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Effect of Ambient Air Pollution on the Lung and Immune System

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

AIR RESOURCES BOARD
Research Division

EFFECT OF AMBIENT AIR POLLUTION ON THE LUNG AND IMMUNE SYSTEM

Final Report

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ABSTRACT

We extended our prior studies of nitrogen dioxide (NO_2) and ozone (O_3) effects on the mouse lung and immune system to tests of the ambient outdoor atmospheres of two communities, Los Angeles (LA) and Santa Barbara (SB). LA often exceeds ozone, nitrogen dioxide, and other pollutant standards, whereas exceedences in Santa Barbara are relatively infrequent. Two additional animal groups were included in the comparisons. One group was maintained in an air conditioned vivarial room in LA, and the other in the same room but in an environmental chamber having an air supply filtered for particulates and oxidants.

During the 1985 summer test period, the LA test site exceeded the 1985 State ozone standard (0.1ppm O_3 one hour average; 1985) 20 of the 43 days and had four O_3 episodes ($\geq$0.2ppm), vs 2 exceedences for Santa Barbara and no episodes. NO_2 levels in LA did not exceed the 0.25 ppm one hour standard for California, but they averaged 0.1 ppm, and there were 9 days when 0.15 ppm one hour maxima averages were reached or exceeded. The on-site O_3 recordings were less due to partial "scrubbing" by the vivarial facility, but there were nevertheless 5 exceedences of the 1985 0.1 pmm O_3 standard. Statistically significant differences in lung measurements were found between the LA and SB animals, including more and larger Type 2 Cells for the LA animals, thicker alveolar walls, increased elastic tissue, and an altered relationship of elastic fibers to the alveolar wall. All of the foregoing are common denominators and early events in many destructive diseases of the human lung. Their greater frequency in the lungs of the LA animals, compared to those of the Santa Barbara group, suggests that LA air pollution accelerates irreversible "wear and tear" injury to the lung, i.e. increases the rate of decline of lung structure and function. The long term effect is to reduce the level of lung reserves. For the human population, an inordinate depletion of lung reserves will cause, promote, facilitate, and/or exacerbate clinical disease in general.

The reserves of the immune system may also have been adversely affected. In particular, the LA animals had smaller spleens and lower percentages of splenic T lymphocytes than did the Santa Barbara animals. Some degree of immunosuppression of the LA animals is implied by the findings, and this would further suggest some increase in susceptibility to disease in general.

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DISCLAIMER

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SUMMARY

We extended our prior studies of nitrogen dioxide (NO_2) and ozone (O_3) effects on the mouse lung and immune system to compare the ambient atmosphere of Los Angeles (LA) with that of Santa Barbara (SB), communities with high and low levels of air pollution, respectively. The mice were exposed continuously to outside air in partially enclosed vivaria. Two additional groups of mice were maintained in an air conditioned room in LA, with one housed in an environmental chamber that excluded particulates and oxidants, i.e. Room Air (RA) and Chamber (C) groups, respectively. Official monitoring stations in LA and in Santa Barbara, each approximately two miles from the respective vivaria, provided air pollution data. Facilities for on-site monitoring were limited to O_3 for the LA animals, and O_3 plus NO_2 for the RA and C groups.

The North Main (Central) station in Los Angeles recorded an O_3 maximum one hour average of 0.11 ppm for the 43 day period, with a high of 0.30ppm. Exceedences of the State standard for ozone (0.1ppm one hour average, 1985) occurred on 20 of the 43 days (46%), and four days reached episode levels ($\geq$ 0.2ppm). On-site LA O_3 recordings were lower, maximum one hour average of 0.05 ppm, a high of 0.23 ppm, and five exceedences. The RA facility maximum one hour recordings averaged 0.04 ppm, with no exceedences, and the C group had $\leq$ 0.01ppm levels of NO_2 and O_3. The Santa Barbara station reported an O_3 maximum one hour average of 0.06 ppm, a high of 0.12ppm, and two exceedences. For NO_2, the maximum one hour average in LA at the monitoring station was 0.10 ppm, and the dosage 57.8 ppm/hrs for the 43 days, vs .03 ppm and 16.5 ppm/hrs for Santa Barbara. There were no exceedences at either station. The maximum one hour NO_2 average at the indoor Los Angeles facility (RA) was .044 ppm (16/43 days), and the highest was 0.1 ppm with no exceedences. For the C animals, levels were $\leq$ 0.01 ppm NO_2. Other regulated pollutants (carbon monoxide, sulfur dioxide, suspended particulates) had maximum hourly averages between two and four times higher in LA.

Image analysis measurements of lungs detected significant differences primarily between the LA and Santa Barbara groups. The LA animal lungs had greater numbers of Type 2 Cells ($p<.05$), larger Type 2 cells (mean field or total cell area, $p<.01$; mean cell area; $p=.06$), and increases in alveolar wall area or thickness ($p=.001$) as well as perimeters (irregular or wavy internal surface area; $p=.001$). Also, total elastin area (per lung field) was greater for the LA animals ($p=.03$), as were number, mean size, intercepts, perimeter ($p<.06$ to $p<.1$), and ratio of elastic fiber perimeter to alveolar wall perimeter ($p<.05$). The RA and C groups also tended to have greater measurements than the SB animals, but to a lesser extent.

The LA animals had the smallest spleens and the lowest **spleen weight to body weight** ratios. The studies of subpopulations of lymphocytes and lung cancer metastasis produced inconclusive results. **Lung volumes** did not differ significantly.

The implication from the lung findings is that air pollution in LA raised both the level of damage and the rate of decline of lung reserves. The Type 2 cell alterations found are known to reflect damage to the alveolar epithelium, and the alveolar wall alterations, including the increase in elastin, are believed to be a mixture of vascular congestion, edema, and connective tissue alterations. Pertinently, reports from studies of animals exposed to ambient levels of NO_2 indicate that both the cell and the wall alterations are not entirely reversible. Of further pertinence, all human adults experience a decremental loss of lung function with time, and there is increasing evidence from lung function surveys and the rising rate of lung disease (especially emphysema) that the rate of loss is rising. The present findings, with the support of prior data, suggest that air pollution is to some extent accelerating the depletion of lung reserves. However, further study is needed to establish the magnitude and pathologic significance of the acceleration.

RECOMMENDATIONS

Our main recommendation is that the animal model system used in the study just completed be applied to a testing of a number of environments in the South Coast Los Angeles County area, as listed under air monitoring sites (e.g. Azusa, Burbank, Hawthorne, North Main Los Angeles, etc.). The basic question asked is: can the animal model system demonstrate a relationship between areas in Los Angeles County of contrasting air quality and alterations of the mouse lung as demonstrated in this study? If differences can be demonstrated, is there a correlation between the amount of lung alteration and one or more of the Criteria Air Pollutants? Are the alterations reversible when the animals are placed in a filtered air environment after being housed in the ambient air of selected communities?

The need for a pathobiological monitor (animal model in particular) was recognized many years ago with the setting up of animal colonies along the various freeways (27,28). However, the methodologies available then (20 years ago) were relatively insensitive, i.e. dependent on the production of damage evident by routine gross and routine histopathologic examination rather than measurements reflecting daily injury and reserve depletion. With the advances made in quantitative image analysis, it is now possible to achieve inventories of cell subpopulations and tissue components, which in effect provide objective quantitation of the cellular ecology as well as the early lesions that are the common denominators of disease in the mammalian lung.

A study of long term effects is needed with respect to the effects of air pollution on both the animal and human lung. The rate of decline of lung function and structure can rise exponentially without present day methods of detection recognizing that rise until the greater part of lung reserves have been irreversibly expended. For the animal lung, an inventory of acinic units and the terminal bronchioles supplying them is the most sensitive means of appraising the magnitude and pathobiologic significance of the rate of lung structural decline. The mouse lung is most appropriate for an initial study. Specific pollutants and ambient air itself can be tested using image analyis quantitation, and sampling is a relatively simple problem considering the relatively small number of alveoli in the mouse lung, an estimated 50.000 vs the estimated 350 million to be found in the human lung. Correlative human studies are nevertheless critically needed since the emphysematous patient, the classical example of severe reserve depletion, may have sustained a loss of 15,000 or more alveoli per day for 30 to 40 years before symptoms appear.

BODY OF REPORT

A. SCOPE, PURPOSE AND BACKGROUND

In earlier studies, exposure of mice to ambient concentrations of nitrogen dioxide (NO_2) and/or ozone (O_3) resulted in lung alterations ("early lesions") that are common to diverse kinds of human lung disease, in particular Type 2 cell hyperplasia (increased number) and hypertrophy (increased size), and also increased alveolar wall thickness (1-5). Undoubtedly, some of the alterations represent irreversible damage since there is a decremental loss of lung function and structure (lung reserves) in all mammalian lungs. Moreover, a number of human epidemiologic and pathologic studies (discussed below) indicate that air pollution does have an adverse impact on the rate of loss of lung reserves. The purpose of the present study was to compare the relative numbers of putative "early lesions" in the lungs of mice in cities of contrasting levels of air pollution, namely Los Angeles and Santa Barbara. The finding of more cell and tissue alterations in the lungs of mice housed in an outdoor Los Angeles environment, compared to those maintained in an equivalent Santa Barbara outdoor facility, would imply a relatively greater amount of lung injury, a greater absolute amount of irreversible damage, and a consequent increase in the "usual" decremental loss of lung reserves. Just how biologically meaningful is the impact of air pollution on the mouse lung is a question that is not a part of the experimental design of this study. However, finding that air pollution does impact on lung reserves is a first and important step towards establishing pathological significance for human health. In effect, the study is a test of the cellular ecology of the mouse lung, and any perturbation found carries the same ominous potential as a perturbation of the macroecology. The finding of no differences between community atmospheres is highly desirable. Conversely, differences that are statistically different raise the question of an inordinate rate of health reserve depletion for that community.

The present study applied a mouse model system to test the effects of ambient air in central Los Angeles on the lungs and immune system, with comparison to tests of Santa Barbara housed animals. Two additional test sites were included: the indoor air of a vivarial room and an environmental chamber in the same vivarial room. The filter system of the chamber removed particulates, NO_2, O_3, and other pollutants.

B. **Rationale and Objectives**

The selection of a six week experimental period during August and September was estimated to provide NO_2 and O_3 dosages comparable to those shown in prior experiments to cause alterations of Type 2 Cells and alveolar walls. More specifically, our prior tests provided total dosages of 63.0 ppm-hours of either NO_2 or O_3, i.e. 0.3 ppm x 7hr/day x 5 days/week x six weeks. The mean dosage for O_3 over a three year period for Los Angeles for the month of August and for the first half of September was 36 ppm-hours (CARB data 1982, 1983 & 1984). For NO_2 during the same period, the mean dosage was 62 ppm-hours, a dosage essentially identical to the 63.0ppm-hours of our earlier report (3).

The significance of testing for Type 2 cell and alveolar wall alterations is their value as indicators of early pathologic lesions. A number of our tests of ambient levels of NO_2 and O_3, singly and in combination, demonstrated cell and tissue alterations of the mouse lung (1-5), and the alterations have been shown to be very early signs of lung damage to the alveolus in general, including Type 1 and Type 2 cells (6-9) and alveolar capillaries (10-12). We also incorporated in the present study measurements of the numbers and size of elastic fibers in the alveolar wall. Regeneration of damaged alveolar linings (epithelial Type 1 and 2 Cells, and endothelium) is contingent on the integrity of the underlying lung scaffolding, and elastic tissue is a major part of that scaffolding. In addition, elastic tissue plays a major role in lung compliance, i.e. the recoil of the inspiratory-expiratory cycle. Therefore the finding of elastic tissue abnormalities implies some increase in the decremental, irreversible loss of lung function and structure taking place in all mammalian lungs. Pertinently, our long term experiments indicated that the cell and tissue alterations induced by NO_2 had not reversed after our longest test period, namely 32 weeks post-exposure (3).

We anticipated that air pollution in Central Los Angeles would cause alterations in the mouse lung that would be significantly greater than those in the lungs of mice exposed to the less heavily polluted atmosphere of Santa Barbara. Further, we expected that the RA animals Air would have changes intermediate between the SB and LA groups, and that the least alterations would be found in the C animals having an air supply filtered for particulates, NO_2 and O_3 in particular. In the latter respect, we did consider the possibility of suboptimal air clearance of bedding contaminants (e.g. ammonia build-up) and some adverse effect on the lungs of the animals.

MATERIALS AND METHODS

a. Animals

Four groups of weanling male Swiss-Webster mice ("Viral antibody free"; COBS CFW; Charles River) were used for the morphometric studies of lungs and T-cell populations. Over 100 animals were housed at each test site in identical plastic cages with open wire tops, and with 4 mice per cage. The animals were fed a standard diet and water ad libitum. The cages were changed three times a week. The food and bedding for the cages in all three Los Angeles sites and in the Santa Barbara facility were derived from a common source. The water supply was that used at each facility. For the lymphocyte bioassay experiment, 110 five week old C57 Bl/6J (Jackson Laboratories) were used.

b. Animal Facilities

1) The Central Los Angeles (LA) animal group was housed in the Livingston Annex Vivarium at the USC-Los Angeles County Hospital Medical Center. The facility afforded a room approximately 20' x 30' where one entire wall could be completely opened to the outside air by removal of two hinged metal doors. A wire enclosure and insect screen served as a protective outside barrier.

2) The Santa Barbara Vivarium (SB) is an approximately 1600 square foot area with a large door-like portal open to the outside air except for a wire mesh insect screen. This facility is located on the grounds of the University of California at Santa Barbara and was used with the cooperation of a member of the faculty, Dr. Steven Horvath.

3) The HMR Vivarium (RA) was located in the Hoffman Research Building on the University of Southern California Health Science Campus. It is an approximately 400 square foot facility supplied with non-recirculating, fiberglas filtered and temperature controlled air.

4) Environmental Chambers (C animal group) were also located in the same room of HMR Vivarium used for housing animals which breathed room air. The 40 ft^3 environmental chambers in the Hoffman facility are constructed of stainless steel. The laminar air flow was maintained at approximately one change per minute. The air in the chambers was doubly filtered, i.e. particle and potassium permanganate impregnated aluminum oxide filters were used in tandem.

Temperature and humidity recordings were made on site within the room housing the animals.

c. **Air Pollution Monitoring**

1) The Los Angeles (LA) Animal Group:

There were two sources of monitoring data, one being a nearby monitoring station and the other an on site detector for ozone. The nearby air monitoring station, located on North Main Street in Los Angeles and two miles east of this facility, provided data on ambient levels of ozone and nitrogen dioxide for the Central Los Angeles area. Recordings of NO_2 and O_3 levels at the North Main monitoring station were obtained from the CARB Aerometric Division publication (Volume XVII). The North Main air monitoring station is the responsibility of the South Coast Air Quality Management District.

Dasibi ozone detector and recorder were used for the monitoring of on site ozone levels. The instrument was regularly standardized and serviced by personnel from the California Air Resources Board at El Monte. The only on site pollutant measured at this facility was ozone.

2) Santa Barbara Outside Air Animal Group (SB)

The data on ambient levels of ozone and nitrogen dioxide came from the Goleta air pollution monitoring station in Santa Barbara which is approximately two miles east of the campus of the University of California at Santa Barbara. Recordings of NO_2 and O_3 levels at the Goleta monitoring station were obtained from the CARB Aerometric Division publication (Volume XVII). The station is the responsibility of the Santa Barbara County Air Pollution Control District.

3) Indoor Los Angeles Animal Group (RA)

Both NO_2 and O_3 were monitored for the final 16 days of the exposure, when instrumentation became available. NO_2 levels were recorded using a liquid Saltzman instrument (Beckman) and a TECO chemiluminescence detector. Several fritted bubbler tests were used to check the readings over the test period. The ozone levels were monitored by a Dasibi unit that was programmed to provide sequential readings of air in the room and air in the two environmental chambers. Dwell time for each reading was 15 minutes, with three readings per cycle. The instruments

were standardized and serviced regularly by personnel from the California Air Resources Board (CARB, El Monte). The RA facility is two miles from the North Central air monitoring station, and is less than one half mile from the facility housing the LA animals.

4) Animals supplied with filtered air (C)

NO_2 and O_3 levels were monitored as described above for the RA animal group. There were two animal groups housed in the environmental chambers, one for the main part of the study (C animals) and one group for mice that were a part of the NK lymphocyte-lung cancer study. The air supplied to the chambers was filtered for particulates as well as for NO_2, O_3 and other oxidants. Some air pollutants are not filtered out, e.g. methane, propane, dimethylamine, and nitrous oxide.

d. **Temperature and Humidity**

Temperature and humidity were recorded continuously at all experimental sites. Humidity was not recorded within the environmental chamber in lieu of the special instrument needed.

e. **Tissue Processing**

(1) Lactate Dehydrogenase (LDH) Study

At the end of the six week exposure period all 441 mice from all four experimental sites were placed in environmental holding chambers. The animals were killed over a four day period with at least 25 animals from each of group by intraperitoneal 0.5 ml sodium pentobarbital (60 mg/ml). The entire lung was removed from the chest cavity with the trachea intact. A miniature bulldog clamp was placed on the left bronchus and the right lung inflated with 6% gelatin (pH 7.2, 37^{o}C) at 25cm water pressure until the volume of the lung approximated the right thoracic cavity volume. Following gelatin inflation, the right bronchus was clamped, sectioned distal to the clamp, and the right lung placed in a refrigerator at 4^{o}C to effect gelation. The separated right lung was then placed on a Mylar sheet, with the hilum down and the apex directed to a marking on the Mylar sheet. The specimen with its Mylar sheet was wrapped in aluminum foil and stored in a deep freeze at -80^{o}C.

The details of the LDH processing have been reported (13). In brief, eight frozen sections of 15uM thickness were obtained from each right lower lobe and stained for LDH. Representative numbers of lungs from each environmental group were sectioned and stained together. The

reaction time of 10 minutes generally converted the tetrazolium salt to an insoluble formazan dye. The sections were fixed in 10% calcium formol, air dried, and mounted in glycerin jelly to provide permanent slide preparations.

2) Elastic Tissue Study

The left lung was inflated with 10% buffered formalin at 25cm water pressure until lung volume approximated the left thoracic cavity volume. After 24 hr. in fixative, the lung volume was measured. The lungs were stored in phosphate buffer. The entire left lung was processed for paraffin embedment, including alcohol dehydration and xylene clearing. The elastic tissue staining procedure has been described earlier. Briefly, four serial sections were stained with aldehyde fuchsin. A Metanil yellow background stain provided the necessary two color high contrast for image analysis. To ensure that lung tissue from different environmental groups was not stained selectively, representative sections from all four animal groups were always stained together.

f) Quantitative Image Analysis

1) Detection

A Cambridge 720 Image Analyzer with a shading corrector and recording terminal (cassette tape and print out) was used to obtain measurements of lung elastin, Type 2 Cells and alveolar wall (14). The levels of detection were set by matching the electronically detected image (electronically "captured" image of the video picture) to the displayed image (simple TV video picture), and by comparing the displayed image with the actual microscopic field. The two image detectors of the analyzer operates on the basis of grey level separation. One detector was set to pick up only very dark objects, i.e. elastic fibers or Type 2 Cells, at a level which resulted in no change between the area of elastic fibers or the Type 2 Cells as seen on the in the video display of the microscopic image, and that observed in the positive electron image displayed by the detector system. The numbers of elastin fibers or Type 2 Cells detected were "fine-tuned" by comparing by manual counting how well the electronic detection matched the "flagging" signals in a selected area of the video display. The detected image was also compared to the field observed through the microscope.

To pick up the lighter objects, a second detector was used for measurement of wall area, its perimeters and linear intercepts. It was set at a level at which it picked up all shades of grey at or above the second setting. Comparisons between video display, electronic image and microscopic fields were made to effect the best representation of the alveolar wall area with minimal background "noise." With the two detector settings set for elastin or Type 2 Cells, and wall area, an automatic program was used for measurement recordings. The quantitative measurements were recorded on cassette tape using a Texas Instrument terminal, were then "captured" on floppy disks through a computer program, and finally formatted and corrected in preparation for statistical analysis. A 10X ocular and 16x objective were used for the quantitation of both the Type 2 cells and elastin fibers. One technician carried out each individual experiment to ensure consistency in the image analysis quantitation.

2) Quantitation

Image analysis quantitation was used to detect and compare lung field architecture in terms of number of Type 2 Cells and elastic fibers, alveolar wall, Type 2 Cells and elastic fiber areas, their perimeters and intercepts, and Type 2 Cell and elastic fiber aggregates.

The criteria for acceptability were absence of pathological lesions, relative absence of bronchovascular structures, and technically satisfactory tissue areas for the most part.

(a) **Image Analysis Quantitation of Type 2 Pneumocytes and Alveolar Walls**

The Type 2 cell and alveolar wall quantitation methodology has been reported earlier (22,35). In brief, a 10x microscope objective provides approximately 400 Type 2 pneumocytes per lung field. There were eight measurements per field, three fields per slide, six slides per lung. The measurements included Type 2 cell number, Type 2 cell area, Type 2 linear intercepts, alveolar wall area, alveolar wall intercepts, and alveolar wall perimeter.

The deposits of formazan reaction product at the sites of LDH activity are essentially selective for the Type 2 pneumocyte under the conditions of the tissue section preparation: the cell is exceptionally large and round, uniquely occupies alveolar corners, and is the only cell in the alveolar wall with a heavy perinuclear cytoplasmic deposit. Other alveolar wall cells, Type 1 pneumocytes, endothelial cells, fibroblasts and macrophages have greatly attenuated cytoplasm, and the finely granular, diffuse LDH reaction products in their cytoplasm are not detected by the Image Analyzer at the grey level value of the detector set for the Type 2 cell. Further, desquamated and rounded macrophages in the alveolar lumina are essentially excluded from the

quantitation by the gelatin washout. The bronchial mucosal cells in the conducting airways are also strongly positive for LDH. However, these structures are readily identifiable and, when present, are excluded from the lung field by an image editor. A weak background staining of the alveolar wall serves to delineate the walls for measurement by a second detector grey value setting of the image analyzer.

In addition, ratio determinations of Type 2 cell number to alveolar area to alveolar area were calculated. The ratio of the cell number to alveolar wall area helps to determine whether the changes in the cell number represent actual increase in the number or merely reflect collapse of air spaces.

For image analysis quantitation, four lung sections from each animal were used. Each lung section was divided into apical, middle and basilar sectors. The first lung field within each quadrant meeting set criteria was selected for analysis. A total of 18 measurements (nine duplicates) were recorded per each field.

Image Analysis Quantitation of Elastin and Alveolar Wall

The entire left lung was used for quantitative measurements of elastin fibers and alveolar walls. Four lung sections were used per each animal. Each lung section was divided into three sectors which represented apical, middle and basilar portions of the right lower lobe. The first field in each sector that contained only alveoli, alveolar sacs, small bronchioles and blood vessels and was free of technical imperfections was used to obtain measurements from control and exposed animals in an alternating sequence. Fields containing medium, large blood vessels and conducting airways in which lining cells could be clearly detected were considered technically unacceptable. The measurements included both the edited fields (in which the blood vessels and their supporting elastic structures were excluded) and unedited fields. The measurements of elastin included number of fibers and elastin area, with and without sizing factors, and also elastin linear intercepts and perimeters. The quantitative measurements of the alveolar wall included area and perimeters.

A metanil yellow counterstain for aldehyde fuchsin stained all of the alveolar wall except for the elastic fibers. The vascular bed and its blood cell content were included in the wall measurements. For the elastin measurements, the image editor was used to exclude bronchovascular structures, and both edited and unedited values were recorded.

g. **Lung volume measurements**

The formalin fixed left lung was submerged in a previously tared volume of water. A balance sensitive to 1.0mg was used for the volume determinations. The weight recorded was translated directly into lung volume (15).

h. **Spleen weights**

In view of our earlier findings (16,17), spleen weights were recorded and spleen to body ratios were calculated.

i. **T Lymphocyte Quantitation**

The relative numbers of T **Lymphocyte subpopulations** (18) were obtained by random sampling of spleens from five animals of each group. Spleen cell suspensions were centrifuged in basal salt solution and the lymphocyte subpopulation labeled by means of three monoclonal antibodies, Lyt 1, Lyt 2, and Thy-1.2. Aliquots of the spleen cell suspensions were analyzed by means of a fluorescence activated cell sorter (FACS).

j. **Bioassay for Spleen Natural Killer (NK) Cells**

The spleens from five animals from each treatment group were tested. The cell suspensions were prepared using stainless 60 mesh screens (18,19). The red cells were lysed using distilled water, the remaining spleen cells from the experimental and control groups were pooled separately for each group, and the cells counted. The spleen cells from each treatment group were added to melanoma cell monolayers in T flasks in a ratio of approximately 50 lymphocytes to one melanoma cell, and incubated for four hours at 37 ^{o} C with gentle mixing every hour. Following incubation, the spleen cells were washed off and melanoma cell suspensions were prepared using versene, and also Ca^{++}, Mg^{++} free balanced salt solution. After washing, the cells were counted and groups of 20 five week old C57Bl/6J unexposed male mice were injected intravenously with $1x10^{4}$ cells from each treatment group. The controls received the same number of untreated melanoma cells, and otherwise were handled identically. This bioassay tested for the ability of melanoma cells to proliferate in vivo, and is considered to be superior to cytotoxicity assays (20) in vitro since it has been shown that NK cells may render melanoma cells cytostatic without lysis (21).

The Melanoma Cell Cultures procedure was as follows: B16F10R4 melanoma cell cultures were carried in this laboratory according to a previously established protocol (19). In brief, all tissue cultures were maintained in plastic tissue culture T flasks in RPMI-1640 medium supplemented with 10% fetal calf serum. The medium was changed twice weekly and the cultures were transferred once a week. For experimental needs the cells were collected from the flasks using Ca^{++} and Mg^{++} free Earle's balance salt solution (EBSS) containing 0.025% EDTA. The single cell suspensions were washed with culture medium and resuspended in EBSS. The animals were injected intravenously, the 10^4 melanoma cells in a 0.2 ml volume suspension being infused via the tail vein. All recipient mice were housed in a filtered air environmental chamber until three weeks after injection, at which time their lungs were examined for the numbers of cancer nodules that had become "seeded" from the IV injection.

The **Evaluation of Melanoma Nodules in the Lungs** was carried out following a three week period. The animals were killed by intraperitoneal pentobarbital. The lungs were removed and inflated with 10% acetate buffered formalin and stored in the same fixative. The melanoma nodules (black) were clearly visible on the surface of each lobe of the lung and were counted utilizing a stereo microscope. Histopathological sections of the lungs were prepared for evaluation.

k. **Statistical analysis and data processing**

The decision to use 100 animals for each test group was based on experience from earlier experiments where large numbers of animals were needed to detect significant differences. On the other hand, past experience with the mouse melanoma metastasis model indicated that a smaller group size, 20 animals each, would suffice.

The statistical analysis was carried out in our own laboratories, using a statistical package (BMDP Statistical Software). The statistical analyses included Student's t test (2 tail p values), one factor analysis of variance, and nonparametric tests (Mann-Whitney U or rank sum test, and Kruskal-Wallis one way analysis of variance). The nonparametric tests were applied when the distribution of the data was not normal. Statistical differences were considered significant at the 95 percentile level, just short of the 95 percentile when p is $\leq .06$, and of borderline significance if $p<.1$. For the most part, results noted with p values between .05 and 0.1 represent trends that have reached significant levels in related studies.

RESULTS

1) Ambient Ozone and Nitrogen Dioxide Levels

NO_2 and O_3 data were obtained from CARB Air Quality Report XVII No.3 for July, August, and September of 1985, and from copies of records received from the Santa Barbara Air Pollution Control District.

The North Main (Central) station in Los Angeles recorded an O_3 maximum one hour average of 0.11 ppm for the 43 day period, with a high of 0.30ppm and a low of 0.02. The 24 hour readings averaged 0.03 ppm for the six weeks, with the highest level 0.09 ppm and the lowest 0.01 ppm. Exceedences of the State standard for ozone (0.1ppm one hour average, 1985) occurred on 20 of the 43 days of the test period (46%). Episodes levels ($\geq$ 0.2ppm) were reached on four days. On-site LA O_3 recordings (partially enclosed vivarium) were lower, a maximum one hour average of 0.09 ppm, a high one hour maximum of 0.23 ppm, and five exceedences of the 0.10 ppm State standard (1985). The RA facility maximum one hour recordings averaged 0.04 ppm, with no exceedences, and recordings for the environmental chambers showed both NO_2 and O_3 to be $\leq$.01 ppm. At the Santa Barbara (Goleta) station, the O_3 maximum one hour average for the 43 day test period was 0.06 ppm (highest 0.12ppm and lowest 0.02ppm), and the mean hourly average was 0.03 ppm. There were 2 days of the 43 with exceedences of the 0.1 ppm 1985 standard (or 5 of 43 by the 0.09 ppm standard).

The maximum one hour NO_2 average for the North Main Los Angeles monitoring station was 0.1ppm, with a range of 0.03 ppm to 0.23 ppm. There were no 0.25 ppm one hour exceedences (State standard) in Los Angeles during the 43 day test period, but there were 9 days when the 0.15 ppm 24 hr average was exceeded. At the Santa Barbara station, the maximum one hour daily average for NO_2 was 0.03 ppm, with a range of 0.02 ppm to 0.07 ppm. There were no State standard (one hour or 24 hour) exceedences. The maximum one hour NO_2 average at the indoor Los Angeles facility (RA) was .044 ppm for the 16 days instrumentation was available, with the highest 0.1 ppm, and no exceedences. Instrumentation for on-site measurements of NO_2 was not available for either the LA or SB animal groups. Other regulated pollutants (carbon monoxide, sulfur dioxide, suspended particulates) were also substantially higher in LA, e.g. one hour maxima averages two to four times greater in LA. Tables 1 & 2 provide details on comparisons of pollutant levels.

2) **Temperature and Humidity**

Temperature readings combined from all sites ranged from 66°F to 92°F, with the lowest mean temperature being 70°F for the RA animals, and the highest 75°F at the LA group site (66°F-92°F). The mean temperature at Santa Barbara was 73°F, with a range of 66°F to 80°F.

Mean humidity ranged from a low of 63% for the LA animals to 74% for the indoor Los Angeles facility (HMR Vivarium; RA) Table 3. The mean humidity for the Santa Barbara facility was 71% and ranged from 45 to 90%.

3) **Animal Survival**

Survival ranged from 94% for the SB animals to 100% for the C group. Pneumonia was grossly evident in three of the C animals, in six RA animals, in six SB animals, and in nine of the LA animals, Table 4.

4) **Type 2 Cell and Alveolar Wall Study (Frozen sections of lung and lactate dehydrogenase enzyme staining of Type 2 Cells)**

Image analysis of 389 mouse lungs was carried out using the right lower lobes from 99 LA mice, 96 SB mice, 98 RA mice, and 96 C mice.

For the outdoor Los Angeles group, 396 lung sections were prepared with 1,021 technically acceptable fields which yielded 18,378 data points. For the Santa Barbara group, 18,486 data points were obtained from an analysis of 1,027 fields derived from 384 lung sections. For the RA group, an analysis of 1,078 fields derived from 392 lung sections gave 19,404 data points. For the C animals, a total of 18,198 data points were obtained by analyzing 1,011 fields in 384 lung sections.

a) **Mean Type 2 Cell Area**

The values for Mean Type 2 Cell Area for the C animals, and for those housed in Santa Barbara, were essentially the same, 71 and 72, respectively (Table 5). The values for Mean Type 2 Cell Area of the LA and RA groups were higher than the other two animal groups, i.e. 75 and 74, respectively. Ratio comparisons of Type 2 Cell Area with Alveolar Wall Area showed no significant differences (Table 6).

A Mean Type 2 Cell difference was found between the LA and SB animals, Los Angeles > Santa Barbara, p=0.06. The difference was at a highly significant level (p=0.01) when Mean Total Field Area measurements (a measure that combines Type 2 Cell Area and Numbers of Type 2 Cells) were compared. Mean Type 2 Cell Area was greater for Santa Barbara animals in comparison to the RA animals, at a borderline level of significance, p=0.0962. Table 5.

b) **Mean Type 2 Cell Numbers**

Type 2 Cell Numbers were quantitated according to size, i.e. the total number of Type 2 cells (>8 um), Type 2 Cells >10 um, and Type 2 Cells >12 um. For all three cell sizings, the Type 2 Cell population was statistically greater in numbers in the lungs of Santa Barbara animals when compared to the C animal group, and this was also the case for the LA animal group when compared to the SB animals, Table 7. The ratio of Type 2 Cell Number to Alveolar Wall Area was highest for the Santa Barbara animals and lowest for the outdoor Los Angeles animals, the difference between the two groups being significant at the level of p<.09 (Table 8). No other paired groups showed significant ratio differences.

c) **Alveolar Wall Area**

Alveolar wall area was lowest for the Santa Barbara housed animals. Statistically significant differences were found between the Santa Barbara group and all other sites, namely LA animals (p=.0012); RA animals, (p<.0507); and C group (p=.0507). The data are summarized in Table 9.

d) **Alveolar Wall Perimeter**

Table 10 summarizes the findings for both perimeters and ratios of perimeter to alveolar wall area. The highest perimeter value was recorded for the LA animals, and the lowest for the SB animals. A number of highly significant statistical differences were found, especially between Santa Barbara and Los Angeles outdoor animals (p=0.001). Ratios of alveolar wall perimeter to alveolar wall area were also significantly different. The highest ratio was found with the Santa Barbara group; the LA and C animals had identical values at the lowest end of the ranking.

e) **Alveolar Wall Linear Intercepts**

Table 11 summarizes data for both linear intercepts and ratios of linear intercepts to alveolar wall area. The outdoor Los Angeles animals had the highest value, and the Santa Barbara group the lowest. The latter difference was at a borderline level of statistical significance, p<.09. However, the reverse was true for the ratio determinations of linear intercepts to alveolar wall

area; the highest value was found in the Santa Barbara group and the lowest, as with the perimeter ratio above, was shared by the LA and C animals. The differences between the Santa Barbara animals and the two lowest groups were highly significant, p=.03 (SB) and p =.02 (C).

5) **Quantitative Image Analysis, Elastic Tissue Study.**

Quantitative image analysis was done on 378 formalin fixed and inflated mouse lungs. Specifically, left lungs from 63 LA mice, 74 SB animals, 72 RA mice, and 75 C mice. The quantitative study was done on paraffin embedded sections that required prior dehydration and then clearing in xylol.

Data were obtained on numbers and perimeters of elastic fibers, the areas of elastic fibers according to seven sizing factors (size categories of fibers), and measurements of the alveolar wall area and its perimeter. Briefly, for the LA group, 400 sections with 1,007 technically acceptable fields yielded 15,105 data points. For the Santa Barbara group, 13,080 data points were obtained from analyzing 1,068 fields in 356 sections. The analysis of 376 sections with 983 fields gave 14,745 data points for the RA animals. For C group, a total of 15,360 data points were obtained by analyzing 1,024 fields in 380 lung sections.

There were a number of elastic tissue measurements showing differences between groups, in particular the comparison of the LA animal group and the animals housed in the in Santa Barbara facility.

a) **Mean Elastic Fiber Area**

The mean elastic fiber area, the ratio of elastic fiber area to elastic fiber number, was greatest for the LA animals, and the lowest for the SB animals. Comparison of elastic fiber areas between the four groups showed that the mean elastic fiber area for the LA animals was greater than for the animals housed in Santa Barbara (p=0.088), Table 12.

b) **Mean Elastic Fiber Field Areas**

Tables 13,14 summarize the data on Mean Elastic Field Area measurements, a combined measure of size and number. For three different fiber size measurements, the outdoor Los Angeles animals consistently had the largest measurements, and the Santa Barbara group the smallest. All of the differences between the outdoor Los Angeles and Santa Barbara groups were statistically significant (p=.03, p<.05, and p=.03, with increasing size of fibers).

c) **Mean Elastic Fiber Number, Mean Elastic Fiber Linear Intercepts, and Mean Fiber Perimeters**

Table 15 summarizes the findings. The elastic fiber numbers varied slightly between groups, and the differences were not statistically significant. Both intercept and perimeter measurements were greatest for the LA animals, and least for those in Santa Barbara, $p<.07$ and $p<.06$ respectively. The ratio of elastic fiber perimeter to alveolar wall perimeter was lowest for the SB animals, and nearly as low for the C animals, $p<.05$ when each was compared with the LA animals.

d) **Mean Unedited Wall Area and Mean Unedited Elastic Fiber Area and Perimeter**

Table 16 and 17 summarize the data for the unedited wall area and elastic fiber area. The analyses of data for all of the foregoing tended to parallel the edited data analyses. As noted earlier, editing is done to exclude bronchovascular structures from the analysis field.

e) **Alveolar Wall Area, based on Paraffin Sections of Lung Tissue**

The lowest alveolar wall area value was derived from measurements of lungs of the RA animals, and was of borderline significance when compared to the findings for the C group ($p<.07$; Table 18). Other paired groups showed no statistically significant differences. Alveolar wall area was also less for the RA animals when elastic tissue area was subtracted from the alveolar wall area. The decrease was at a borderline level of statistical significance when compared to the alveolar wall areas in lungs from C and SB animals ($p=0.07$ and $p=0.06$, respectively), Table 18.

f) **Alveolar Wall Perimeter**

Table 18 summarizes the data on the Alveolar Wall Perimeter. No significant differences were observed.

6) **Natural Killer (NK) Lymphocytes**

The NK cell activity assay showed that Santa Barbara animals had more active NK cells. This was particularly true when compared to NK cell activity for RA animals, Table 19.

7) **Subpopulations of Lyt-1 lymphocytes**

In general, there were lower percentages of T cells and their subtypes for LA and RA animals. However, the only significant difference was observed between RA and C animals with respect to Lyt2 lymphocytes (Table 20).

8) **Mean spleen weights** were essentially the same for all groups except for the LA animals, the latter being 77.8 mgs as opposed to 88.3 mgs for both the RA and C groups, and 92.6 mgs for the Santa Barbara animals, Table 21.

9) **Spleen Weight to Body Weight Ratio**

The ratio of **spleen weight to body weight** ratios was also lowest for the LA animals, and highest for the Santa Barbara animals. The differences were statistically significant for all groups compared except the RA vs C comparison, Table 21.

10) **Lung volumes** varied from a low of 141.9 ul (LA) to a high of 149.5 ul(RA). The mean lung volume for the Santa Barbara animals was 143.0 ul, and for the C group 146.7 ul, Table 22.

11) **Mean Body Weight**

Mean body weight at the end of the experiment ranged from 27.8 Gms (SB) to 29.3 Gms (C). The environmental chamber animals also had the highest average weight gain over the six week period, 14.01 gms (92%), and the Santa Barbara animals the lowest weight gain, 12.45 gms (81%), Table 23.

DISCUSSION:

The main findings of the study, alterations of lung alveolar cells and tissues, follow trends demonstrated in studies of the mammalian lung in general. The trends were demonstrated in tests of individual pollutants that we and others have carried out (1-8) and in studies of human lung disease (22). Specifically, measurements made with frozen section preparations (right lung) showed that the Los Angeles (LA) animal lungs had greater numbers of Type 2 Cells ($p<.05$), an increase in Type 2 cell field (total) area ($p=.01$), an increase in alveolar wall area ($p=.001$), and an increase in the perimeter of the alveolar wall ($p=.001$). Several differences that were just short of the 95 percentile level warrant attention since they too are in line with trends demonstrated repeatedly in several earlier studies of exposures to ambient levels of NO_2 and/or O_3. In particular, there were increases for the LA animals relative to those in Santa Barbara (SB) in both mean Type 2 cell number ($p=.06$) and mean Type 2 cell size ($p=.06$). While the latter two measurements were just short of the 95 percentile, each tends to be reinforced by the fact that the combined measurement of number and area of the Type 2 cells, i.e. total Type 2 cell area per lung field, was highly significant ($p=.01$). The Type 2 cell changes are now widely accepted as a sign of damage and/or loss of the more delicate Type 1 cell (6,22) and the alveolar wall area increase is at least partly due to congestion and edema (9) in association with increased capillary permeability (10-12). The increase in overall area of the Type 2 cells indicates that gas exchange is to some extent less efficient due to the greater amount of cytoplasm now present between the air spaces and capillaries.

With respect to other group comparisons, few significant differences were found. In line with the expected intermediate response for the Room Air (RA) animals, we found an increase in alveolar wall area that was less than that for the LA animals (NS) but greater than that for the SB group ($p=.05$). We anticipated that the animals in the environmental Chamber (C) would also have less alterations than all three of the other groups. This was true for the C vs LA comparison, but C and RA measurements were essentially equal, and C vs SB was greater rather than less at a significant level ($p<.02$). A relative increase in Type 2 cell numbers for the C animals, compared to the SB group, was near significance ($p=.06$), but no other Type 2 cell difference was found. The increase in number and not mean size may mean less injury, i.e. when both size and number increase, the cells tend to coalesce, accentuating mean size and reducing mean number. Why the environmental chamber group (C) had more lung alteration than did the Santa Barbara animals cannot be explained by the data. Since cage cleaning was carried out every forty eight hours, excessive ammonia build-up is unlikely. Neither infection nor infestation was evident, and no animals were lost to attrition.

The elastic tissue data, derived from the paraffin embedded lung tissue preparations as opposed to the frozen section data above, also suggested that ambient air pollution of Los Angeles altered the mouse lung, i.e. all elastin measurements (number, size, intercepts, and perimeter) were greater for the LA animals. While significance was obtained only with two measurements, total area per field ($p=.03$) and the **ratio** of elastic fiber perimeter to alveolar wall perimeter ($p<.05$), most differences were at borderline levels of significance, e.g. average fiber size ($p<.09$), fiber perimeter ($p<.06$), and fiber intercept ($p<.07$). All of the foregoing are in accord with elastin increases that follow diverse kinds of lung injury, including drug induced fibrosis (23-25). They are also consistent with the elastin fragmentation and defective linkage of resynthesis that has been reported (26-27). In the latter respect, little is known about metabolic disturbances in elastin synthesis and pulmonary fibrosis (28). Of further pertinence, elastase models of emphysema have shown an increase in lung elastin consequent to an initial decrease (29), and the aging skin shows a continuous increase in elastic fiber surface area and elastin itself as elasticity paradoxically decreases (30). The fiber perimeter/wall perimeter ratio difference has special significance in that it is evidence against atelectasis as the sole cause of the increase in elastin. The lung volume measurements also argue against a predominant role for atelectasis since no significant differences were found between the LA and SB animal groups. However, some atelectasis may be involved, especially considering the predominance of the injury at the proximal acinar site where cell and tissue swelling can readily obstruct air flow to the distal portions of the acinus. It is also pertinent to note that the Type 2 cell itself may be altered by the air pollution, and that an alteration of surfactant production and/or lung content by injury to the Type 2 cell would contribute to atelectasis.

The lack of a difference in alveolar wall area with the formalin fixed tissue (in contrast to the results of the frozen section study) is most likely related to a procedural difference, i.e. the extraction of edema fluid by the paraffin embedment procedure. Whereas frozen sections preserve protein rich edematous fluid as well as a variety of lipid substances, paraffin embedment shrinks tissue by as much as 50% through the removal of water soluble and fatty substances. As we and others have shown, exposure to ambient levels of air pollutants leads to an increase in capillary permeability and a protein-rich type of edematous change. Edema of cells and alveolar walls following exposure of animals to ambient levels of NO_2 has also been observed directly by electron microscopic studies (9,31). Edematous change that tends to predominate in the proximal portion of the acinus might explain the reversal in the frozen section based ratio of alveolar wall perimeter to alveolar wall area (SB> LA; $p<.02$). NO_2 and O_3 preferentially injure the proximal portions of the acinus and edema tends to predominate in peribronchiolar areas

(32,33). Both would tend to favor greater increases in the alveolar wall area than in alveolar wall perimeters, thereby reducing the ratio. However, we emphasize the need for additional data as well as confirmation.

The magnitude of the NO_2 dosage that the animals would have received if they had breathed LA air at the North Main air monitoring station would have been comparable to that for the earlier studies, i.e. 58.2 ppm-hrs (.0564 ppm hourly average x 24hrs x 43 days) for LA vs 63 ppm-hrs (92%) for the NO_2 exposure experiments (0.3 ppm NO_2 x 7 hr/day x 5 days/wk x 6 wk) (3). The comparable O_3 dosage would have been 32.3 ppm for the present study vs 63 ppm-hrs (57%) for the prior experiment (4). Interestingly enough, we had anticipated 36 ppm/hrs O_3 dosage based on records for the past three years, and we experienced 32.3 ppm/hrs. For NO_2, the expected was 62 ppm/hrs and the actual 58.2 ppm/hrs. Since the monitoring station was two miles away from the LA vivarial site, and on-site monitoring was not a part of this preliminary study, the actual dosages of NO_2 and O_3 received by the animals is not known. However, the lower on-site O_3 recordings for the LA animals (e.g. one hour maximum O_3 in LA of .10 ppm vs .05 ppm on-site average) confirms the expected reduction in actual O_3 dosage for the LA animals. A comparable reduction should have occurred at the Santa Barbara on-site facility, and perhaps a greater reduction because of the lower initial level. Scrubbing of pollutants by the partial enclosure of both the LA and SB vivaria was especially expected for O_3 considering its highly reactive nature. The reduction was not due to lower ambient levels of air pollutants since monitoring station ringing the LA test site had either equal or higher pollutant levels.

From the foregoing it is evident that air pollution was relatively high at the LA monitoring station, in particular a ten fold greater number of O_3 exceedences compared to Santa Barbara as well as four vs no O_3 episodes ($\geq$.20 ppm). While exceedences of the one hour State standard for NO_2 (0.25 ppm) did not occur in LA, there were nine days when a 0.15 ppm hourly average was reached or exceeded, vs none for Santa Barbara. However, a 24 hour average of .15 ppm, a marker for episode declaration, was not reached. Nevertheless, the NO_2 dosage for the 43 days at the monitoring station site was 58.2 ppm/hrs, which compares to the 63 ppm/hrs (92%) applied in prior NO_2 experiments where similar cell and tissue alterations were produced. At the Santa Barbara station, the dosage was 15.6 ppm/hrs, or roughly one fourth. In the absence of on-site O_3 data for the SB animals, comparisons with the LA on-site recordings cannot be made, but scrubbing of the pollutants at the SB vivarium should have been comparable, and perhaps even greater considering the lower ambient levels. Most importantly, reduced pollutant levels for the SB animals implies there were few if any high peak values. Pertinently, experimental animal data have linked the "spikes" of pollutant exposures to the degree of injury

that occurs. In the latter respect, we noted that the mean hourly dosage for NO_2 over the 43 day test period was 0.056 ppm for LA and 0.016 ppm for Santa Barbara, an elevation that on average was 3.5 times greater. Of further pertinence, the mean hourly value for O_3 differed little between LA and SB (0.031 ppm vs 0.029 ppm, respectively), but there were marked disparities between LA and SB in maximum hourly averages (0.10 ppm vs 0.06 ppm, respectively) and especially in exceedences of the State standard. The foregoing add support to the concept that repeated high concentrations dictate the greater part of the adverse effect.

The overall significance is that all of the alterations are commonly found in human lung disease in general. They represent early events in the evolution of pathologic lesions and they contribute to a decremental depletion of lung reserves. The rate of depletion varies according to environmental circumstances and host susceptibility. Finding a greater amount of cell and tissue alteration in the lungs of mice exposed to a high level of air pollution (Los Angeles), in comparison to animals housed in a community with a much lower level of air pollution (Santa Barbara), suggests that air pollution in Los Angeles has caused an increase in the daily decremental loss of lung structure and function. In support of some degree of reserve depletion are prior animal studies showing that comparable levels of NO_2 exposure had not entirely reversed several months to as long as 27 months after exposure had been stopped (4,9). Just how significant a portion of murine lung reserve has been depleted cannot be answered without further studies for confirmation and expansion of the findings. The answers that can be obtained with the animal model have an immediate bearing on human lung reserves. A decremental loss of lung function and structure is ubiquitous in the well population, and there is recent evidence suggesting that air pollution may be accelerating the decremental loss in Los Angeles County (34). Moreover, a pathologic study has shown a relationship between human emphysema and air pollution in a comparison of two midwestern states (35).

The immunologic findings of significance were found mainly in the spleen data. The LA animals had exceptionally small spleens compared to all other groups, and the differences in mean spleen weights for the LA animals were statistically significant when paired with all other groups. In addition, the ratios of **spleen weight to body weight** were lowest for the Los Angeles animals and highest for the Santa Barbara animals ($p=.0005$).

Splenic lymphocytes from each animal group, which are comprised in part of Natural Killer lymphocytes (NK cells), were mixed with mouse melanoma (pigmented tumor) cells and tested for an enhancing or inhibiting effect on lung seeding (metastasis or colonization). No significant differences were found although the comparison between the Santa Barbara and RA groups was just short of the 95 percentile ($p<.06$). There were also near significant differences between un-

treated melanoma cells (no prior addition of lymphocytes) and melanoma cells exposed to lymphocytes from the Santa Barbara and LA groups ($p<.06$ and $p=.07$, respectively), with the least colonization by cancer cells (metastasis) in the lungs of the Santa Barbara animals. The trend towards the reverse of that expected, enhanced anti-tumor activity of lymphocytes, is very uncertain in view of the wide variation of the data in general. However, a related study, 10 ppm NO_2 exposure of mice and the effect on immune function has shown a biphasic response, i.e. enhanced immunological activity for short term exposure but suppression for long term the reverse over the long term (36). The short term exposure period also has critical meaning for the cancer cell metastasis model. In the several prior experiments with exposure of mice to comparable levels of NO_2, differences in lung metastases were not observed before 12 weeks of exposure (37,38), whereas the experimental design for the present experiment required testing at the end of six weeks of exposure. There is the possibility that a test of a larger number of animals may afford the statistical strength to demonstrate effects earlier than the 12 weeks so far required.

We also found that the differences observed in the study of subpopulations of lymphocytes were inconclusive, which contrasts with our earlier experience with NO_2 exposure (39). However, two differences warrant mention: 1) the percentage of the **Lyt-1** subpopulation varied from a high of 46% for Santa Barbara to a low of 36% and 37% for the indoor (RA) and outdoor (LA) Los Angeles animals, with the environmental chamber animals (C) intermediate at 42%. 2) The Santa Barbara animals also had the highest percentage of **Lyt-2** lymphocytes, with the indoor and outdoor Los Angeles groups the lowest and next to the lowest respectively. The difference between the indoor Los Angeles (RA) and chamber (C) groups was statistically significant ($p=.05$), whereas significance between the LA and Santa Barbara animals appeared to be precluded by the wide variation of the data for the Santa Barbara animals.

Animal attrition generally occurs to some extent. Attrition for the Santa Barbara colony amounted to 7 animals, with the precise cause of death undetermined. While the latter was greater than observed for the other groups, the variation is nevertheless within the range found with past experience. Similarly, the incidence of pneumonia was within the variation generally encountered.

Lung volumes, which were determined with formalin fixed left lungs, tended to be lower for the LA and SB groups (141.9 ul and 143.0 uL, respectively) and higher for the RA and C groups (149.5 ul and 146.7 ul, respectively). The LA group, but not the Santa Barbara animals,

differed significantly from both the RA and C groups. The study is considered inconclusive, but warrants additional attention with respect to volume determinations of the unfixed lung and the question of some degree of air pollution induced atelectasis.

In summary, all of the foregoing cell and tissue changes in the lung following Los Angeles air pollution exposure are a part of the "wear and tear" injury that takes place in all mammalian lungs daily. Since there is also a daily decremental loss of function and structure in all mammalian lungs, the implication of added "wear and tear" is an incremental increase in lung reserve depletion. More specifically, the present study, with the support of similar findings from individual air pollution studies, suggests that the alveolar lining cells have suffered some degree of irreversible damage, that the Type 2 cell replacement and alveolar wall thickening have led to some impairment of gas exchange, that the altered elastin has to some extent adversely influenced lung compliance, and that the immune system has also been adversely affected. The damage is presumably very minor considering the relatively short period of exposure and the immense reserves of the lung. However, the results raise the more fundamental question of the effect of relatively minor adverse effects over the long term. Further, the decremental loss of lung structure and function is periodically punctuated by episodes of serious injury, as for example viral infections. This raises the broader question of a relatively minor increment in lung injury having a very large amplifying effect on concomitant injurious events of diverse nature. For the human well population, the cost-benefit assessment of air quality control hinges more on the interactive role that air pollution plays in disease rather than on direct causation, i.e. the magnitude of its effect in promoting, facilitating, and/or exacerbating the decremental loss of lung reserves. The contribution of the present study is that the preliminary findings at the very least indicate that the mouse model system has a high level of sensitivity for detecting small increments in the acceleration of the rate of injury, and should have value as an indicator of relative air quality of community atmospheres. With the further development of inventories through image analysis (Type 2 cells and alveoli in particular), a means of measuring absolute losses (i.e. lung reserve depletion) should be possible.

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ABREVIATIONS

NO_2	- Nitrogen Dioxide
O_3	- Ozone
ppm	- parts per million
pphm	- parts per hundred million
hrs	- hours
C	- Centigrade
mg	- milligram
gms	- grams
ml	- milliliter
um	- micrometer (micron)
ul	- microliter
LA	- Los Angeles
SB	- Santa Barbara
RA	- HMR Vivaria
C	- Environmental Chamber
HMR	- Hoffman Research Building
USC	- University of Southern California
LDH	- Lactic acid dehydrogenase
T2 Cell	- Type 2 Cell
T2A	- Type 2 Cell Area
AW	- Alveolar Wall
AWA	- Alveolar Wall Area
P	- Perimeter
LI	- Linear Intercept
SD	- Standard Deviation from the Mean
Lyt-1	- Helper inducer lymphocyte marker
Lyt-2	- Cytotoxic and suppressor lymphocyte marker (maturation antigen)
Thy-1.2	- Thymus derived lymphocyte marker
NK	- Natural Killer lymphocytes
Ca	- Calcium
Mg	- Magnesium
EDTA	- Ethylene diamine tetraacetic acid
EBSS	- Earle's Balanced Salt Solution

Table 1

OZONE: MAXIMUM HOURLY CONCENTRATIONS (pphm**)
August 2, 1985 - September 13, 1985

Monitoring site	AUGUST																															SEPTEMBER												
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13
Los Angeles*	11	12	12	10	13	14	9	8	10	7	6	7	9	10	7	6	12	13	12	16	13	17	24	30	6	13	17	16	25	21	9	9	2	2	2	5	5	4	2	3	5	13	14
Goleta*	6	9	5	6	7	5	6	7	7	6	6	5	5	6	5	5	5	4	5	7	7	7	8	8	11	9	6	12	10	10	7	7	3	4	4	4	3	2	3	4	4	5	10
On-site LA	9	9	9	8	9	8	1	1	1	1	1	1	1	1	1	1	1	1	7	2	2	1	1	3	1	1	1	11	23	18	9	10	2	1	2	3	3	2	2	3	6	14	13
On-site SB	No on-site O_3 measurements																																										
On-site RA	5	6	6	5	7	6	4	-	-	-	-	-	-	-	3	4	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	4	6	8
On-site C	0	0	0	0	0	0	0	-	-	-	-	-	-	-	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0

State Standard = 0.1 ppm

* From California Air Quality Data, July-August-September 1985, California Air Resources Board Vol XVII(3), pp42, 44

** from the official records; $x10^{-2}$ = ppm

LA - Los Angeles
SB - Santa Barbara
RA - Room Air
C - Environmental Chamber

Table 1A

OZONE CONCENTRATIONS
August 2, 1985 - September 13, 1985

Monitoring Site	Hourly Average ppm		Maximum Hourly Average ppm		Exceedence(s)
	Mean	Range/24 hrs	Mean	Range/1 hr	
*LOS ANGELES, NORTH MAIN STATION	0.03	0.006 - 0.086	0.1	0.02 - 0.3	20
*GOLETA	0.03	0.011 - 0.047	0.06	0.02 - 0.12	2
On-site Los Angeles (LA)	0.01	0.005 - 0.044	0.05	0.01 - 0.23	5

State Standard = 0.1 ppm

* California Air Quality Data, July-August-September 1985
California Air Resources Board Vol XVII(3), pp 42, 44

Table 2

NITROGEN DIOXIDE: MAXIMUM HOURLY CONCENTRATIONS (pphm**)
August 2, 1985 - September 13, 1985

Monitoring site	AUGUST																															SEPTEMBER												
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13
Los Angeles*	13	8	7	13	17	13	9	7	6	3	5	6	8	10	6	5	5	11	11	11	19	21	23	18	8	19	17	21	14	14	8	4	4	5	4	8	4	4	4	5	6	13	17
Goleta*	3	2	2	3	3	2	3	3	3	2	2	3	3	3	3	2	2	3	3	2	3	4	4	4	4	5	-	-	4	6	3	2	2	2	2	4	2	2	2	2	4	5	7
On-site IA	No on-site NO_2 measurements																																										
On-site SB	No on-site NO_2 measurements																																										
On-site RA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	6	6	3	2	3	4	5	4	3	3	3	3	4	7	10
On-site C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* California Air Quality Data, July-August-September 1985, California Air Resources Board Vol XVII(3), pp 42, 44
State Standard = 0.25 ppm

** from the official records; $x10^{-2}$ = ppm

LA - Los Angeles
SB - Santa Barbara
RA - HMR Vivaria
C - Environmental Chamber

Table 2A

NITROGEN DIOXIDE CONCENTRATIONS
August 2, 1985 - September 13, 1985

Monitoring Site	Hourly Average ppm		Maximum Hourly Average ppm		Exceedence(s)
	Mean	Range/24 hrs	Mean	Range/1 hr	
*LOS ANGELES, NORTH MAIN STATION	0.056	0.024 - 0.108	0.1	0.03 - 0.23	0
*GOLETA	0.016	0.011 - 0.047	0.03	0.02 - 0.07	0

State Standard = 0.25 ppm; maximum hourly average
= 0.15 ppm; 24 hour average

* California Air Quality Data, July-August-September 1985
California Air Resources Board Vol XVII(3), pp 42, 44

Table 3

MEAN TEMPERATURE AND HUMIDITY
August 1 - September 13, 1985
(175)

LOCATION		Temperature F			Humidity		
		Mean	SD	Range	Mean	SD	Range
LOS ANGELES	(LA)	75	6.26	66 - 92	63	3.8	30 - 89
SANTA BARBARA	(SB)	72	3.14	66 - 80	71	7.6	45 - 90
HMR VIVARIA	(RA)	70	3.81	67 - 85	74	10.3	38 - 89
ENVIRONMENTAL CHAMBER	(C)	*			*		

* temperature and humidity data not available

Table 4

Animal Status: Ambient Air Exposure Experiment
Swiss Webster Weanling Male Mice
(175)

Experimental Groups		Animal Number			
		Experiment		Percent Survival	Lungs with Pneumonia
		Start	End		
LOS ANGELES	(LA)	121	120	99	9
SANTA BARBARA	(SB)	116	109	94	6
HMR VIVARIA	(RA)	109	108	99	6
ENVIRONMENTAL CHAMBER	(C)	108	108	100	3

Table 5

MEAN TYPE 2 CELL AREA
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 Weeks
(M175)

LOCATION		No Mice	Field Area Mean ± SD	Cell Area Mean ± SD
LOS ANGELES	(LA)	99	11505 ± 3059	75 ± 16
SANTA BARBARA	(SB)	96	10511 ± 2311	71 ± 13
HMR VIVARIA	(RA)	98	11065 ± 2303	74 ± 13
ENVIRONMENTAL CHAMBER	(C)	96	10908 ± 2565	72 ± 15

	p Value (2-tail)	
LA vs C	p=0.1411	p=0.1235
RA vs C	NS	NS
SB vs C	NS	NS
LA vs SB	p=0.0114	p=0.0608
LA vs RA	NS	NS
SB vs RA	p=0.0962	NS

SD - standard deviation

Table 6

MEAN RATIO OF TYPE 2 CELL AREA/ALVEOLAR WALL AREA
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 Weeks
(M175)

LOCATION		No Mice	T2A/AWA Mean ± SD
LOS ANGELES	(LA)	99	1.019 ± 0.300
SANTA BARBARA	(SB)	96	1.005 ± 0.235
HMR VIVARIA	(RA)	98	1.007 ± 0.229
ENVIRONMENTAL CHAMBER	(C)	96	0.984 ± 0.258

	p Value (2-tail)
LA vs C	NS
SB vs C	NS
RA vs C	NS
LA vs SB	NS
LA vs RA	NS
SB vs RA	NS

T2A/AWA - Type 2 Cell Area/Alveolar Wall Area

SD - standard deviation

Table 7

MEAN TYPE 2 CELL NUMBERS
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 Weeks
(M175)

LOCATION	No Mice	Type 2 Cell ≥8 um M ± SD	Type 2 Cell ≥10 um M ± SD	Type 2 Cell ≥12 um M ± SD
LOS ANGELES (LA)	99	153 ± 19	118 ± 13	107 ± 12
SANTA BARBARA (SB)	96	148 ± 18	116 ± 11	104 ± 10
HMR VIVARIA (RA)	98	151 ± 18	117 ± 12	106 ± 11
ENVIRONMENTAL CHAMBER (C)	96	153 ± 19	118 ± 12	107 ± 11

	p Value (2-tail)		
LA vs C	NS	NS	NS
SB vs C	p=0.0639	p=0.0689	p=0.0933
RA vs C	NS	NS	NS
LA vs SB	p=0.0479	p=0.0843	p=0.0962
LA vs RA	NS	NS	NS
SB vs RA	NS	NS	NS

SD - standard deviation

Table 10

MEAN ALVEOLAR WALL PERIMETER
(LDH Study)
Swiss Webster Weanling Male Mice
Ambient air exposure period: 6 weeks
(M175)

LOCATION	No Mice	Perimeter Mean ± SD	P/AW Mean ± SD
LOS ANGELES (LA)	99	35074 ± 4251	3.12 ± 0.46
SANTA BARBARA (SB)	96	33923 ± 4521	3.28 ± 0.49
HMR VIVARIA (RA)	98	34007 ± 5038	3.14 ± 0.55
ENVIRONMENTAL CHAMBER (C)	96	34308 ± 4492	3.12 ± 0.47

	p Values (2-tail)	
LA vs C	NS	NS
SB vs C	p=0.0146	p=0.0201
RA vs C	NS	NS
LA vs SB	p=0.0012	p=0.0178
LA vs RA	NS	NS
SB vs RA	p=0.0507	p=0.0704

P/AW - Perimeter/Alveolar wall area

SD- standard deviation

Table 11

MEAN ALVEOLAR WALL LINEAR INTERCEPT
(LDH Study)
Swiss Webster Weanling Male Mice
Ambient air exposure period: 6 weeks
(M175)

LOCATION	No Mice	LI	LI/AW
		Mean ± SD	Mean ± SD
LOS ANGELES (LA)	99	11035 ± 1493	0.98 ± 0.15
SANTA BARBARA (SB)	96	10656 ± 1605	1.03 ± 0.16
HMR VIVARIA (RA)	98	10679 ± 1713	0.99 ± 0.18
ENVIRONEMENTAL CHAMBER (C)	96	10737 ± 1607	0.98 ± 0.16

	p Value (2-tail)	
LA vs C	NS	NS
SB vs C	NS	p=0.0231
RA vs C	NS	NS
LA vs SB	p=0.0887	p=0.0306
LA vs RA	p=0.1209	NS
SB vs RA	NS	p=0.0933

LI - Linear Intercept
LI/WA - Linear Intercept/Wall Area

SD- standard deviation

Table 12

MEAN ELASTIC FIBER AREA
(Elastic Fiber Area/Elastic Fiber Number)
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 Weeks
(M175)

LOCATION		No Mice	Fiber Area / Fiber Number Mean ± SD
LOS ANGELES	(LA)	53	19.87 ± 5.64
SANTA BARBARA	(SB)	42	18.51 ± 5.23
HMR VIVARIA	(RA)	54	18.62 ± 6.05
ENVIRONMENTAL CHAMBER	(C)	62	19.10 ± 6.61

	p Value (2-tail)
LA vs C	NS
SB vs C	NS
RA vs C	NS
LA vs SB	p=0.0880
LA vs RA	NS
SB vs RA	NS

SD - standard deviation

Table 13
MEAN ELASTIC FIBER FIELD AREAS
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 Weeks
(M175)

LOCATION	No Mice	Elastic Fiber Field Area		
		≥8 um	≥9 um	≥10 um
		Mean ± SD	Mean ± SD	Mean ± SD
LOS ANGELES (LA)	100	18675 ± 6309	13500 ± 4798	10209 ± 3925
SANTA BARBARA (SB)	89	16812 ± 5464	12142 ± 4403	9019 ± 3564
HMR VIVARIA (RA)	94	17515 ± 6815	12156 ± 5385	9346 ± 4257
ENVIRONMENTAL CHAMBER (C)	95	17501 ± 5947	12438 ± 4676	9406 ± 4001

	p Value (2-tail)		
LA vs C	NS	p=0.1191	NS
SB vs C	NS	NS	NS
RA vs C	NS	NS	NS
LA vs SB	p=0.0323	p=0.0449	p=0.0658
LA vs RA	NS	NS	NS
SB vs RA	NS	NS	NS

SD - standard deviation

Table 14
MEAN ELASTIC FIBER FIELD AREAS
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 Weeks
(M175)

LOCATION	No Mice	Elastic Fiber Field Area		
		≥12 um	≥14 um	≥16 um
		Mean ± SD	Mean ± SD	Mean ± SD
LOS ANGELES (LA)	100	5887 ± 2620	4560 ± 2174	704 ± 421
SANTA BARBARA (SB)	89	5198 ± 2503	3998 ± 2087	605 ± 465
HMR VIVARIA (RA)	94	5365 ± 2932	4187 ± 2510	634 ± 516
ENVIRONMENTAL CHMABER (C)	95	5423 ± 2809	4225 ± 2380	638 ± 528

	p Value (2-tail)		
LA vs C	NS	NS	NS
SB vs C	NS	NS	NS
RA vs C	NS	NS	NS
LA vs SB	p=0.0658	p=0.0723	p=0.1253
LA vs RA	NS	NS	NS
SB vs RA	NS	NS	NS

SD - standard deviation

Table 15

MEAN ELASTIC FIBER NUMBERS, PERIMETER AND INTERCEPTS
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 Weeks
(M175)

LOCATION		No Mice	Fiber Number	Fiber Intercepts	Fiber Perimeter	Fiber Perimeter / Wall Perimeter
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
LOS ANGELES	(LA)	100	954 ± 195	5058 ± 1400	16961 ± 4283	0.3315 ± 0.0828
SANTA BARBARA	(SB)	89	922 ± 201	4699 ± 1273	15809 ± 3879	0.2961 ± 0.0836
HMR VIVARIA	(RA)	94	954 ± 234	4819 ± 1497	16257 ± 4519	0.3242 ± 0.0902
ENVIRONMENTAL CHAMBER	(C)	95	948 ± 221	4806 ± 1368	16289 ± 4057	0.3007 ± 0.0784

	p Value (2-tail)			
LA vs C	NS	NS	NS	0.0430
SB vs C	NS	NS	NS	NS
RA vs C	NS	NS	NS	NS
LA vs SB	NS	p=0.0680	p=0.0552	0.0418
LA vs RA	NS	NS	NS	NS
SB vs RA	NS	NS	NS	NS

SD - standard deviation

Table 16

MEAN UNEDITED ELASTIC FIBER MEASUREMENTS
Swiss Webster Weanling Male Mice
Ambient air exposure period: 6 weeks
(M175)

LOCATION		No Mice	Elastic Fiber Area Mean ± SD	Elastic Fiber Perimeter Mean ± SD
LOS ANGELES	(LA)	100	27155 ± 8800	21216 ± 5016
SANTA BARBARA	(SB)	87	24703 ± 7885	19954 ± 7489
HMR VIVARIA	(RA)	94	25006 ± 9827	20298 ± 5623
ENVIRONMENTAL CHAMBER	(C)	94	25609 ± 8423	20399 ± 4665

	p Value (2-tail) Area	p Value (2-tail) Perimeter
LA vs C	p=0.0461	p=0.0822
SB vs C	NS	NS
RA vs C	p=0.1098	NS
LA vs SB	NS	NS
LA vs RA	NS	NS
SB vs RA	NS	NS

Table 17

MEAN UNEDITED ALVEOLAR WALL MEASUREMENTS
(Elastic Fiber Study)
Swiss Webster Weanling Male Mice
Ambient air exposure period: 6 weeks
(M175)

LOCATION		No Mice	Alveolar Wall	
			Area	Perimeter
			Mean ± SD	Mean ± SD
LOS ANGELES	(LA)	100	134781 ± 44042	59012 ± 9802
SANTA BARBARA	(SB)	87	134991 ± 38197	59613 ± 11162
HMR VIVARIA	(RA)	94	125499 ± 39668	59487 ± 8513
ENVIRONMENTAL CHAMBER	(C)	94	138511 ± 50746	58670 ± 10668

	p Value (2-tail)	
LA vs C	NS	NS
SB vs C	NS	NS
RA vs C	p=0.0427	NS
LA vs SB	NS	NS
LA vs RA	p=0.1062	NS
SB vs RA	p=0.0844	NS

SD - standard deviation

Table 18

MEAN ALVEOLAR WALL AREA AND PERIMETER
(Elastic Fiber Study)
Swiss Webster Weanling Male Mice
Ambient air exposure period: 6 weeks
(M175)

LOCATION		No Mice	Wall Area	Wall Area minus Elastic Fiber Area	Perimeter
			Mean ± SD	Mean ± SD	Mean ± SD
LIVINGSTON	(LA)	100	115534 ± 40790	96859 ± 41044	56112 ± 10849
SANTA BARBARA	(SB)	87*	115447 ± 36946	99979 ± 35763	58673 ± 12464
HMR VIVARIA	(RA)	94	107659 ± 36045	90144 ± 35351	55224 ± 7845
ENVIRONMENTAL CHAMBER	(C)	94	118188 ± 44091	100643 ± 44304	56945 ± 12831
			p Value (2-tail)		
LA vs C			NS	NS	NS
SB vs C			NS	NS	NS
RA Vs C			p=0.0747	p=0.0742	NS
LA vs SB			NS	NS	NS
LA vs RA			p=0.1568	NS	NS
SB vs RA			p=0.1530	p=0.0622	p=0.1218

SD - standard deviation

* 86 animals for Wall Area minus Elastic Fiber Area

TABLE 19

INCIDENCE OF MELANOMA NODULES IN LUNG*

Influence of Ambient Air on NK Cell Activity
(175)

Source of Mouse Splenic Lymphocytes** Used for Pretreating B16 Melanoma Cells		Number of Animals	Number Melanoma Nodules	
			Total per Lung	Average per Lung
LOS ANGELES	(LA)	20	567	28.35 + 19.07
SANTA BARBARA	(SB)	20	663	33.15 + 41.45
ROOM AIR	(RA)	20	831	41.55 + 25.80
ENVIRONMENTAL CHAMBER	(C)	19	921	48.47 + 45.67
B16 MELANOMA CELLS	(Untreated)	20	846	42.30 + 23.50

* C57B1/6J female mice - 12 weeks old
** Swiss Webster male mice - 9 weeks old

MANN WHITNEY U-TEST

LA vs C	NS
SB vs C	NS
RA vs C	NS
LA vs SB	NS
LA vs RA	NS
SB vs RA	0.0564

TABLE 20

INFLUENCE OF AMBIENT AIR ON SPLENIC T-LYMPHOCYTE SUBPOPULATIONS

Swiss Webster Male Mice
(175)

LOCATION		N	% T-Lymphocyte Subtypes		
			Thy-1.2	Lyt-1	Lyt-2
LOS ANGELES	(LA)	10	28.88 (2.00)	37.13 (2.07)	6.29 (1.05)
SANTA BARBARA	(SB)	10	34.02 (3.82)	46.44 (4.40)	7.96 (1.26)
ROOM AIR	(RA)	10	29.71 (2.58)	36.13 (3.60)	5.24 (0.85)
ENVIRONMENTAL CHAMBER	(C)	10	33.81 (3.57)	41.98 (2.62)	7.66 (0.95)

Thy-1.2 - marker for all T-lymphocytes
Lyt-1 - marker mainly for helper/inducer T cells
Lyt-2 - marker for suppressor/cytotoxic T cells

MANN WHITNEY U TEST

	Thy1.2	Lyt 1	Lyt 2
LA vs C	NS	NS	NS
SB vs C	NS	NS	NS
RA vs C	NS	NS	0.05
LA vs SB	NS	NS	NS
LA vs RA	NS	NS	NS
SB vs RA	NS	NS	NS

( ) - Standard error of the mean
N - Number of animals

Table 21
Mean Values of Mouse Spleen Weights
and
Mouse Spleen Weight/Body Weight Ratio
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 weeks
(M175)

LOCATION		No Mice	Weight (mgs) Mean + SD	Spleen weight Body weight Mean + SD
LOS ANGELES	(LA)	93	77.8 ± 13.8	0.271 ± 0.042
SANTA BARBARA	(SB)	82	92.6 ± 43.6	0.338 ± 0.163*
HMR VIVARIA	(RA)	87	88.3 ± 17.4	0.305 ± 0.052
ENVIRONMENTAL CHAMBER	(C)	87	88.3 ± 13.0	0.302 ± 0.043

	p Value (2-tail)	
LA vs C	0	0
SB vs C	NS	0.0778
RA vs C	NS	NS
LA vs SB	0.0021	0.0005
LA vs RA	0	0
SB vs RA	NS	0.0778

SD - standard deviation
* ratios of 81 mice

Table 22
Mean Lung Volume
Swiss Webster Weanling Male Mice
Ambient Air Exposure Period: 6 weeks
(M175)

LOCATION		No Mice	Lung Volume (uL) Mean + SD
LOS ANGELES	(LA)	110	141.9 ± 13.5
SANTA BARBARA	(SB)	102	143.0 ± 14.0
HMR VIVARIA	(RA)	105	149.5 ± 18.1
ENVIRONMENTAL CHAMBER	(C)	107	146.7 ± 14.1

	p Value (2-tail)
LA vs C	0.0103
SB vs C	0.0549
RA vs C	NS
LA vs SB	NS
LA vs RA	0
SB vs RA	0.0044

SD - standard deviation

Table 23

MEAN BODY WEIGHTS
Weights at the Start and Termination of the Experiment
Swiss Webster Male Mice
(M175)

LOCATION		ANIMAL WEIGHTS			
		Start of Experiment		End of Experiment	
		No Mice	Mean + SD	No Mice	Mean + SD
LOS ANGELES	(LA)	121	15.2 + 1.9	120	28.5 + 2.6
SANTA BARBARA	(SB)	116	15.4 + 2.9	105	27.8 + 2.9
HMR VIVARIA	(RA)	109	15.3 + 2.0	108	28.9 + 2.2
ENVIRONMENTAL CHAMBER	(C)	108	15.3 + 2.0	108	29.3 + 2.2

End of Experiment	p value (2-tail)
LA vs C	0.02
SB vs C	0
RA vs C	NS
LA vs SB	0.05
LA vs RA	NS
SB vs RA	0.002

SD - standard deviation