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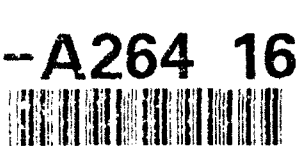

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The second year of this project concentrated on using a "high" dose of JP-8 jet fuel in our exposure regimen. We selected a target dose of approximately 1,000 mg/m^3 based on a published epidemiological study conducted at NATO Air Force Bases that demonstrated jet fuel concentrations as high as 1,020 mg/m^3 during refueling operations. The rats in the "high" dose studies were exposed to an average of 813.8 mg/m^3 for one hour/day for 7 and 28 days. In our previous work, a "low" dose concentration of JP-8 jet fuel (500 mg/m^3) for one hour/day for 7 and 28 days did not show any significant changes in lung structures by light microscopy. However, when light microscopy was performed on lung sections from rats exposed to JP-8 jet fuel for 7 and 28 days at the "high" dose concentration, the evidence for injury to the alveolar-capillary barrier was overwhelming. In these rats, we observed red blood cells in the alevolar air spaces, distortion of the bronchial airways, and loss of epithelial cells in the alveoli. These findings were substantiated by electron microscopy which showed epithelial cells missing their basement membrane, airways devoid of cilia, and alterations of type II alveolar epithelial cells.

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THE CHRONIC EFFECTS OF JP-8 JET FUEL EXPOSURE ON THE LUNGS

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SUMMARY ABSTRACT

The second year of this project concentrated on using a "high" dose of JP-8 jet fuel in our exposure regimen. We selected a target dose of approximately 1,000 mg/m^3 based on a published epidemiological study conducted at NATO Air Force Bases that demonstrated jet fuel concentrations as high as 1,020 mg/m^3 during refueling operations. The rats in the "high" dose studies were exposed to an average of 813.8 mg/m^3 for one hour/day for 7 and 28 days. In our previous work, a "low" dose concentration of JP-8 jet fuel (500 mg/m^3) for one hour/day for 7 and 28 days did not show any significant changes in lung structures by light microscopy. However, when light microscopy was performed on lung sections from rats exposed to JP-8 jet fuel for 7 and 28 days at the "high" dose concentration, the evidence for injury to the alveolar-capillary barrier was overwhelming. In these rats, we observed red blood cells in the alveolar air spaces, distortion of the bronchial airways, and loss of epithelial cells in the alveoli. These findings were substantiated by electron microscopy which showed epithelial cells missing their basement membrane, airways devoid of cilia, and alterations of type II alveolar epithelial cells.

Rats pretreated for six days with capsacin (25 mg/kg, s.c.) before an extremely "low" dose of JP-8 jet fuel (190 mg/m^3) for one hour/day for 7 days demonstrated the same pathological evidence of lung injury as the "high" dose rats. We have previously demonstrated that capsaicin pre-treatment before 500 mg/m^3 JP-8 jet fuel for one hour/day for 7 days caused a huge increase in airway sensitivity to histamine. Thus, our pathological evidence confirms our earlier findings that capsaicin pre-treatment before JP-8 jet fuel exposure potentiates the lung injury induced by jet fuel exposure.

We also were interested in investigating the effects of chronic JP-8 jet fuel inhalation exposure on secondary organ injury, primarily the liver. There was a significant increase in relative liver/body weight ratio between the 7 and 28 day "high" JP-8 jet fuel-exposed groups and their longitudinal controls. Furthermore, there was a significant increase in relative liver/body weight ratio between the "low" JP-8 and "high" JP-8 groups at 7 and 28 days.

We have continued to develop the congenic mouse model for use in our JP-8 jet fuel exposure studies. Specifically, we have developed histamine challenges for measurement of airway sensitivity and adapted our computerized pulmonary function system to measure pulmonary function parameters in the mice.

STATEMENT OF WORK

There will be a total of 372 rats utilized in the study. The rats will be divided into the following groups-

(1) Baseline Control, (N=12). These rats will be killed at the start of the study to establish baseline values on all the parameters to be examined in the study.

(2) Longitudinal Control, (N=180). These rats will undergo exposure to sham air.

(3) JP-8 Jet Fuel-Exposed, (N=180). These rats will be exposed to either of the three concentrations of JP-8 jet fuel (30 mg/m^3, 300 mg/m^3, or 1020 mg/m^3) for one of the four exposure time periods (1 day, 7 days, 28 days, or 56 days).

The parameters we will study are the following-

(1) ^{99m}TcDTPA pulmonary epithelial permeability of each rat.

(2) Lung mechanics of dynamic and static lung compliance, work of breathing, power of breathing, respiratory time constant, and lung resistance on each rat.
(3) Measure lung eicosanoids, TNF, IL-1, and Substance P in nine rats in each group.
(4) Pathologic studies of wet lung weight/body weight ratio, electron and light microscopy in three rats in each group.
(5) Alveolar macrophage studies in nine rats in each group.
(6) Pharmacological blocker studies of Substance P, N=6 for each JP-8 jet fuel exposure group and their corresponding longitudinal control group, N=6.

The proposed study will take three years to complete. We believe it is essential to standardize our study timetable as much as possible to minimize the effects of variables such as seasonal variations of temperature, humidity, and pollen count on our rat population. Consequently, we propose to complete 124 rats/year for the three years of the study with equal numbers of rats from each group completed in each of the yearly time sequences.

WORK IN PROGRESS

Light microscopic analysis of rats breathing at "high" dose exposure JP-8 levels of 813.8 mg/m^3 show overt signs of airway and parenchymal damage. Bronchii are convoluted and red blood cells are observed filling many of the alveolar air spaces. Electron microscopy showed segments of the alveolar-capillary membrane devoid of endothelial cells as well as segments of epithelial loss (Figure 1). Type II cells are also observed in various stages of degeneration and with increased vacuolization. Lungs of rats with capsaicin pretreatment and a very "low" ,190 mg/m^3, JP-8 jet fuel dose also demonstrate light and electron microscopic evidence of lung injury. Interstitial thickening is observed in light micrographs. Electron microscopy demonstrates interstitial edema, endothelial injury, and alveolar type II cells in degeneration (Figure 2). In addition, segments of the alveolar epithelium are lifted from the basement membrane. There were no significant differences between JP-8 exposed groups for wet lung weight/body weight ratio or pulmonary epithelial clearance of the radioactive tracer technetium-labeled diethylenetriamine pentaacetate (^{99m}TcDTPA, physical half-life of 6.02 hours, MW= 492 daltons). Our Substance P consultant, Dr. Susan E. Leeman, is presently conducting assays for neuropeptides including Substance P on the "high" dose rats who underwent bronchoalveolar lavage instead of lung fixation for pathology studies.

We also conducted histamine challenges on a group of "high" dose rats and their PC150% was 9.1 X 10^2 mg/ml histamine. This PC150% was similar to that of rats pretreated with capsaicin before JP-8 jet fuel exposure. The PC150% for the capsaicin pretreated "low" dose JP-8 jet fuel exposed rats was 7.0 X 10^2 mg/ml histamine. In other words, it is the combination of capsaicin pretreatment with JP-8 jet fuel inhalation or "high" dose JP-8 jet fuel alone that causes the greatest histamine responsiveness, as determined by PC150%.

We also were interested in investigating the effects of chronic JP-8 jet fuel inhalation exposure on secondary organ injury, primarily the liver. There was a significant increase in relative liver/body weight ratio between the 7 and 28 day "high" (813.8 mg/m^3) JP-8 jet fuel dose and their longitudinal control groups. Furthermore, there was a significant increase between the "low" (500 mg/m^3) and high dose JP-8 jet fuel groups at 7 and 28 days. The data are the following-

Group	Liver/Body Weight Ratio
Baseline Control (N=18)	0.04093 (0.00104)
7 Day Low Dose JP-8 (N=22)	0.36705 (0.00035)
28 Day Low Dose JP-8 (N=17)	0.32038 (0.00029)
7 Day Longitudinal Control (N=14)	0.36510 (0.00058)
28 Day Longitudinal Control (N=8)	0.31537 (0.00036)
7 Day High Dose JP-8 (N=11)	0.38755 (0.00063)*
28 Day High Dose JP-8 (N=8)	0.36376 (0.00059)**

Data are mean (SEM). $^{*}p < 0.0001$ between 7 day High Dose JP-8 group and 7 day Longitudinal Control and 7 day Low Dose JP-8 groups. $^{**}p < 0.0001$ between 28 day High Dose JP-8 group and 28 day Longitudinal Control and 28 day Low Dose JP-8 groups.

Similar changes in organ/body weight ratios were observed for the kidney and spleen.

Our work in Year 2 with the congenic mouse model has been to develop histamine airway challenge and pulmonary function tests by modifying our techniques used in the Fischer 344 rats. We have demonstrated that the congenic mice have significantly less variability in their pulmonary function measurement of inspiratory resistance compared to their parent strain C57BL6 mice by a statistical technique called bootstrap. The data are the following-

Group	Inspiratory Resistance (cm H_2O/L/sec)
Congenic Mice (N=21)	3227 (193)
C57BL6 Mice (N=8)	3313 (594)

Data are mean (SEM).

The statistical analysis demonstrated that the substantially lower SEM in the congenic mice was from a different population than the SEM for the C57BL6 group despite the differences in sample size. Thus, we conclude that the congenic mice have less variability in their inspiratory resistance measurements due to their similar genetic make-up.

PUBLICATIONS FROM SECOND YEAR OF PROJECT

(1) Witten ML, Pfaff JK, Lantz RC, Parton KH, Chen H, Hays A, Kage R, Leeman SE: Capsaicin pretreatment before JP-8 jet fuel exposure causes a large increase in airway sensitivity in rats. REGULATORY PEPTIDES, 1992, S1:S176.

(2) Witten ML: Chronic effects of JP-8 jet fuel exposure on the lungs. GOVERNMENT REPORTS, ANNOUNCEMENTS, & INDEX, Issue 17, 1992.

(3) Witten ML, Figueroa JT, McKee JL, Lantz RC, Quan SF, Sobonya RE, Lemen RJ: Fractal and morphometric analysis of lung structures after canine adenovirus-induced bronchiolitis in beagle puppies. PEDIATRIC PULMONOLOGY (in press).

(4) Pfaff JK, Parton K, Lantz RC, Chen H, Hays AM, Witten ML: Chronic exposure to JP-8 jet fuel causes lung injury in Fischer 344 rats. JOURNAL OF APPLIED TOXICOLOGY (accepted pending minor revisions).

(5) Parton KH, Pfaff J, Hays AM, Witten M: Effects of JP-8 jet fuel inhalation on the liver of F-344 rats. THE TOXICOLOGIST, 1993, 13:83.

(6) Pfaff J, Parliman G, Parton K, Lantz R, Chen H, Hays A, Witten M: Pathologic changes after JP-8 jet fuel inhalation in Fischer 344 rats. THE FASEB JOURNAL, 1993, 7:A408.

(7) Pfaff J, Parton K, Lantz R, Chen H, Carter D, Witten M: Large dose JP-8 inhalation causes decreased pulmonary compliance in Fischer 344 rats. AMERICAN REVIEW OF RESPIRATORY DISEASE (in press).

PARTICIPATING PROFESSIONALS

(1)	Mark L. Witten, Ph.D. University of Arizona College of Medicine	Principal Investigator
(2)	Susan E. Leeman, Ph.D. Boston University College of Medicine	Consultant
(3)	Robert C. Lantz, Ph.D. University of Arizona College of Medicine	Co-Investigator
(4)	Dean E. Carter, Ph.D. University of Arizona College of Pharmacy	Consultant
(5)	John K. Pfaff, M.D. Lt. Commander, U.S. Navy Medical Corps	Fellow
(6)	Kathy H. Parton, D.V.M. University of Arizona College of Pharmacy	Master's Student
(7)	Huizhong Chen, M.D. Jiangxi Medical College, Nanchang, China	Visiting Scientist
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(9)	Dr. James R. Halpert University of Arizona College of Pharmacy	Consultant
(10)	Richard E. Sobonya, M.D. University of Arizona College of Medicine	Consultant
(11)	Jason T. Figueroa University High School	High School Student
(12)	Brian J. Tollinger University of Arizona College of Pharmacy	Research Associate

(13) Robert P. Erickson, M.D. Consultant
University of Arizona College of Medicine

Advanced Degrees Awarded:

(1) Dr. Parton is currently pursuing a Master's degree in the Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona. She is scheduled to defend her Master's thesis on the effects of JP-8 jet fuel on secondary organs on April 27, 1993.

(2) Allison M. Hays is currently pursuing a Doctoral degree in the Department of Anatomy, College of Medicine, University of Arizona.

COUPLING ACTIVITIES

We have assisted Captain Donald R. Tocco, a research toxicologist, from the Armstrong Aerospace Medical Research Laboratory at Wright-Patterson Air Force Base in setting up his laboratory to perform ^{99m}TcDTPA pulmonary clearance studies and computerized pulmonary function tests in rats.

DISCOVERIES, INVENTIONS, PATENT DISCLOSURES, AND SPECIFIC APPLICATIONS

There have been no discoveries, inventions, patent disclosures and specific applications generated from the Air Force project at this point in time.

RESEARCH ACCOMPLISHMENTS AND PLANS FOR YEAR 3 OF THE CURRENT PROJECT

We believe that we have accomplished a large amount of work related to the important findings of Year 1 of the project despite losing a group of rats and approximately 1.5 months of time devoted to their exposure regimen due to the outbreak of salivary gland virus in the rat colony at the University of Arizona. We have conducted the "large" dose studies of JP-8 jet fuel exposure for both 7 and 28 days of exposure. In contrast to the "low" JP-8 jet fuel studies of Year 1, we found a "high" dose of JP-8 jet fuel exposure to cause significant lung injury. We are very interested in the dose-response relationship between JP-8 jet fuel exposure and lung injury. The difference between our "low" and "high" jet fuel concentrations was only a 60% increase. We also find it very interesting that capsaicin pre-treatment before an extremely low JP-8 jet fuel exposure seems to mimic the lung injury observed in the "high" dose JP-8 jet fuel groups. We believe this is more indirect evidence that Substance P and other related neuropeptides may play very significant roles in the maintenance of pulmonary epithelial integrity both in the airway and alveolar airspaces. We plan to examine lung neutral endopeptidase concentrations in Year 3 of the current project. To this end, we have reconditioned our high pressure liquid chromatography system to conduct these assays.

We will conduct the long-term (56 days) exposures to JP-8 jet fuel in Year 3 of the current project both at the "low" and "high" dose levels. In addition, we have talked

with Dr. Walter J. Kozumbo, Program Manager of the Directorate of Life and Environmental Sciences of the U.S. Air Force Office of Scientific Research, about the possibility of developing techniques to measure JP-8 jet fuel metabolites in the blood and urine of our 56 day exposure groups. We will attempt to develop these techniques for possible use in Air Force personnel.

We have purchased an exposure system almost identical to our present rat set-up for use in our congenic mouse model of jet fuel exposure. Previously, we used our present rat exposure chamber for the congenic mouse studies. Consequently, these studies were whole-body exposures for the mice compared to nose-only studies for the rats. Our new exposure system will allow us to use a nose-only exposure for our congenic mice. We have also demonstrated that the congenic mice have significantly less variability in their pulmonary function measurements compared to their parent strain C57BL6 mice. Thus, we will continue to develop the congenic mouse model in our JP-8 jet fuel experiments in Year 3 of the current project.

FIGURE LEGENDS

FIGURE 1. Transmission electron micrograph. Alveolar septum (A) in a Fischer 344 rat after "high" dose exposure to JP-8 jet fuel. Proteinaceous material and red blood cells (R) are observed in the alveolar space. The epithelial surface is missing, as exposed basement membrane is observed (large arrow). The capillary endothelium is disorganized (small arrow).

FIGURE 2. Transmission electron micrograph. The alveolar space (A) in a "high" dose JP-8 jet fuel exposed Fischer 344 rat contains darkly stained protein and red blood cells (R). Within the type II alveolar epithelial cell, we observed mitochrondria in various stages of disintegration (arrows). Interstitial edema is observed in the area adjacent to the type II cell (IE).

Figure 1

Figure 2