

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #5 AND #2
4. EXPOSURE CHAMBER: 4.2-16
5. EXPOSURE TECHNICIANS: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	11/15/86	08:50	12/15/86	08:52	30	0	2	4236	0.38	0.02	0.28	0.48

EXPOSURE REPORT ON CALIFORNIA-LONDON #7 (CHAMBER 4.2-14)

OZONE PLUS "CALIFORNIA" AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
20-JAN-87

EXPOSURE REPORT ON CALIFORNIA-LONDON #7 (CHAMBER 4.2-14)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN ON 09/06/86 WERE CONTINUOUSLY EXPOSED TO 0.4 PPM OZONE PLUS "CALIFORNIA" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF 80 PERCENT FOR THIRTY DAYS.

(NOTE: THE UV PHOTOMETRIC STANDARD WAS USED FOR THIS EXPOSURE.)

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: 10 MINUTES
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: DASIBI OZONE ANALYZER #5 AND #2
4. EXPOSURE CHAMBER: 4.2-14
5. EXPOSURE TECHNICIANS: TIM DUVALL, STEVE TEAGUE
6. DATA HANDLING: MARY BREEN
7. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	11/15/86	08:50	12/15/86	08:52	30	0	2	4229	0.37	0.02	0.29	0.42

EXPOSURE REPORT ON CALIFORNIA-LONDON #7 (Ch 4.2-15)

SULFUR DIOXIDE (1.0 PPM) PLUS "LONDON"
AEROSOL GROUP

AIR POLLUTION EXPOSURE FACILITY
CALIFORNIA PRIMATE RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, DAVIS
05-JAN-87

EXPOSURE REPORT ON CALIFORNIA-LONDON #7 (Ch 4.2-15)

EXPOSURE PROTOCOL
****

BANTIN & KINGMAN F344 MALE RATS BORN ON 09/06/86 WERE CONTINUOUSLY EXPOSED TO 1.0 PPM SULFUR DIOXIDE PLUS "LONDON" AEROSOL AT AN ELEVATED RELATIVE HUMIDITY OF APPROXIMATELY 80 PERCENT FOR THIRTY DAYS.

EXPOSURE DETAILS
****

1. POLLUTANT CONCENTRATION SAMPLING INTERVAL: AVERAGE EVERY HOUR
2. CHAMBER VOLUME CHANGES PER HOUR: 30
3. POLLUTANT MONITORING EQUIPMENT: MELOY SULFUR ANALYZER #5
4. EXPOSURE CHAMBER: 4.2-15
5. EXPOSURE TECHNICIANS: TIM DUVALL, STEVE TEAGUE
6. ANIMAL CARE: SCOTT MERLINO & MARY BREEN
7. DATA HANDLING: MARY BREEN
8. STEVE TEAGUE, L.E.H.R., HAS AEROSOL EXPOSURE DATA.

EXPOSURE STATISTICS BY GROUP
****

G	EXPOSURE PERIOD				ELAPSED TIME			POLLUTANT CONCENTRATIONS				
R	START		STOP					SAMP	MEAN	STD	MIN	MAX
P	DATE	TIME	DATE	TIME	DAYS	HRS	MIN	SIZE	PPM	DEV	PPM	PPM
1	11/15/86	09:00	12/15/86	08:52	29	23	52	698	0.96	0.05	0.70	1.13

APPENDIX B

Hematology and Clinical Chemistry Data

Table B-1: EXPOSURE CL-1 HEMATOLOGY DATA

Gas = Ozone at 0.4 ppm
Aerosol = California Type at 0.55 mg/m^3

Treatment:	None		Ozone		CA Aerosol		Ozone + CA Aerosol	
Impaired	-	+	-	+	-	+	-	+
RBC	5.45	5.46	4.98	5.15	5.24		4.91	5.85
Hbg	13.6	13	12.4	13.2	12.5		11.8	13.5
PCV	40	39	37	38	38	37	36	40
WBC	4.5	3.8	0.375	4.6	4.2		4.2	4.2
Bands	0.5	0.5	0.5	0.5	1	0	0	0.5
PMN	20	24	28	29	34	33	20	33
Lymph	77	74	67	67	57	63	76	61
Mono	2	2	2	3	7	1	2	3
Eos	2	0.5	2	2	2	2	2	2

Average of samples from 2 animals per group. Abbreviations: RBC = red blood cell count (x 106 / ul), Hbg = hemoglobin (gm/dl), PCV = packed cell volume (%), WBC = White cell count (/ul), Bands = Band neutrophil (%) PMN = Neutrophils (%) Lymph = lymphocytes (%), mono = monocyte (%), eos = eosinophils (%).

Table B-2: EXPOSURE CL-1 BLOOD CHEMISTRY DATA

Treatment	None		Ozone		CA Aerosol		Ozone + CA Aerosol	
Impaired	-	+	-	+	-	+	-	+
SGOT	256	182	194	186	222	200	220	200
SGPT	80	79	78	80	70	92	113	98
LDH	4420	1008	4340	1063	1268	1029	969	914
ALK P	317	558	579	596	565	511	504	506
BUN	20	18	18	18	20	19	20	17
GLU	166	146	179	151	145	135	132	143
CHOL	56	52	52	53	51	49	46	52
PHOS	9.7	9.3	8.8	9.9	10.2	10.3	9.3	9.8
Ca	10.6	9.9	10	9.9	8	10	9.7	10
ALB	4.1	3.5	3.9	3.3	4	3	3.1	3.5
T.P.	5.7	5.5	5.4	5.4	5.8	5.3	5	5.4

Average of 2 animals per group. Abbreviations: SGOT = serum glutamic oxalic transaminase ((U/L), SGPT = serum glutamic pyruvate transaminase (U/L), LDH = lactate dehydrogenase (U/L) ALK P = alkaline phosphotase ((U/L), BUN = blood urea nitrogen (mg/dl), GLU = glucose (mg/dl), Chol = cholesterol (mg/dl), PHOS = phosphorus (mg/dl), Ca = calcium (mg/dl), ALB = albumin (gm/dl) T.P. = total protein (gm/dl)

Table B-3: EXPOSURE CL-2 HEMATOLOGY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	CA AEROSOL	CA+I	OZONE+CA	OZONE+CA+I
RBC	6.94	7.53	7.08	6.23	6.52	6.74	6.72	6.56
Hbg	14.5	16.0	14.7	13.3	14.4	14.2	14.2	14.2
PCV	44	48	45	40	43	44	43	43
WBC	4.10	4.15	3.30	3.50	4.00	3.50	3.30	3.70
Bands								
PMN	34	24	34	34	30	28	33	30
Lymph	63	71	65	64	68	67	61	69
Mono	3	5	1	2	2	5	3	1
Eos	0	0.5	0	0.5	0	1	0.5	1

Table B-4: EXPOSURE CL-2 BLOOD CHEMISTRY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	CA AEROSOL	CA+I)	OZONE+CA	OZONE+CA+I
SGOT	217	248	318	230	238	234	220	192
SGPT	82	116	112	82	125	86	89	89
LDH	800	2391	1521	800	800	1186	800	724
ALK P	487	573	508	473	533	505	504	477
BUN	19	20	23	19	19	20	21	20
GLU	144	293	157	144	125	145	149	147
CHOL	41	39	35	41	44	37	44	40
PHOS	8.5	12.1	8.4	8.8	10.0	9.6	11.0	9.7
Ca	10.2	10.7	9.6	10.1	10.4	9.9	9.9	9.9
ALB	3.8	4.0	3.7	3.6	3.7	3.9	3.8	3.7
T.P.	5.8	5.8	5.7	5.5	5.5	5.8	5.7	5.6

Table B-5: EXPOSURE CL-3 HEMATOLOGY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	CA AEROSOL	CA+I	OZONE+CA	OZONE+CA+I
RBC	6.38	6.36	5.81	5.54	6.05	5.80	6.45	6.40
Hbg	14.6	14.2	13.7	13.2	14.8	13.8	14.5	14.6
PCV	44	43	41	40	45	43	43	44
WBC	5.35	4.50	5.90	4.50	5.00	4.50	4.45	3.90
Bands								
PMN	38	42	25	37	32	34	32	49
Lymph	59	56	72	60	65	62	65	49
Mono	2	2	2	3	3	5	3	2
Eos	0.5	1	1.5	0.5	1	0.5	1	1

Table B-6: EXPOSURE CL-3 BLOOD CHEMISTRY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	CA AEROSOL	CA+I	OZONE+CA	OZONE+CA+I
SGOT	238	231	210	190	233	198	275	229
SGPT	91	86	83	89	88	88	101	118
LDH	800	800	800	800	800	800	800	788
ALK P	410	397	391	366	389	403	389	412
BUN	22	21	21	20	21	24	25	24
GLU	177	156	154	154	143	155	135	142
CHOL	44	42	42	42	46	42	42	38
PHOS	9.7	8.4	8.4	8.7	9.1	9.1	9.9	9.4
Ca	10.1	9.7	10.0	9.7	9.9	9.8	10.0	9.7
ALB	4.3	4.2	4.0	3.9	4.1	4.1	4.4	4.2
T.P.	6.1	6.0	5.9	5.7	6.0	5.9	6.1	6.3

Table B-7: EXPOSURE CL-4 HEMATOLOGY DATA

	HEALTHY	IMPAIRED	SO_2	SO_2+I	LT AEROSOL	LT+I	SO_2+LT	SO_2+LT+I
RBC	6.91	6.37	6.30	6.75	6.75	6.34	7.18	6.62
Hbg	14.3	13.7	13.4	14.1	13.8	13.6	14.5	14.0
PCV	42	40	41	42	41	42	43	42
WBC	4.6	4.9	3.3	4.8	4.2	3.5	3.1	4.1
Bands								
PMN	32	43	32	32	28	28	33	31
Lymph	64	54	65	66	69	67	63	66
Mono	3	2	3	2	3	2	5	4
Eos	3	2	1	1	1	3	1	0

Table B-8: EXPOSURE CL-4 BLOOD CHEMISTRY DATA[1]

	HEALTHY	IMPAIRED	SO_2	SO_2+I	LT AEROSOL	LT+I	SO_2+LT	SO_2+LT+I
SGOT	199	168	201	161	158	153	194	123
SGPT	132	114	94	106	135	93	95	101
LDH	800	752	800	800	800	800	695	741
ALK P	398	399	384	334	369	346	369	362
BUN	24	22	22	22	21	22	22	22
GLU	223	194	230	163	182	148	187	139
CHOL	44	41	41	42	44	39	45	43
PHOS	9.2	9.2	8.3	9.9	7.5	9.3	8.0	8.7
Ca	10.3	10.0	10.1	9.9	9.8	9.4	10.0	9.8
ALB	4.4	4.2	4.1	4.1	4.1	3.7	4.2	4.1
T.P.	6.3	5.9	5.8	5.9	5.9	5.5	6.2	6.0

Table B-9: EXPOSURE CL-5 HEMATOLOGY DATA

	HEALTHY	IMPAIRED	SO_2	SO_2+I	LT AEROSOL	LT+I	SO_2+LT	SO_2+LT+I
RBC	7.09	6.91	7.11	6.69	6.71	6.78	7.07	6.86
Hbg	14.2	14.6	14.5	14.3	13.8	14.3	15.0	14.1
PCV	41	41	42	41	41	40	43	40
WBC	5.0	5.0	6.0	3.8	3.7	4.5	7.1	4.3
Bands								
PMN	32	34	20	33	23	25	24	34
Lymph	64	65	77	64	73	73	74	64
Mono	4	2	3	2	4	2	2	1
Eos	1	1	1	2	1	1	1	2

Table B-10: EXPOSURE CL-5 BLOOD CHEMISTRY DATA

	HEALTHY	IMPAIRED	SO_2	SO_2+I	LT AEROSOL	LT=+I	SO_2+LT	SO_2+LT+I
SGOT	197	219	214	264	197	232	215	193
SGPT	77	81	72	85	82	72	85	74
LDH	478	418	505	671	435	405	361	379
ALK P	344	396	369	375	331	361	398	374
BUN	21	21	21	21	23	21	20	20
GLU	172	171	153	158	154	153	208	154
CHOL	46	43	46	42	42	42	44	43
PHOS	8.2	9.0	8.5	8.5	9.1	9.5	9.2	10.1
Ca	10.0	10.0	10.2	10.0	9.8	9.9	10.5	9.8
ALB	3.9	3.9	4.1	4.1	3.8	3.9	4.1	3.7
T.P.	5.9	5.9	6.0	5.9	5.7	5.7	5.8	5.5

Table B-11: EXPOSURE CL-6 HEMATOLOGY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	LT AEROSOL	LT+I	OZONE+LT	OZONE+LT+I
RBC	7.32	6.49	6.13	6.24	6.75	6.52	6.35	6.16
Hbg	14.3	13.1	12.8	13.1	13.5	13.3	12.9	12.6
PCV	43	39	38	39	41	41	39	38
WBC	3.85	3.00	4.00	3.75	4.00	3.45	3.50	3.15
Bands								
PMN	16	28	29	28	28	32	19	34
Lymph	77	65	65	72	68	63	79	61
Mono	7	7	4	0	4	4	3	4
Eos	2	0	3	1	3	3	0	2

Table B-12: EXPOSURE CL-6 BLOOD CHEMISTRY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	LT AEROSOL	LT+I	OZONE+LT	OZONE+LT+I
SGOT	198	230	392	187	193	167	192	182
SGPT	106	121	299	108	113	94	107	101
LDH	953	776	815	870	836	775	910	942
ALK P	311	360	265	357	336	329	322	289
BUN	21	24	19	19	23	22	20	23
GLU	194	168	163	167	156	152	165	197
CHOL	51	47	56	48	50	41	47	46
PHOS	6.8	8.8	6.5	7.2	7.5	7.9	6.8	6.3
Ca	10.1	9.7	9.9	9.6	9.8	9.8	9.7	9.7
ALB	4.1	3.8	3.9	3.9	3.9	3.9	3.9	3.9
T.P.	6.1	5.6	5.8	5.9	5.8	5.9	5.7	6.0

Table B-13: EXPOSURE CL-7 HEMATOLOGY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	CA AEROSOL	CA+I
RBC	7.00	7.13	6.41	7.52	7.02	7.02
Hbg	14.5	15.5	14.2	15.2	14.5	15.2
PCV	42	44	41	43	42	44
WBC	3.95	5.64	5.10	5.30	4.24	5.60
Bands						
PMN	20	24	22	21	28	26
Lymph	76	73	74	76	69	70
Mono	3	2	3	2	3	2
Eos	2	1	1	1	1	2

	OZONE+CA AEROSOL	OZONE+CA+I	SO_2& LT AEROSOL	SO_2&LT+I
RBC	6.86	7.25	6.59	7.30
Hbg	14.5	15.1	14.5	15.2
PCV	42	44	42	43
WBC	3.90	6.13	4.20	4.86
Bands				
PMN	27	21	23	17
Lymph	69	76	73	79
Mono	4	3	4	2
Eos	2	2	1	2

Table B-14: EXPOSURE CL-7 BLOOD CHEMISTRY DATA

	HEALTHY	IMPAIRED	OZONE	OZONE+I	CA AEROSOL	CA+I
SGOT	156	153	178	159	173	155
SGPT	78	67	93	86	79	71
LDH	690	830	829	546	907	770
ALK P	324	287	311	279	319	300
BUN	21	21	21	21	21	20
GLU	184	177	172	191	171	191
CHOL	52	53	54	49	52	49
PHOS	8.6	7.6	8.1	8.1	9.1	8.1
Ca	10.3	10.1	10.0	10.1	10.4	10.4
ALB	4.1	4.1	4.1	4.1	4.0	4.1
T.P.	6.3	6.2	6.2	6.3	6.3	6.3

	OZONE+CA AEROSOL	OZONE+CA+I	SO_2&LT AEROSOL	SO_2&LT+I
SGOT	179	175	198	144
SGPT	94	104	94	72
LDH	878	545	1019	679
ALK P	341	287	317	336
BUN	21	20	22	21
GLU	173	210	173	183
CHOL	53	50	50	51
PHOS	8.5	7.8	8.8	8.2
Ca	10.4	10.0	10.4	10.2
ALB	4.1	4.1	4.1	4.0
T.P.	6.5	6.1	6.1	6.2

APPENDIX C

Lung Pathology and Morphometric Data

Table C-1: Exposure CL-1 Gross Pathology Data
(Averages for six rats per group)

Gas = Ozone at 0.4 ppm
Aerosol = California type at 0.53 mg/m^3

Treatment	None		Ozone		CA Aerosol		Ozone + Aerosol	
Impaired	-	+	-	+	-	+	-	+
Body Weight (g)								
Mean	181	173	181	175	182	175	178	175
SE	13	10	12	9	14	10	12	9
Lung Weight (g)								
Mean	1.24	1.21	1.27	1.28	1.35	1.4	1.27	1.4
SE	0.1	0.13	0.11	0.1	0.15	0.14	0.08	0.08
Lung Volume (ml)								
Mean	6.07	5.95	5.05	4.45	5.23	6.25	5.95	6.22
SE	0.55	0.47	0.46	0.59	0.73	0.81	0.49	0.56
Lung Weight/Body Weight (%)								
Mean	0.67	0.7	0.7	0.73	0.76	0.81	0.75	0.78
SE	0.05	0.08	0.03	0.06	0.1	0.09	0.07	0.04
Lung Volume/Body Weight (%)								
Mean	3.38	3.46	2.8	2.58	2.83	3.58	3.4	3.59
SE	0.32	0.29	0.22	0.37	0.25	0.43	0.33	0.34

Table C-2: Exposure CL-2 Gross Pathology Data
(Averages for six rats per group)

Treatment	None		Ozone		CA Aerosol		Ozone + Aerosol	
Impaired	-	+	-	+	-	+	-	+
Body Wt (g)								
Mean	206	202	202	205	209	204	206	208
SE	6	7	5	5	3	7	4	5
Lung Weight (g)								
Mean	1.18	1.23	1.16	1.35	1.23	1.26	1.20	1.04
SE	0.07	0.07	0.08	0.04	0.02	0.09	0.05	0.26
Lung Volume (ml)								
Mean	9.05	9.52	7.88	10.18	9.25	10.12	8.15	9.65
SE	0.36	0.67	0.34	0.39	0.33	0.48	0.62	0.61
Lung Weight/Body Weight (%)								
Mean	0.57	0.61	0.57	0.66	0.59	0.62	0.58	0.49
SE	0.03	0.03	0.03	0.02	0.01	0.04	0.02	0.12
Lung Volume/Body Weight (%)								
Mean	4.40	4.71	3.91	4.98	4.43	4.97	3.98	4.62
SE	0.17	0.30	0.15	0.18	0.14	0.14	0.32	0.22

Table C-3: Exposure CL-3 Gross Pathology Data
(Averages for six rats per group)

Treatment	None		Ozone		CA Aerosol		Ozone + Aerosol	
Impaired	-	+	-	+	-	+	-	+
Body Wt (g)								
Mean	238	229	235	232	234	230	230	228
SE	4	6	5	4	4	4	5	4
Lung Weight (g)								
Mean	1.38	1.51	1.46	1.65	1.38	1.42	1.40	1.67
SE	0.11	0.11	0.09	0.10	0.07	0.02	0.06	0.04
Lung Volume (ml)								
Mean	8.85	11.4	10.00	10.68	8.78	10.18	9.58	11.0
SE	0.25	0.58	0.50	0.52	0.62	0.75	0.38	0.50
Lung Weight/Body Weight (%)								
Mean	0.57	0.66	0.62	0.71	0.59	0.62	0.61	0.72
SE	0.04	0.04	0.03	0.04	0.02	0.01	0.03	0.02
Lung Volume/Body Weight (%)								
Mean	3.73	4.98	4.25	4.60	3.75	4.41	4.16	4.82
SE	0.12	0.20	0.16	0.17	0.24	0.27	0.10	0.21

Table C-4: Exposure CL-4 Gross Pathology Data
(Averages for six rats per group)

Treatment	None		Sulfur Dioxide		LT Aerosol		SO_2 + Aerosol	
Impaired	-	+	-	+	-	+	-	+
Body Wt (g)								
Mean	253	235	248	245	255	242	249	243
SE	5	4	6	2	3	3	6	4
Lung Weight (g)								
Mean	1.34	1.47	1.35	1.60	1.46	1.62	1.39	1.57
SE	0.06	0.08	0.07	0.05	0.04	0.05	0.08	0.08
Lung Volume (ml)								
Mean	10.20	10.48	10.67	11.70	10.78	11.32	10.15	11.08
SE	0.68	0.39	0.33	0.48	0.43	0.46	0.63	0.19
Lung Weight/Body Weight (%)								
Mean	0.53	0.62	0.55	0.65	0.57	0.67	0.56	0.65
SE	0.02	0.03	0.03	0.02	0.02	0.02	0.03	0.04
Lung Volume/Body Weight (%)								
Mean	4.03	4.48	4.29	4.77	4.23	4.68	4.09	4.57
SE	0.21	0.24	0.07	0.17	0.15	0.20	0.24	0.13

Table C-5: Exposure CL-5 Gross Pathology Data
(Averages of six rats per group)

Treatment	None		Sulfur Dioxide		LT Aerosol		SO_2 + Aerosol	
Impaired	-	+	-	+	-	+	-	+
Body Wt (g)								
Mean	232	229	228	220	221	219	233	228
SE	3	5	3	6	4	5	2	4
Lung Weight (g)								
Mean	1.54	1.39	1.19	1.31	1.22	1.27	1.24	1.38
SE	0.25	0.07	0.04	0.04	0.05	0.10	0.05	0.06
Lung Volume (ml)								
Mean	10.50	11.35	10.42	11.28	10.12	10.35	9.75	11.32
SE	0.40	0.41	0.66	0.30	0.41	0.53	0.59	0.49
Lung Weight/Body Weight (%)								
Mean	0.66	0.61	0.52	0.59	0.55	0.58	0.53	0.61
SE	0.10	0.04	0.02	0.01	0.03	0.03	0.03	0.03
Lung Volume/Body Weight (%)								
Mean	4.53	4.95	4.57	5.13	4.57	4.75	4.19	4.95
SE	0.17	0.14	0.29	0.10	0.15	0.28	0.27	0.19

Table C-6: Exposure CL-6 Gross Pathology Data
(Averages for six rats per group)

Treatment	None		Ozone		LT Aerosol		Ozone + Aerosol	
Impaired	-	+	-	+	-	+	-	+
Body Wt (g)								
Mean	239	232	242	239	243	239	241	237
SE	5	12	5	8	5	6	4	6
Lung Weight (g)								
Mean	1.42	1.64	1.52	1.74	1.52	1.58	1.59	1.82
SE	0.04	0.06	0.04	0.08	0.06	0.05	0.04	0.08
Lung Volume (ml)								
Mean	10.07	11.56	8.37	9.84	9.92	11.06	9.42	11.13
SE	1.08	0.79	0.61	0.76	0.88	0.95	0.57	0.68
Lung Weight/Body Weight (%)								
Mean	0.59	0.71	0.63	0.73	0.62	0.66	0.66	0.77
SE	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.03
Lung Volume/Body Weight (%)								
Mean	4.24	5.12	3.42	4.15	4.08	4.67	3.91	4.70
SE	0.34	0.67	0.26	0.30	0.29	0.38	0.24	0.26

Table C-7: Exposure CL-7 Gross Pathology Data
(Six animals per group)

Treatment	None		Ozone		CA Aerosol	
Impaired	-	+	-	+	-	+
Body Wt (g)						
Mean	288	278	274	269	288	286
SE	9	7	7	5	5	4
Lung Weight (g)						
Mean	1.84	1.94	1.84	1.97	1.88	1.91
SE	0.08	0.05	0.04	0.08	0.05	0.06
Lung Volume (ml)						
Mean	12.74	12.80	12.50	12.90	12.37	13.04
SE	0.48	0.37	0.31	0.27	0.18	0.19
Lung Weight/Body Weight						
Mean	0.64	0.70	0.67	0.73	0.65	0.67
SE	0.02	0.01	0.01	0.03	0.02	0.02
Lung Volume/Body Weight (%)						
Mean	4.42	4.61	4.58	4.80	4.30	4.56
SE	0.06	0.12	0.16	0.09	0.07	0.10

Treatment	CA Aerosol + Ozone		LT Aerosol + SO_2	
Impaired	-	+	-	+
Body Wt (g)				
Mean	291	285	287	298
SE	4	9	6	9
Lung Weight (g)				
Mean	1.92	2.08	1.82	1.94
SE	0.06	0.06	0.07	0.08
Lung Volume (ml)				
Mean	12.80	13.63	11.78	12.76
SE	0.16	0.20	0.23	0.33
Lung Weight/Body Weight (%)				
Mean	0.66	0.73	0.64	0.65
SE	0.02	0.02	0.03	0.01
Lung Volume/Body Weight (%)				
Mean	4.41	4.80	4.11	4.29
SE	0.07	0.12	0.10	0.13

Table C-8: Effects of Elastase Pretreatment and Aerosol/Gas Exposure on Alveolar Size and Surface/Volume Ratios as Average Area (AA) and Area/Perimeter (A/P), Quantimet analysis of Exposure CL-1 (Averages for 6 rats per group)

	HEALTHY (H)		IMPAIRED (I)	
Treatment	AA (um^2)	A/P	AA (um^2)	A/P
None	3960	10.6	4301	12
Ozone	2528	9.2	2806	10.2
CA Aerosol	4076	11.4	4953	12.8
CA Aerosol + Ozone	4928	11.6	4391	12.9
Mean	3873	10.7	4112.8	11.9
SD	862	0.9	794.7	1.1

Table C-9: Effects of Elastase Pretreatment and Aerosol/Gas Exposure on Alveolar Size and Surface-to-Volume Ratios as Average Area (AA) and Area/Perimeter (A/P), Quantimet Analysis of Exposure CL-3 (Averages for six rats per group)

	HEALTHY (H)		IMPAIRED (I)	
Treatment	AA (um^2)	A/P	AA (um^2)	A/P
None	4327.8	11.8	6538.4	14.5
Ozone	6281.5	12.7	11593.3	16.6
CA Aerosol	4877.2	12.2	6369.4	15.3
CA Aerosol + Ozone	6429.2	13.4	9485.2	16.9
Mean	5478.9	12.5	8496.6	15.8
SD	899.2	0.6	2175.2	1.0

Table C-10:Effects of Elastase Pretreatment and Aerosol/Gas Exposure on Alveolar Size and Surface-to-Volume Ratios as Average Area (AA) and Area/Perimeter (A/P), Quantimet Analysis of Exposure CL-7 (Averages for six rats per group)
Gas = ozone at 0.4 ppm
Aerosol = California type

	HEALTHY (H)		IMPAIRED (I)	
Treatment	AA (um^2)	A/P	AA (um^2)	A/P
None	1764	8.48	1765	8.97
Ozone	1960	9.46	1669	8.07
CA Aerosol	1894	9.61	1456	8.11
CA Aerosol + Ozone	1637	8.90	1731	9.04
Mean	1814	9.11	1655	8.55
SD	124	0.45	120	0.46

Table C-11: Exposure CL-1 Cell Counts In Alveolar Ducts
Inflammatory Cells /Alveolus by LM (Averages for six rats per group)

	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.020176	0.019537	0.025901	0.043378
Ozone	0.108455	0.118983	0.082103	0.065590
CA Aerosol	0.024656	0.022083	0.045754	0.039255
CA Aerosol + Ozone	0.143688	0.120427	0.245390	0.266612

Table C-12: Exposure CL-2 Cell Counts In Alveolar Ducts
(Averages of six rats per group)

	Inflammatory Cells /Alveolus by LM			
	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.017090	0.021898	0.024700	0.022190
Ozone	0.087467	0.078378	0.100157	0.164555
CA Aerosol	0.013053	0.027723	0.025524	0.025214
CA Aerosol + Ozone	0.154134	0.126712	0.221676	0.191406

Table C-13: Exposure CL-3 Cell Counts In Alveolar Ducts
(Averages for six rats per group)

	Inflammatory Cells /Alveolus by LM			
	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.009927	0.018153	0.048918	0.049708
Ozone	0.137386	0.122509	0.080044	0.076888
CA Aerosol	0.002315	0.007414	0.048106	0.041080
CA Aerosol + Ozone	0.162109	0.103870	0.120877	0.108122

Table C-14: Exposure CL-4 Cell Counts In Alveolar Ducts
(Averages for six rats per group)

	Inflammatory Cells /Alveolus by LM			
	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.017177	0.022625	0.028024	0.030540
Sulfur Dioxide	0.015683	0.024660	0.038089	0.034670
LT Aerosol	0.012965	0.022464	0.056896	0.053933
LT Aerosol + SO_2	0.021845	0.031077	0.044693	0.031599

Table C-15: Exposure CL-5 Cell Counts In Alveolar Ducts
(Averages for six rats per group)

	Inflammatory Cells /Alveolus by LM			
	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.016187	0.020862	0.048152	0.045281
Sulfur Dioxide	0.007780	0.013245	0.065829	0.099271
LT Aerosol	0.011274	0.014582	0.021283	0.027604
LT Aerosol + SO_2	0.004577	0.010279	0.043235	0.046907

Table C-16: Exposure CL-6 Cell Counts In Alveolar Ducts
(Averages for six rats per group)

	Inflammatory Cells /Alveolus by LM			
	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.024474	0.034009	0.060895	0.053233
Ozone	0.108126	0.111475	0.088223	0.079986
LT Aerosol	0.010980	0.017272	0.011433	0.020114
LT Aerosol + Ozone	0.110653	0.081285	0.171191	0.132537

Table C-17: Exposure CL-7 Cell Counts In Alveolar Ducts
(Averages for six rats per group)

	Inflammatory Cells /Alveolous by LM			
	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.010	0.015	0.058	0.019
Ozone	0.449	0.216	0.650	0.270
CA Aerosol	0.000	0.000	0.087	0.050
CA Aerosol + Ozone	0.389	0.081	1.044	0.549
LT Aerosol + SO_2	0.017	0.010	0.007	0.010

Table C-18: Exposure CL-1 SEM Cell Counts In Alveolar Ducts
(Averages for six rats per group)

	Inflammatory Cells / Alveolus by SEM			
	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.047501	0.048046	0.075157	0.069872
Ozone	0.131099	0.071046	0.069099	0.061826
CA Aerosol	0.060245	0.047544	0.150504	0.101899
CA Aerosol + Ozone	0.156875	0.079234	0.219436	0.156933

Table C-19: Exposure CL-2 SEM Cell Counts In Alveolar Ducts
Inflammatory cells / Alveolus by SEM
(Averages for six rats per group)

	HEALTHY (H)		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	0.042	0.042	1.359	2.984
Ozone	0.076	0.048	0.140	0.081
CA Aerosol	0.053	0.042	0.081	0.065
CA Aerosol + Ozone	0.121	0.065	0.061	0.042

Table C-20: Exposure CL-7 SEM Cell Counts In Alveolar Ducts
(Averages for six rats per group)

Inflammatory cells /Alveolus by SEM

	Healthy		IMPAIRED (I)	
Treatment	Mean	SD	Mean	SD
None	2.454	1.901	1.588	0.669
Ozone	4.072	1.149	4.702	1.978
CA Aerosol	1.491	0.209	1.737	0.218
CA Aerosol + Ozone	4.191	1.877	3.585	1.986
LT Aerosol + SO_2	1.752	0.623	2.802	2.735

Table C-21: Histopathology Data for Lung H&E Sections
(Averages for siz rats per group)

EXPOSURE#	TREATMENT	LESION TYPES: THICKENING OF WALL Bronchiolar	THICKENING OF WALL Alveolar	INFLAMMATORY CELLS Alveolar Space
CL-1 to 6	NONE/HEALTHY	0	0	0
CL-1 to 6	IMPAIRED	0	0	0
CL-1	OZONE/HEALTHY	2.0	1.5	1.0
CL-2	OZONE/HEALTHY	2.5	2.5	1.5
CL-3	OZONE/HEALTHY	2.5	2.0	1.0
CL-6	OZONE/HEALTHY	2.5	2.0	1.0
CL-1	OZONE/IMPAIRED	2.0	1.5	1.0
CL-2	OZONE/IMPAIRED	2.5	1.5	1.0
CL-3	OZONE/IMPAIRED	2.0	1.5	1.0
CL-6	OZONE/IMPAIRED	2.0	1.5	1.0
CL-1 to 6	AEROSOL/HEALTHY	0	0	0
CL-1 to 6	AEROSOL/IMPAIRED	0	0	0
CL-1	OZONE/AERO./HEALTHY	2.0	1.5	1.0
CL-2	OZONE/AERO./HEALTHY	2.5	2.5	1.0
CL-3	OZONE/AERO./HEALTHY	2.0	2.0	1.0
CL-6	OZONE/AERO./HEALTHY	2.5	2.0	1.0
CL-1	OZONE/AERO./IMPAIRED	2.0	1.5	1.0
CL-2	OZONE/AERO./IMPAIRED	2.5	2.5	1.5
CL-3	OZONE/AERO./IMPAIRED	1.5	1.0	1.5
CL-6	OZONE/AERO./IMPAIRED	1.0	0.5	0.5
CL-4 & 5	SO_2/HEALTHY	0	0	0
	SO_2/IMPAIRED	0	0	0

0 : NO Lesion
0.5: Healthy to Mild
1.0: Mild
1.5: Mild to Moderate
2.0: Moderate
2.5: Moderate to Severe
3.0 Severe

Table C-22: Subjective Analysis of Exposure 7
30 Day Exposure to Indicated Atmospheres
(Nominal averages for six rats per group less unusables)

Treatment	Lesion	Mural Changes	Fibrosis	Pigmentation	Emphysema
None	0.0	0.0	0.0	0.0	0.8
Impaired	0.0	0.0	0.4	0.3	2.0
Ozone	1.8	1.7	0.9	0.6	0.8
Ozone + Impaired	2.3	2.2	1.4	1.7	1.0
CA Aerosol	0.2	0.1	0.0	0.8	0.4
CA Aerosol + Impaired	0.1	0.1	0.1	0.8	1.6
CA Aerosol + Ozone	2.4	2.4	1.3	2.1	0.9
CA Aerosol + Ozone + Impaired	2.4	2.3	1.9	2.9	2.1
LT Aerosol + SO_2	0.1	0.1	0.1	1.2	0.3
LT Aerosol + SO_2 + Impaired	0.3	0.3	0.3	1.5	1.6

0 = no lesion or response.
1 = equivocal lesion or response
2 = moderate but definite lesion or response.
3 = moderately severe.
4 = severe.

MANN-WHITNEY ANALYSIS OF VARIANCE FOR SUBJECTIVE OBSERVATIONS IN EXPOSURE 7

Treatment	Lesion		Mural Changes		Fibrosis		Pigmentation		Emphysema	
CA Aerosol	-	+	-	+	-	+	-	+	-	+
N	22	33	22	33	22	33	22	33	22	33
Median	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	1.0
P	ns		ns		ns		0.0005		ns	
Ozone	-	+	-	+	-	+	-	+	-	+
N	33	22	33	22	33	22	33	22	33	22
Median	0.0	2.0	0.0	2.0	0.0	1.0	0.0	1.5	0.0	1.0
P	<0.00005		<0.00005		<0.00005		0.03		ns	
Impaired	-	+	-	+	-	+	-	+	-	+
N	30	25	30	25	30	25	30	25	30	25
Median	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	2.0
P	ns		ns		ns		ns		0.0005	

Significant Interactions:

CA Aerosol x Ozone (Fibrosis) p = 0.0183

Table C-23: Parenchymal components of lungs of rats exposed to "London" or "California" smog for 30 days - Exposure CL-7.
(Values are percentage of total lung volume; averages for six rats per group.)

Treatment		TERMINAL BRONCHIOLES	ALVEOLAR DUCTS	ALVEOLI
None	Mean	1.53	20.79	69.22
	SD	0.71	5.64	10.57
Impaired	Mean	2.06	18.09	56.50
	SD	1.23	9.52	25.44
Ozone	Mean	3.05	18.92	68.34
	SD	1.15	3.58	3.60
Ozone + Impaired	Mean	2.33	17.57	59.72
	SD	1.77	9.15	27.36
CA Aerosol	Mean	2.53	16.72	69.99
	SD	1.09	2.76	3.39
CA Aerosol + Impaired	Mean	1.11	14.77	58.32
	SD	0.88	6.94	26.16
CA Aerosol + Ozone	Mean	3.08	22.28	64.39
	SD	1.65	7.48	10.03
CA Aerosol + Ozone + Impaired	Mean	1.47	17.37	71.69
	SD	1.00	3.06	5.22
LT Aerosol & SO_2	Mean	2.27	17.57	80.14
	SD	1.82	4.53	4.55
LT Aerosol & SO_2 + Impaired	Mean	1.56	20.71	77.72
	SD	0.78	2.53	2.49

Table C-24: Nested analysis of variance of inflammatory cell counts for rats in exposure set CL-1. A comparison of the results of inflammatory cell counts done on 8 regions per lung per animal in each group of six rats was made with similar counts done on one slide per animal to determine the relative apportionment of variance to region counted, treatment group, and variability associated with the individual animals.

Analysis of Variance: Inflammatory cell counts
Exposure CL 1: 8 slides per animal

Variance Source	D.F.	Sum of Squares	Mean Squares	Variance Component	Percent
Total	641	1779.	2.8	3.12	100.0
Treatment	2	447.	223.3	1.01	32.3
Animal	15	118.	7.8	0.16	5.0
Region	124	275.	2.2	0.08	2.4
Error	500	940.	1.9	1.88	60.2

Analysis of Variance: Inflammatory cell counts
Exposure CL 1: 1 slide per animal.

Variance Source	D.F.	Sum of Squares	Mean Squares	Variance Component	Percent
Total	142	0.625	0.0044	0.00525	100.0
Treatment	2	0.251	0.1257	0.00248	47.2
Animal	15	0.113	0.0075	0.00068	13.0
Error	125	0.261	0.0021	0.00209	39.8

Conclusions: Treatment variance is greatest followed by variance between animals. Variance within regions in a single animal is the smallest component of variance and is insignificant when compared with animal to animal variation in response. Therefore, one slide evaluated per animal should provide sufficient data to detect any statistical significant trends.

APPENDIX D

Lung Biochemistry Data

Table D-1: Hydroxyproline micrograms per gram lung protein (Hpr/Pro) and protein micrograms per gram dry lung weight. (Averages of six rats)

Exposure CL-1

Treatment	(mass ratios) Hpr/Pro	Pro/Drywt
None	25.72	643.40
	4.17	84.83
Impaired	30.73	562.17
	5.70	84.23
Ozone	17.98	808.25
	2.21	64.77
Ozone + Impaired	14.68	885.00
	1.74	150.83
CA Aerosol	25.58	748.20
	5.46	139.98
CA Aerosol + Impaired	30.53	506.67
	9.65	95.64
CA Aerosol + Ozone	36.28	398.00
	9.53	29.48
CA Aerosol + Ozone + Impaired	34.53	281.83
	4.46	18.27

Exposure CL-4

Treatment	(mass ratios) Hpr/Pro	Pro/Drywt
None	5.38	1714.17
	0.83	126.84
Impaired	6.72	1613.83
	1.03	289.15
SO2	9.17	1169.00
	0.77	210.43
SO2 + Impaired	9.34	924.17
	1.69	78.59
LT Aerosol	10.04	859.67
	1.41	101.20
LT Aerosol + Impaired	8.19	920.83
	1.65	80.58
LT Aerosol + SO2	9.95	964.17
	1.39	75.60
LT Aerosol + SO2 + Impaired	12.56	830.33
	1.08	91.19

Table D-2: Lung DNA, RNA, and Protein Content for Rats in Acute Exposure CL-2
(Averages for six rats per group)

		LUNG WT.	(mg) PER LUNG			AS % OF CONTROL		
TREATMENT	PARAMETER	(g)	DNA	RNA	PROTEIN	DNA	RNA	PROTEIN
NONE	MEAN	1.30	3.18	17.4	143	100	100	100
	SE	0.07	0.28	1.9	15	9	11	11
IMPAIRED	MEAN	1.51	4.08	21.5	192	128	124	135
	SE	0.07	0.33	1.6	9	10	9	6
OZONE	MEAN	1.40	4.23	22.2	196	132	128	137
	SE	0.07	0.30	1.3	15	9	8	11
OZONE + IMPAIRED	MEAN	1.52	4.34	23.3	198	136	134	139
	SE	0.08	0.30	1.5	12	9	9	9
CA AEROSOL	MEAN	1.27	3.61	17.0	158	113	98	111
	SE	0.06	0.58	2.3	15	18	13	11
CA AEROSOL + IMPAIRED	MEAN	1.56	5.43	23.7	250	171	137	175
	SE	0.09	0.81	3.3	28	26	19	20
CA AEROSOL + OZONE	MEAN	1.48	4.63	21.5	229	146	124	160
	SE	0.04	0.30	1.8	13	9	10	9
CA AEROSOL + OZONE + IMPAIR	MEAN	1.70	5.57	25.0	221	175	144	155
	SE	0.11	0.43	2.5	18	14	15	12

Table D-3: Lung DNA, RNA and Protein Concentrations for Rats in Acute Exposure CL-2 (Averages of six rats per group)

TREATMENT	PARAMETER	LUNG WT. (g)	LUNG CONCENTRATION (mg per gram lung) DNA	RNA	PROTEIN	RNA/ DNA	PROTEIN / DNA
NONE	MEAN	1.30	2.46	13.4	109	5.51	45.0
	SE	0.07	0.18	1.2	7	0.59	3.3
IMPAIRED	MEAN	1.51	2.72	14.3	128	5.31	48.0
	SE	0.07	0.20	1.1	7	0.30	2.4
OZONE	MEAN	1.40	3.02	16.0	139	5.39	46.4
	SE	0.07	0.13	0.9	5	0.51	1.8
OZONE + IMPAIRED	MEAN	1.52	2.89	15.5	131	5.49	46.2
	SE	0.08	0.26	1.1	7	0.47	2.3
CA AEROSOL	MEAN	1.27	2.81	13.2	124	5.04	46.8
	SE	0.06	0.42	1.5	11	0.68	4.5
CA AEROSOL + IMPAIRED	MEAN	1.56	3.42	14.9	158	4.48	47.6
	SE	0.09	0.35	1.2	10	0.34	2.9
CA AEROSOL + OZONE	MEAN	1.48	3.13	14.5	155	4.65	49.8
	SE	0.04	0.19	1.1	9	0.23	1.5
CA AEROSOL + OZONE + IMPAIR	MEAN	1.70	3.34	14.7	132	4.52	40.0
	SE	0.11	0.32	1.1	11	0.38	2.0

Table D-4: Lung DNA, RNA and Protein Content for Rats in Acute Exposure CL-5
(Averages for six rats per group)

TREATMENT	PARAMETER	LUNG WT. (g)	(mg) PER LUNG DNA	RNA	PROTEIN	AS % OF CONTROL DNA	RNA	PROTEIN
NONE	MEAN	1.59	5.10	19.3	232	100	100	100
	SE	0.05	0.46	1.3	18	9	7	8
IMPAIRED	MEAN	1.69	4.93	18.6	208	97	97	90
	SE	0.06	0.26	1.6	18	5	8	8
SO_2	MEAN	1.63	4.74	17.0	220	93	88	95
	SE	0.07	0.15	0.4	10	3	2	4
SO_2 + IMPAIRED	MEAN	1.73	5.34	17.4	218	105	90	94
	SE	0.05	0.24	0.4	8	5	2	3
LT AEROSOL	MEAN	1.66	4.24	18.7	271	83	97	117
	SE	0.05	0.31	1.3	16	6	7	7
LT AEROSOL + IMPAIRED	MEAN	1.79	6.21	21.3	242	122	110	105
	SE	0.07	0.42	1.8	28	8	9	12
SO_2 + LT AEROSOL	MEAN	1.44	4.04	16.7	188	79	87	81
	SE	0.09	0.24	1.2	17	5	6	7
SO_2 + AEROSOL + IMPAIRED	MEAN	1.75	5.81	21.4	244	114	111	105
	SE	0.04	0.44	1.0	10	9	5	5

Table D-5: Lung DNA, RNA and Protein Concentrations for Rats in Acute Exposure CL-5 (Averages for six rats per group)

TREATMENT	PARAMETER	LUNG WT. (g)	LUNG CONCENTRATION (mg per gram lung) DNA	RNA	PROTEIN	RNA/ DNA	PROTEIN / DNA
NONE	MEAN	1.59	3.18	12.1	145	3.84	46.3
	SE	0.05	0.23	0.5	8	0.17	3.1
IMPAIRED	MEAN	1.69	2.93	11.0	122	3.78	42.1
	SE	0.06	0.16	0.8	8	0.25	3.0
SO_2	MEAN	1.63	2.94	10.5	136	3.59	46.7
	SE	0.07	0.21	0.6	9	0.07	3.1
SO_2 + IMPAIRED	MEAN	1.73	3.10	10.1	127	3.29	41.4
	SE	0.05	0.14	0.3	7	0.16	3.1
LT AEROSOL	MEAN	1.66	2.55	11.2	162	4.43	64.7
	SE	0.05	0.17	0.6	6	0.16	3.9
LT AEROSOL + IMPAIRED	MEAN	1.79	3.47	11.9	135	3.43	38.6
	SE	0.07	0.18	0.9	13	0.20	2.8
SO_2 + LT AEROSOL	MEAN	1.44	2.81	11.6	130	4.12	46.1
	SE	0.09	0.04	0.2	5	0.08	1.9
SO_2 + AEROSOL + IMPAIRED	MEAN	1.75	3.34	12.3	140	3.73	43.1
	SE	0.04	0.25	0.5	4	0.18	3.5

Table D-6: Lung Hydroxyproline Content for Rats in Subchronic Exposure CL-7
(Averages for six rats per group)

TREATMENT	PARAMETER	LUNG WT. (g)	(mg) HYDROXYPROLINE PER (g) LUNG	(mg) HYDROXYPROLINE PER LUNG	AS % OF CONTROL	OH-PROLINE / DNA
NONE	MEAN	1.75	0.91	1.60	100	0.40
	SE	0.07	0.08	0.17	10	0.03
IMPAIRED	MEAN	1.83	1.11	1.98	124	0.43
	SE	0.08	0.11	0.13	8	0.02
OZONE	MEAN	1.88	0.90	1.64	103	0.39
	SE	0.15	0.09	0.09	6	0.02
OZONE + IMPAIRED	MEAN	2.11	0.93	1.88	118	0.40
	SE	0.20	0.12	0.22	13	0.04
CA AEROSOL	MEAN	1.72	1.08	1.83	115	0.44
	SE	0.07	0.14	0.19	12	0.06
CA AEROSOL + IMPAIRED	MEAN	1.85	0.96	1.77	111	0.43
	SE	0.10	0.06	0.14	9	0.02
CA AEROSOL + OZONE	MEAN	1.99	0.96	1.89	118	0.52
	SE	0.11	0.08	0.10	6	0.01
CA AEROSOL + OZONE + IMPAIR	MEAN	2.07	1.22	2.53	159	0.62
	SE	0.06	0.09	0.19	12	0.04
LT AEROSOL WITH SO_2	MEAN	1.80	0.99	1.75	110	0.44
	SE	0.08	0.12	0.16	10	0.03
LT AEROSOL WITH SO_2 + IMPAIRED	MEAN	1.76	1.21	2.14	134	0.57
	SE	0.05	0.08	0.15	9	0.06

Table D-7: Lung DNA, RNA and Protein Content for Subchronic Exposure CL-7
(Averages for six rats per group)

TREATMENT	PARAMETER	LUNG WT. (g)	(mg) PER LUNG			AS PERCENT OF CONTROL		
			DNA	RNA	PROTEIN	DNA	RNA	PROTEIN
NONE	MEAN	1.75	3.98	20.5	250	100	100	100
	SE	0.07	0.27	1.2	19	7	6	8
IMPAIRED	MEAN	1.83	4.61	20.3	259	116	99	104
	SE	0.08	0.31	0.5	11	8	2	4
OZONE	MEAN	1.88	4.29	20.1	257	108	98	103
	SE	0.15	0.40	0.9	21	10	4	8
OZONE + IMPAIRED	MEAN	2.11	4.69	22.6	290	118	110	116
	SE	0.20	0.24	1.2	33	6	6	13
CA AEROSOL	MEAN	1.72	4.17	20.5	262	105	100	105
	SE	0.07	0.14	0.8	12	4	4	5
CA AEROSOL + IMPAIRED	MEAN	1.85	4.12	19.0	247	103	93	99
	SE	0.10	0.19	1.1	21	5	6	8
CA AEROSOL + OZONE	MEAN	1.99	3.65	19.3	262	92	94	105
	SE	0.11	0.12	0.3	7	3	1	3
CA AEROSOL + OZONE + IMPAIR	MEAN	2.07	4.08	20.1	257	102	98	103
	SE	0.06	0.20	0.8	21	5	4	8
LT AEROSOL WITH SO_2	MEAN	1.80	3.98	20.4	253	100	100	101
	SE	0.08	0.22	0.7	12	6	4	5
LT AEROSOL WITH SO_2 + IMPAIRED	MEAN	1.76	3.88	21.1	247	98	103	99
	SE	0.05	0.33	0.5	8	8	2	3

Table D-8: Lung DNA, RNA and Protein Concentrations for Subchronic Exposure CL-7 (Averages for six rats per group)

TREATMENT	PARAMETER	LUNG WT. (g)	LUNG CONCENTRATION (mg per gram lung) DNA	RNA	PROTEIN	AS PERCENT OF CONTROL DNA	RNA	PROTEIN
NONE	MEAN	1.75	2.28	11.7	142	100	100	100
	SE	0.07	0.12	0.4	6	5	3	4
IMPAIRED	MEAN	1.83	2.58	11.2	143	113	96	100
	SE	0.08	0.31	0.4	8	14	4	5
OZONE	MEAN	1.88	2.29	10.9	138	101	93	97
	SE	0.15	0.14	0.6	8	6	5	6
OZONE + IMPAIRED	MEAN	2.11	2.29	11.0	137	101	94	96
	SE	0.20	0.16	0.8	6	7	7	4
CA AEROSOL	MEAN	1.72	2.44	11.9	153	107	102	107
	SE	0.07	0.08	0.4	6	4	3	4
CA AEROSOL + IMPAIRED	MEAN	1.85	2.24	10.3	134	98	88	94
	SE	0.10	0.11	0.4	11	5	3	8
CA AEROSOL + OZONE	MEAN	1.99	1.86	9.8	132	82	84	93
	SE	0.11	0.12	0.4	4	5	3	3
CA AEROSOL + OZONE + IMPAIR	MEAN	2.07	1.97	9.7	124	86	83	87
	SE	0.06	0.06	0.2	8	3	2	6
LT AEROSOL WITH SO_2	MEAN	1.80	2.26	11.4	141	99	98	99
	SE	0.08	0.25	0.5	4	11	4	3
LT AEROSOL WITH SO_2 + IMPAIRED	MEAN	1.76	2.22	12.0	141	97	103	99
	SE	0.05	0.21	0.4	7	9	3	5

APPENDIX E

Lung Clearance and Permeability Nuclear Medicine Data

Table E-1: ^{99m}Tc Imaging Activity and Rat Uptake

EXPOSURE	ACTIVITY(mCi)		Initial Lung Burden (CPM) X ± SE	
	DTPA	Fe_2O_3	DTPA ± SE	Fe_2O_3 ± SE
CL-1	200	350	1663 ± 181	5236 ± 230
CL-2	300	250	15906 ± 1427	24439 ± 254
CL-3	760	240	38957 ± 1063	15979 ± 1799
CL-4	277	370	15796 ± 1362	27714 ± 3743
CL-5	300	275	18682 ± 1982	22627 ± 2656
CL-6	353	369	23368 ± 1548	36276 ± 3367
CL-7	400	377	26135 ± 1934	15355 ± 2132

Table E-2: Exposures CL-2 to CL-6 DTPA and Iron Oxide Clearance Half Times (hours) (MEAN ± S.E., N=3)

TREATMENT		EXPOSURE#(*)	DTPA	IRON OXIDE
		CL-2	69.6±13.3	26.6±5.6
NONE		CL-3	52.5±6.2	52.1±2.6
(UNEXPOSED)		CL-4	65.1±6.1	32.5±19.8
(HEALTHY)		CL-5	48.5±5.7	120.1±87.7
		CL-6	75.6±12.0	50.9±17.9
		CL-2	48.5±4.7	27.9±9.5
		CL-3	58.4±1.2	32.1±4.2
IMPAIRED		CL-4	55.0±15.0	44.0±21.7
(UNEXPOSED)		CL-5	59.8±8.4	54.4±9.1
		CL-6	58.8±14.1	70.2±21.0
		CL-2	50.8±5.2	18.9±3.6
OZONE		CL-3	77.3±26.1	44.4±24.9
(HEALTHY)		CL-6	69.5±22.0	28.4±4.3
		CL-2	94.6±37.2	24.4±8.1
OZONE		CL-3	64.6±10.1	13.6±5.4
(IMPAIRED)		CL-6	70.3±24.8	40.5±9.1
	CA	CL-2	47.1±2.0	25.3±5.3
	CA	CL-3	62.4±9.6	81.0±24.8
AEROSOL	LT	CL-4	63.9±3.1	29.4±5.7
(HEALTHY)	LT	CL-5	58.6±5.0	218.3±123.8
	LT	CL-6	54.1±12.1	52.7±2.7
	CA	CL-2	50.8±2.2	34.1±10.2
	CA	CL-3	62.4±14.3	27.0±3.5
AEROSOL	LT	CL-4	65.3±3.8	35.4±10.5
(IMPAIRED)	LT	CL-5	49.1±6.7	76.6±15.2
	LT	CL-6	56.3±11.3	57.5±5.7
	CA	CL-2	42.6±3.1	20.3±4.3
AEROSOL + OZONE	CA	CL-3	61.8±4.1	48.2±35.1
(HEALTHY)	LT	CL-6	59.4±14.9	49.7±9.8
	CA	CL-2	62.1±21.1	24.2±5.7
AEROSOL + OZONE	CA	CL-3	65.5±6.2	33.5±1.0
(IMPAIRED)	LT	CL-6	52.1±3.3	75.9±23.7
SO2 (1 ppm)		CL-4	125.5±65.3	47.0±17.9
(HEALTHY) (21 ppm)		CL-5	62.1±10.1	60.0±22.1
SO2 (1 ppm)		CL-4	61.5±3.1	66.1±24.8
(IMPAIRED) (21 ppm)		CL-5	74.0±7.4	72.7±58.3
AEROSOL + SO2	LT	CL-4	71.6±9.6	43.4±10.7
(HEALTHY)	LT	CL-5	89.2±16.9	409 ±230
AEROSOL + SO2	LT	CL-4	54.5±10.9	43.4±10.7
(IMPAIRED)	LT	CL-5	49.3±7.5	76.5±32.3

(*) IRON OXIDE IN EXPOSURE CL-3 & CL-5 N=2

Table E-3: Exposure CL-7 (30 day) DTPA and Iron Oxide Clearance Half Times (MEAN ± S.E., N=3)

*TREATMENT	DTPA (minutes)	IRON OXIDE (**) LONG (hours)	IRON OXIDE (**) SHORT
NONE (H)	45.3±4.2	90.2±27.5	0.44±0.03
IMPAIRED (I)	48.2±5.0	97.0±18.8	0.56±0.38
OZONE (H)	51.4±15.5	64.1±24.8	0.30±0.20
OZONE (I)	68.0±6.9	40.6±4.9	0.37±0.09
CA AEROSOL (H)	53.3±8.3	126 ±47	0.70±0.25
CA AEROSOL (I)	59.5±13.0	274 ±165	1.03±0.27
OZONE & CA AEROSOL (H)	45.5±1.6	48.0±9.1	0.36±0.15
OZONE & CA AEROSOL (I)	53.4±6.4	58.2±4.5	0.47±0.15
SO2 & LT AEROSOL (H)	53.6±4.3	126 ±50	0.90±0.40
SO2 & LT AEROSOL (I)	53.2±3.5	118 ±51	0.50±0.40

* (H) : HEALTHY, (I): IMPAIRED
**LONG: LONG RETENTION PART OF FITTED DOUBLE-EXPONENTIAL FUNCTION.
SHORT: SHORT RETENTION PART OF FITTED DOUBLE-EXPONENTIAL FUNCTION.

NON PARAMETRIC MANN WHITNEY ANALYSIS OF VARIANCE
FOR THE LONG COMPONENT HALF TIME FOR INSOLUBLE IRON OXIDE PARTICLES

Treatment		N	MEDIAN	P
Ozone	-	18	113.6	
	+	12	47.8	0.0056
Aerosol	-	12	63.5	
	+	18	66.4	ns
(without ozone)		12	143.0	0.096
Impaired	-	15	66.0	
	+	15	66.9	ns

Significant interactions:

Aerosol x Ozone