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IN VIVO COMPARISON OF EPITHELIAL RESPONSES FOR S-8 VERSUS JP-8 JET FUELS BELOW PERMISSIBLE EXPOSURE LIMIT

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Abstract

This study was designed to characterize and compare the pulmonary effects in distal lung from a low-level exposure to jet propellant-8 fuel (JP-8) and a new synthetic-8 fuel (S-8). It is hypothesized that both fuels have different airway epithelial deposition and responses. Consequently, male C57BL/6 mice were nose-only exposed to S-8 and JP-8 at average concentrations of 53 mg/m³ for 1 hour/day for 7 days. A pulmonary function test performed 24 hr after the final exposure indicated that there was a significant increase in expiratory lung resistance in the S-8 mice, whereas JP-8 mice had significant increases in both inspiratory and expiratory lung resistance compared to control values. Neither significant S-8 nor JP-8 respiratory permeability changes were observed compared to controls, suggesting no loss of epithelial barrier integrity. Morphological examination and morphometric analysis of airway tissue demonstrated that both fuels showed different patterns of targeted epithelial cells: bronchioles in S-8 and alveoli/terminal bronchioles in JP-8. Collectively, our data suggest that both fuels may have partially different deposition patterns, which may possibly contribute to specific different adverse effects in lung ventilatory function.

Keywords

Jet fuel; Inhalation; Epithelial injury; Morphometry; Respiratory permeability; Lung resistance

INTRODUCTION

Jet propellant-8 fuel (JP-8) is currently the primary fuel for the U.S. military and NATO (North Atlantic Treaty Organization) forces where it is used to fuel jet aircraft, tanks, fighting vehicles, ships, helicopters, and portable heating/air conditioning units. It is

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estimated that over 2 million people worldwide are exposed to 60 billion gallons of JP-8 annually. However, the problematic long-term supplies of oil and increasing knowledge of health effects have recently spurred the U.S. military to develop a new synthetic-8 fuel (S-8), an alternative to JP-8. S-8 is derived from synthetic gas through the Fischer-Tropsch (FT) synthetic fuel process (Personal communication with Tim Edwards, Air Force Fuel Laboratory, Wright-Patterson AFB, Ohio, U. S. A.). Currently, S-8 has been certified by the U. S. Department of Defense for use in B-52H Stratofortress aircraft when blended with JP-8 and possibly support vehicles or other aircraft in the future. It is reported that S-8 could reduce engine exhaust by 80% and particulate emission by 90% compared with JP-8 fossil fuel (Inman *et al.*, 2007)

As the current primary fuel, JP-8 has numerous adverse health effects including developmental (Cooper *et al.* 1996; Koschier 1999), hepatic (Anand *et al.* 2007; Dossing *et al.* 1985), immunological (Harris *et al.* 1997; Keil *et al.* 2003; 2004), neurological (Knave *et al.* 1979; Smith *et al.* 1997; Struwe *et al.* 1983), pulmonary (Robledo *et al.* 1999a; Wang *et al.* 2001), and dermal (Ullrich, *et al.* 2000; McDougal *et al.* 2004; Chao *et al.* 2006). Because jet fuel vapors and aerosol are mainly an inhalational hazard, the major route of jet fuel exposure to flight and ground crew personnel is via the respiratory tract. Previous studies on JP-8 pulmototoxicology have found significant physiological (Hays *et al.* 1995; Robledo *et al.* 1999b; Wong *et al.* 2004), cellular (Robledo *et al.* 1999a), and biochemical changes (Espinoza *et al.* 2006; Witzmann *et al.* 1999) resulting from jet fuel exposure. Changes in airways were characterized by loss of epithelial barrier integrity and alterations of ventilatory function in bronchial and bronchiolar airways (Hays *et al.* 1995; Robledo *et al.* 1999a; Wang *et al.* 2001). These injury parameters at the high jet fuel levels may be important indicators for lung injury at the low level as well. For example, acute 1-hour inhalation exposures to aerosolized JP-8 have been shown to induce cellular and morphological indications of pulmonary toxicity that were associated with increased respiratory permeability to 99mTc-DTPA (Robledo and Witten, 1998). Most recently, morphological examination and morphometric analysis of distal lung tissue demonstrated that alveolar type II epithelial cells showed a notable increase in the volume density of lamellar bodies (vacuoles), which is indicative of increased surfactant production at 45 mg/m³ (Herrin *et al.* 2006). The morphometric analysis techniques appear to provide an increased sensitivity for detecting the deleterious effects of JP-8 as compared to the evidence offered by physiological and biochemical tests.

Little information for jet fuels is available about its toxicological effects at or below the current permissible exposure limit (PEL, 350 mg/m³) and the short-term exposure limit (STEL, 1800 mg/m³). Both limits were based on the risks of vapor-only exposures of more-volatile petroleum distillates. The risks of aerosol plus vapor exposure of less-volatile kerosene-based JP-8 or aliphatic hydrocarbon fuel S-8 were not taken into consideration. Based on our previous dose-effect (45–406 mg/m³) JP-8 study (Herrin *et al.* 2006), the present study was specifically formulated to examine whether toxic effects or lung injury occurred after a low level exposure to S-8 compared to an identical exposure to JP-8 at 53 mg/m³ for seven consecutive days for one hour/day. The mouse model, established by a simulated flightline exposure protocol, was utilized to examine the effects of both mixtures of jet fuel vapors/aerosols. The results revealed that differences in epithelial response between S-8 and JP-8 may generate respective adverse effects in the distal airways.

MATERIALS AND METHODS

A total of 18 specific-pathogen-free male C57BL/6 mice (25–30 g, 6 weeks old, Harlan, Indianapolis, IN, USA) were utilized for this study. Mice were randomly assigned to receive either a low dose S-8 exposure of 53 mg/m³, a low dose JP-8 exposure of 53 mg/m³, or

controls (ambient air). All mice were housed in the Association for Accreditation and Assessment of Laboratory Animal Care (AAALAC)-approved animal facility at the University of Arizona College of Medicine. Animals were housed two per cage and were fed and watered *ad libitum*.

S-8 and JP-8 Inhalation Exposure Regimen

This method has been duplicated from the previous jet fuel exposure studies performed in our laboratory (Herrin *et al*, 2006). Both fuel (Syntroleum, Tulsa, OK, USA) vapor/aerosol mixture were generated using a Lovelace jet nebulizer (Model 01–100, IN-TOX, Albuquerque, New Mexico, USA). A total hydrocarbon (THC) analysis system (VIG Industries, Anaheim, CA, USA) and a seven-stage cascade impactor (range of 0.25 to 5 μm , IN-TOX, Albuquerque, NM, USA) were used to measure jet fuel vapor/aerosol concentrations. Previous research in our laboratory has determined that when jet fuel is aerosolized, the animal exposure chamber contains 5–15% aerosol to vapor ratios (Dietzel *et al*, 2005).

Mice were exposed to either JP-8 or S-8 using a nose-only exposure chamber (IN-TOX, Albuquerque, NM, USA) under constant vacuum flow (25 L/min) through the chamber. Animals were exposed over a period of seven consecutive days for one hour/day at 53 mg/m³ (80 ppm THC, Figure 1) concentration for both JP-8 and S-8. The exposure protocol was chosen based on our recent study that initially indicated cellular alterations in terminal airways at this exposure level (Herrin *et al.*, 2006). Control mice were exposed to ambient air for one hour/day each day for seven consecutive days. These jet fuel exposures simulated the intermittent exposures of military personnel during a seven-day work week. Nose-only exposures were used to more closely simulate occupational exposures, in addition to being utilized to minimize oral ingestion of jet fuel during post-exposure grooming.

Pulmonary Function and Respiratory Permeability

Following 24 hours after the last S-8 or JP-8 exposure, the mice were anesthetized with an intramuscular injection mixture of ketamine HCl (80 mg/kg), xylazine (10 mg/kg) and acepromazine maleate (3 mg/kg). Subsequently, a tracheostomy was performed by inserting a teflon IV catheter (20 gauge, Critikon, Tampa Bay, FL, USA) as an endotracheal tube into the trachea. The mice were placed under pressure-controlled ventilation from a small animal ventilator (Kent Scientific, Litchfield, CT, USA). A pneumotachograph (Fleisch, #0000, Instrumentation Associates, New York, NY, USA) measured airflow while connected to a differential pressure transducer (Validyne, Northridge, CA, USA). A computerized pulmonary function system (PEDS-LAB, Medical Associated Services, Hatfield, PA, USA) was used to measure pulmonary function and record airflow and pressure signals, while normalizing them to each animal's individual weight. After recording the pulmonary function for each animal, respiratory permeability was measured using the pulmonary clearance of ^{99m}Tc-labeled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) over a period of 10 minutes using a gamma counter (Ludlum, Sweetwater, TX, USA). The ^{99m}Tc-DTPA lung clearance was expressed as k (% clearance/minute).

Morphological Lung Analysis

After the respiratory permeability tests, animals per group were randomly assigned for morphological lung analyses (Robledo *et al*, 2000; Wong *et al*, 2004). The mice were killed by exsanguination of the abdominal aorta and the lungs were removed and filled with Karnovsky's fixative (2% paraformaldehyde, 2% glutaraldehyde, and 0.01% picric acid in 0.1 M HEPES buffer solution) at a constant pressure of 20 cm H₂O for one hour. The lungs were then immersed in Karnovsky's fixative for 24 hours at 4 °C. Many sagittal sections (2–3 mm) were taken from all lobes of both the right and left fixed lungs were minced into 1

mm³ pieces for electron microscopy, osmicated, and dehydrated through alcohol series and then embedded in Spurr's resin. The electron microscopy sections (silver to gold interference colors, ~80 nm) were prepared by sectioning, and staining with lead citrate and uranyl acetate. A Philips CM-12 transmission electron microscope (Mahwah, NJ, USA) was used to examine the sections by blinded techniques.

Morphometric Procedures

Standard point counting morphometric techniques adapted to the lung were used for this study (Weibel, 1979). Analysis of electron micrographs was performed as outlined previously (Lantz and Hinton, 1984). Briefly, for point counting, electron micrographs were enlarged to 1,300 $\times$ and 3,000 $\times$. The test grid, a 19 $\times$ 25-square lattice (520 points per field) with a distance between the points of 0.20 μ m and 0.12 μ m, respectively, was placed over each micrograph. The number of points falling on structures of interest was used to estimate the volume density (V_v). In alveolar type II epithelial cells, V_v of the lamellar bodies and mitochondrial vacuoles were determined. In Clara cells located in small airways, V_v of secretory granule and mitochondrial vacuoles was determined. A total of 75 bronchiolar/alveolar septa were randomly chosen for controls or each of fuel groups.

Statistical Analyses

All data are presented as mean $\pm$ standard error of the mean (SEM). Comparisons of means between groups were made using one-way ANOVA and t-tests in a log₁₀ scale. Since the measures are independent variables, mean changes were evaluated when appropriate using post-hoc linear contrasts with adjustment for multiple comparisons made using both Bonferroni- and Fisher's PLSD-corrected significance levels. Statistical analyses were performed using SPSS version 14 (Chicago, Illinois), and p values < 0.05 were considered significant (2-tailed).

RESULTS

Pulmonary Function

No significant effects or changes in total lung compliance (including inspiratory and expiratory) were observed either in S-8 or in JP-8 group. There was a significant increase in expiratory lung resistance in S-8 group compared to control values. Additionally, JP-8 group had significant increases in both inspiratory and expiratory lung resistance compared to control values.

Respiratory Permeability

Exposure of mice to S-8 had a 20.1% increase in respiratory permeability compared to controls and JP-8 exposure had a 31.2% increase in respiratory permeability compared to the control group (Table 1). However, neither S-8 nor JP-8 induced change were statistically significant when compared to controls.

Histopathological Evaluation

Ultrastructural examination of distinct lung tissue focused on epithelial cells of alveolar and bronchiolar regions (Figures 2–4). In the air control group, alveolar area appeared normal for intracellular and extracellular structures (Figure 2B). There were many typical structures of surfactant-producing lamellar bodies throughout alveolar type II epithelial cells. The majority of mitochondria exhibited a normal shape with obvious cristae in the cytoplasm. JP-8 group had apparent alterations in the content and size of lamellar bodies within alveolar type II epithelial cells (Figure 2C). Under higher magnification, there was the appearance of lamellar inclusion bodies that appeared to be secondary lysosomes containing intracellular

debris (not shown). Severely swollen mitochondria with decreased granulation in the matrix occurred throughout the cytoplasm. However, exposure to S-8 appeared to have normal intracellular structures observed in control group except round mitochondria with few cristae.

Also the alteration in JP-8 group was Clara cells of terminal bronchioles, opening into the alveolar duct. The membrane blebbing (B) on apical surface of Clara cells frequently occurred, a sign of cell injury after JP-8 exposure (Figure 3C). In S-8 group, there was no membrane bleb damage in Clara cells.

In bronchiolar (small airways) area, conversely, S-8 group showed more swollen mitochondria and vacuolization of endoplasmic reticulum in Clara cells than those of JP-8 group (Figure 4B). Sloughing mucus lining and disrupted epithelial cilia were occasionally observed in S-8 group, but there was no consistent pattern to warrant evaluation (Figure 4B). However, JP-8 group had no such appearances observed in S-8 group (Figure 4C). To further characterize the epithelial response patterns after two jet fuel exposure, we determined the major organelle volume density by a morphometric method (Table II):

Alveolar Type II Epithelial Cells—In S-8 group, there was not a significant alteration in the vacuole volume density of lamellar bodies (0.174 ± 0.104 , Mean $\pm$ SEM) when compared with controls (0.197 ± 0.024). In contrast, JP-8 exposure had a significant increase in the vacuole volume density of lamellar bodies of 0.327 ± 0.018 compared to controls. There was no difference in the volume density of mitochondria between any fuel group and the control group.

Bronchiolar Clara cells—In the control group, the vacuole volume density of secretory granules was 7.615 ± 2.038 (Mean $\pm$ SEM). S-8 exposure significantly decreased the vacuole volume density of secretory granules with a mean value of 3.481 ± 0.651 . JP-8 exposure caused a large significant decrease in the vacuole volume density of secretory granules, 2.480 ± 0.560 . There was also a significant decrease in the volume density of mitochondria in S-8 when compared to JP-8 group.

DISCUSSION

We compared the cytotoxic or injury effects of the distal lungs after nose-only exposure of C57BL/6 mice to S-8 and JP-8 at 53 mg/m^3 (80 ppm THC) one hour per day for 7 days, respectively. The results showed that there was a significant increase in expiratory lung resistance in the S-8 group. In the JP-8 mice, there were significant increases in both inspiratory and expiratory lung resistance compared to control values. However, neither the S-8 nor JP-8 respiratory permeability values were statistically significant compared to controls, suggesting no loss of epithelial barrier integrity (Robledo *et al.*, 1999a). Collectively, we showed that exposure to both fuels below the permissible exposure limit could induce a small, but significant change in lung function, but did not exhibit permeability injury observed at the high levels of JP-8 exposure (Robledo and Witten, 1998; Wang *et al.*, 2001; Wong *et al.*, 2004). More importantly, these data suggest that there may be partial different effects of epithelial deposition and responses responsible for changes of lung function induced by S-8 or JP-8 exposure, respectively.

In ultrastructure examination, differences were also observed in cellular injury or organelle alterations of the Clara cells and alveolar type II cells for the two groups. JP-8 inhalation resulted in structural damage to the extended alveolar area involving cytoplasmic vacuolization and apical membrane blebs. These alterations, especially bleb formation,

represented an early sign of cell injury which may precede changes in respiratory permeability after JP-8 exposure.

This finding is similar with a previous study that bleb formation preceded increases in membrane permeability after acute naphthalene injury to Clara cells in vivo (Van Winkle et al., 1999). This finding suggests that JP-8-induced Clara cell blebbing may be a uniform response observed in other P-450 bioactivated toxicants through one or several possible mechanisms, such as transformation of preexisting microvilli, changes in the cortical cytoskeleton, and disturbances in both thiol and calcium homeostasis within the injured cell (Hinshaw, et al., 1986; Van Winkle et al., 1996; 1999). However, these alveolar alterations were not observed in S-8 group which exhibited mild ultrastructure alterations in bronchiolar Clara cells. Our findings suggest that there were site-selective and cell-specific epithelial responses after the two jet fuel exposure.

Morphometric analysis provided further evidence for differences in targeted cellular effects of the two fuels: bronchioles in S-8 and alveoli/terminal bronchioles in JP-8. These data suggest that surfactant synthesis and/or secretory processes were affected by JP-8 exposure, as indicated by an increase in volume density of surfactant-producing lamellar bodies. Consequently, the type II epithelial cells may compromise their normal capabilities to control the volume and composition of the epithelial lining fluid and to maintain the integrity of the alveolar wall (Juvin *et al.*, 2002). However, S-8 exposure significantly decreases the volume density of mitochondria, as indicative of the onset of cell swelling, when compared to JP-8 group,

It is noted that the two jet fuels also exhibited a similar bronchiolar epithelial response, as indicated by the decreases of vacuole volume density of secretory granules in Clara cells. This phenomenon was similar with previous data that Clara cells' secretory granules disappeared 1 hour after propranolol plus isoproterenol administration, and the granules reappeared at 4 hours (Aryal *et al.*, 2003). As we know, Clara cells represent the predominant secretory cell containing characteristic secretory granules and constitute up to 80% of the epithelial cell populations of the distal airways. Clara cell secretory protein deficiency after jet fuel exposure could lead to alterations in the composition of epithelial lining fluid and to enhanced susceptibility to environmental agents (Stripp *et al.*, 2002). As for relevance of human toxicity, however, the relevance of functional alteration of Clara cells remains to be addressed since there are species dependent differences histologically and physiologically, such as cell density and metabolizing capacity.

The differences for two jet fuels may be attributed to the chemical differences between the two fuels *per se* or their dynamics in airways such as diffusion and deposition, consequently resulting in differences of ventilatory function of the small respiratory tract between S-8 and JP-8 exposure. JP-8 consists of a hydrocarbon-rich kerosene base commercial jet fuel (Jet-A) plus additives [corrosion inhibitor DC1-4A, antistatic compound Stadis 450, and an icing inhibitor diethylene glycol monomethyl ether (DIGME)] that make up less than 2% of the formulation (Inman *et al.*, 2007). S-8, an aliphatic hydrocarbon (HC) fuel, is synthesized using the Fischer-Tropsch (FT) synthetic fuel process. Synthetic gas produced from natural gas, coal, or biomass is converted using heat and pressure into a clean burning liquid fuel made up of aliphatic HC (Inman *et al.*, 2007).

In summary, the present study demonstrated that exposure of mice to both S-8 and JP-8 caused the morphological/morphometric and functional alterations at 53 mg/m³, well below the permissible exposure limit. We also showed that the relative differences in epithelial deposition and response between the two jet fuels leads to different adverse effects in the distal lungs, possibly through their deposition patterns and chemical compositions.

However, additional experiments with the dose-effect design are needed to confirm the effects generated by these two fuels over a wider range of jet fuel concentrations. Moreover, it is important to correlate human studies with actual field exposure in order to develop the best formulation of S-8 for both Air Force personnel and aircraft.

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References

- Anand SS, Campbell JL, Fisher JW. In vitro rat hepatic metabolism of n-alkanes: nonane, decane, and tetradecane. *Int J Toxicol* 2007;26:325–329. [PubMed: 17661223]
- Aryal G, Kimula Y, Koike M. Ultrastructure of Clara cells stimulated by isoproterenol. *J Med Dent Sci* 2003;50:195–202. [PubMed: 15074357]
- Chao YC, Kupper LL, Serdar B, Egeghy PP, Rappaport SM, Nylander-French LA. Dermal exposure to jet fuel JP-8 significantly contributes to the production of urinary naphthols in fuel-cell maintenance workers. *Environ Health Perspect* 2006;114:182–185. [PubMed: 16451852]
- Chaturvedi A, Lee Z. Three-dimensional segmentation and skeletonization to build an airway tree data structure for small animals. *Physics in Medicine and Biology* 2005;50:1405–1419. [PubMed: 15798332]
- Cooper JR, Mattie DR. Developmental toxicity of JP-8 jet fuel in the rat. *J Appl Toxicol* 1996;16:197–200. [PubMed: 8818858]
- Dietzel KD, Campbell JL, Bartlett MG, Witten ML, Fisher JW. Validation of a gas chromatography mass spectroscopy (GC/MS) method for quantification of aerosolized jet propellant (JP-8). *Journal of Chromatography Analysis* 2005;1093:11–20.
- Dossing M, Loft S, Schroeder E. Jet fuel and liver function. *Scand J Work Environ Health* 1985;11:433–437. [PubMed: 4095521]
- Espinoza LA, Tenzin F, Cecchi AO, Chen Z, Witten ML, Smulson ME. Expression of JP-8-induced inflammatory genes in AEII cells is mediated by NF-kappaB and PARP-1. *Am J Respir Cell Mol Biol* 2006;35:479–487. [PubMed: 16690985]
- Harris DT, Sakiestewa D, Robledo RF, Witten M. Immunotoxicological effects of JP-8 jet fuel exposure. *Toxicol Ind Health* 1997;13:43–55. [PubMed: 9098949]
- Hays AM, Parlman G, Pfaff JK, Lantz RC, Tinajero J, Tollinger B, Hall JN, Witten ML. Changes in lung permeability correlate with lung histology in a chronic exposure model. *Toxicol Ind Health* 1995;11:325–336. [PubMed: 7482572]
- Herrin BR, Haley JE, Lantz RC, Witten ML. A reevaluation of the threshold exposure level of inhaled JP-8 in mice. *J Toxicol Sci* 2006;31:219–228. [PubMed: 16960432]
- Hinshaw DB, Sklar LA, Bohl B, Schraufstatter IU, Hyslop PA, Rossi MW, Spragg RG, Cochrane CG. Cytoskeletal and morphologic impact of cellular oxidant injury. *Am J Pathol* 1986;123:454–464. [PubMed: 3717299]
- Inman AO, Monteiro-Riviere NA, Riviere JE. Inhibition of jet fuel aliphatic hydrocarbon induced toxicity in human epidermal keratinocytes. *J Appl Toxicol* 2008;28:543–553. [PubMed: 17966119]
- Juvin P, Fournier T, Grandsaigne M, Desmonts JM, Aubier M. Diesel particles increase phosphatidylcholine release through a NO pathway in alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L1075–L1081. [PubMed: 11943673]
- Keil DE, Warren DA, Jenny MJ, EuDaly JG, Smythe J, Peden-Adams MM. Immunological function in mice exposed to JP-8 jet fuel in utero. *Toxicol Sci* 2003;76:347–356. [PubMed: 14514957]
- Keil D, Dudley A, EuDaly J, Dempsey J, Butterworth L, Gilkeson G, Peden-Adams M. Immunological and hematological effects observed in B6C3F1 mice exposed to JP-8 jet fuel for 14 days. *J Toxicol Environ Health A* 2004;67:1109–1129. [PubMed: 15205027]

- Knave B, Mindus P, Struwe G. Neurasthenic symptoms in workers occupationally exposed to jet fuel. *Acta Psychiatr Scand* 1979;60:39–49. [PubMed: 474176]
- Koschier FJ. Toxicity of middle distillates from dermal exposure. *Drug Chem Toxicol* 1999;22:155–164. [PubMed: 10189576]
- Lantz RC, Hiton DE. Pulmonary toxicity associated with fly ash from fluidized bed coal combustion. *Toxicol Appl Pharm* 1984;75:44–51.
- McDougal JN, Rogers JV. Local and systemic toxicity of JP-8 from cutaneous exposures. *Toxicol Lett* 2004;149:301–308. [PubMed: 15093277]
- Robledo RF, Witten ML. NK1-receptor activation prevents hydrocarbon-induced lung injury in mice. *Am J Physiol* 1999b;276:L229–L238. [PubMed: 9950884]
- Robledo RF, Barber DS, Witten ML. Modulation of bronchial epithelial cell barrier function by in vitro jet propulsion fuel 8 exposure. *Toxicol Sci* 1999a;51:119–125. [PubMed: 10496683]
- Robledo RF, Witten ML. Acute pulmonary response to inhaled JP-8 jet fuel in mice. *Inhal Toxicol* 1998;10:531–553.
- Robledo RF, Young RS, Lantz RC, Witten ML. Short-term pulmonary response to inhaled JP-8 jet fuel aerosol in mice. *Toxicol Pathol* 2000;28:656–663. [PubMed: 11026600]
- Smith LB, Bhattacharya A, Lemasters G, Succop P, Puhala E, Medvedovic M, Joyce J. Effect of chronic low-level exposure to jet fuel on postural balance of US Air Force personnel. *J Occup Environ Med* 1997;39:623–632. [PubMed: 9253723]
- Stripp BR, Reynolds SD, Boe IM, Lund J, Power JH, Coppens JT, Wong V, Reynolds PR, Plopper CG. Clara cell secretory protein deficiency alters clara cell secretory apparatus and the protein composition of airway lining fluid. *Am J Respir Cell Mol Biol* 2002;27:170–178. [PubMed: 12151308]
- Struwe G, Knave B, Mindus P. Neuropsychiatric symptoms in workers occupationally exposed to jet fuel--a combined epidemiological and casuistic study. *Acta Psychiatr Scand Suppl* 1983;303:55–67. [PubMed: 6575584]
- Ullrich SE, Lyons HJ. Mechanisms involved in the immunotoxicity induced by dermal application of JP-8 jet fuel. *Toxicol Sci* 2000;58:290–298. [PubMed: 11099641]
- Van Winkle LS, Johnson ZA, Nishio SJ, Brown CD, Plopper CG. Early events in naphthalene-induced acute Clara cell toxicity: comparison of membrane permeability and ultrastructure. *Am J Respir Cell Mol Biol* 1999;21:44–53. [PubMed: 10385592]
- Van Winkle LS, Isaac JM, Plopper CG. Repair of naphthalene-injured microdissected airways *in vitro*. *Am J Respir Cell Mol Biol* 1996;15:1–8. [PubMed: 8679213]
- Wang S, Young RS, Witten ML. Age-related differences in pulmonary inflammatory responses to JP-8 jet fuel aerosol inhalation. *Toxicol Ind Health* 2001;17:23–29. [PubMed: 12004922]
- Weibel, ER. *Precritical method for biological morphometry*. Vol. 1. Academic Press; New York: 1979. Stereological method.
- Witzmann FA, Bauer MD, Fieno AM, Grant RA, Keough TW, Kornguth SE, Lacey MP, Siegel FL, Sun Y, Wright LS, Young RS, Witten ML. Proteomic analysis of simulated occupational jet fuel exposure in the lung. *Electrophoresis* 1999;20:3659–3669. [PubMed: 10612293]
- Wong SS, Hyde J, Sun NN, Lantz RC, Witten ML. Inflammatory responses in mice sequentially exposed to JP-8 jet fuel and influenza virus. *Toxicology* 2004;197:139–147. [PubMed: 15003324]

Figure 1.

Total hydrocarbon (THC) concentrations of S-8 and JP-8 at exposure chamber. THC levels were continuously monitored by Heated THC Analyzer (F.I.D. Model-20 Heated, VIG Industries, Anaheim, CA, USA) during 60-minute exposure (A) for 7 days (B).

Figure 2.

Representative transmission electron micrographs of alveolar epithelial cells from mice following ambient air (A), S-8 (B), and JP-8 (C) exposure. Note the apparent alteration in surfactant-producing lamellar bodies (arrows) of alveolar type II epithelial cells with swollen mitochondria (arrow heads) in the JP-8 group (C). N: Nucleus. Uranyl acetate and lead citrate. Magnification: 8,000 $\times$.

Figure 3.

Representative transmission electron micrographs of terminal bronchiole epithelial cells from mice following ambient air (A), S-8 (B), and JP-8 (C) exposure. Note Clara cells with apical membrane blebbing (arrows) and the vacuolated mitochondria (arrows head) in the JP-8 group (C). M: macrophages; N: Nucleus. Uranyl acetate and lead citrate. Magnification: 1,800 $\times$.

Figure 4. Representative transmission electron micrographs of small airway epithelial cells from mice following ambient air (A), S-8 (B), and JP-8 (C) exposure. Note vacuolated mitochondria (arrows head) with occasional occurrence of sloughing of mucosal lining (arrow) in the S-8 group (B). Uranyl acetate and lead citrate. N: Nucleus. Magnification: 1,800 $\times$.

TABLE I

Changes of Lung Function and Respiratory Permeability Following JP-8 and S-8 Jet Fuel Exposure

	Control	S-8	JP-8
<i>Total lung compliance (ml·cmH₂O⁻¹·kg⁻¹)</i>			
Inspiratory	2.83 ± 0.13	3.97 ± 0.95	2.96 ± 0.12
Expiratory	2.90 ± 0.13	2.84 ± 0.10	2.97 ± 0.29
Total	2.88 ± 0.12	2.82 ± 0.08	2.70 ± 0.06
<i>Lung resistance (cmH₂O·kg·L⁻¹·s)</i>			
Inspiratory	62.84 ± 18.74	129.60 ± 31.29	110.86 ± 8.28*
Expiratory	128.24 ± 9.52	167.93 ± 8.81*	243.76 ± 43.49*
Total	96.00 ± 21.09	118.20 ± 15.47	140.84 ± 23.67
<i>Respiratory Permeability</i>			
[^{99m} Tc-DTPA (%/min)]	1.89 ± 0.66	2.27 ± 0.39	2.48 ± 0.69

Note: Data expressed as Mean ± SE (N=6).

* p<0.05 compared to control.

TABLE II

Morphometric Alterations in the organelle volume density of Epithelium Following JP-8 and S-8 Jet Fuel Exposure

	Control	S-8	JP-8
<i>Alveolar type II epithelial cells</i>			
Lamellar bodies	0.197 ± 0.024	0.174 ± 0.014	0.327 ± 0.018*
Mitochondria	5.85 ± 1.11	5.80 ± 1.34	6.00 ± 0.98
<i>Bronchiolar Clara cells</i>			
Secretory granules	7.615 ± 2.038	3.481 ± 0.651*	2.480 ± 0.560*
Mitochondria	41.85 ± 3.73	30.30 ± 2.36**	55.00 ± 5.91

Note: Data expressed as Mean ± SE (N = 6).

* p<0.05 compared to control.

** p<0.05 compared to JP-8.