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TOXICOLOGIC AND ONCOGENIC POTENTIAL OF JP-4 VAPOR: 90-DAY CONTINUOUS INHALATION EXPOSURE

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Male and female Fischer 344 rats, female C57BL/6 mice, and male and female beagle dogs were divided into three treatment groups and exposed nearly continuously (23 h/day, 7 days/wk) to JP-4 jet fuel vapor at concentrations of 0, 500, and 1000 mg/m^3 for 90 days. At exposure termination, all dogs and one-third of the rodents were euthanized and the remaining animals were held for either a 19- or 21-mo postexposure tumorigenesis observation period. Pathologic findings in male rats revealed treatment-related renal toxicity consistent with male rat $\alpha_{2\mu}$-globulin nephropathy. No treatment-related respiratory toxicity was noted in any species. This study did not demonstrate target-organ toxicity or carcinogenesis that could be extrapolated to other species.

JP-4 is a complex mixture of aliphatic and aromatic hydrocarbon compounds with physicochemical properties similar to gasoline. It is the principal fuel used in U.S. Air Force flight operations and meets Military Specification MIL-J-5624E. JP-4 fuel accounts for approximately 85% of the turbine engine fuel used by the Department of Defense (Bruner et al., 1993). Because of its widespread use within the military, investigations were conducted to determine the acute, subchronic, and chronic toxicity of JP-4 vapor.

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This inhalation study was performed in 1979 and was completed under U.S. Department of the Air Force contracts F33615-76-C-5005 (1976), F33615-80-C-0532 (1980), F33615-85-C-0532 (1985), and F33615-90-C-0532 (1990). Reprints of this article are identified by the Armstrong Laboratory, Occupational and Environmental Toxicology Division, Wright-Patterson Air Force Base, OH, as AL/OE-TR-1994-0088. The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on the Care and Uses of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHS, National Institute of Health Publication 86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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Single oral doses of neat fuel were administered to Sprague-Dawley rats at 8 g JP-4/kg. No deaths were observed. In CF_1 mice, deaths occurred at lower doses (JP-4 diluted in corn oil) of 0.5 g/kg, but only 33% mortality was achieved at the highest gavage dose of 1.0 g JP-4/kg. An inhalation study of 6 h duration to an estimated JP-4 concentration of 38 mg/L (based on weight loss) resulted in poor coordination and convulsions in several rats, but no mortality (Kinkead et al., 1993). Dermal sensitization studies with guinea pigs indicated that JP-4 was not a skin sensitizer. Rabbit eye irritation tests were negative, while dermal application of the jet fuel resulted in slight skin irritation in that same species (Kinkead et al., 1992). An 8-mo inhalation study (6 h/day, 5 days/wk at 5000 mg JP-4/m^3 to beagle dogs, rhesus monkeys, Sprague-Dawley rats, and C57BL/6 mice produced increased organ-to-body weight ratios of liver, kidney, lung, and splenic tissue, bronchial irritation in male rats, and a transient increase in osmotic erythrocyte fragility in female dogs. Histopathologic findings in exposed monkeys, dogs, and mice showed no treatment-related effects (MacEwen & Vernot, 1975).

In a subsequent study, male and female Fischer 344 (F344) rats and male and female C57BL/6 mice were exposed to 1000 and 5000 mg JP-4/m^3, 6 h/day, 5 days/wk, for 12 mo. The inhalation exposure period was followed by a 12-mo holding/observation period for possible oncogenic effects (Bruner et al., 1993). Pathologic findings in male rats revealed treatment-related renal toxicity and neoplasia consistent with male rat $\alpha_{2\mu}$-globulin nephropathy syndrome. The study did not produce any additional target-organ toxicity or tumor formation that was considered treatment related.

Results of inhalation exposure to fuels similar in composition to JP-4 have been reported. Kinkead et al. (1991a) reported the effects of a 1-yr (6 h/day, 5 days/wk) study with the fuels JP-TS, a high-altitude jet fuel, and JP-7, a fuel similar to JP-5 jet fuel, on F344 rats and C57BL/6 mice. The most significant finding was an increased incidence of renal disease in male rats similar to changes reported with other hydrocarbon inhalation exposures (Carpenter et al., 1975a, 1975b). Continuous exposure (24 h/day, 7 days/wk) of F344 rats, C57BL/6 mice, and beagle dogs to 750 and 150 mg JP-5/m^3 vapor for 90 days also resulted in similar renal changes in male rats (Gaworski et al., 1984). A 90-day continuous exposure (24 h/day, 7 days/wk) of F344 rats and C57BL/6 mice to 500 and 1000 mg/m^3 JP-8 vapor only resulted in the renal changes in male rats (Mattie et al., 1991).

This study was designed to determine the toxicologic effects of a 90-day nearly continuous (approximately 23 h/day) exposure of dogs, rats, and mice to atmospheric concentrations of 500 and 1000 mg JP-4/m^3. This exposure regimen simulates worst-case working conditions that may be experienced by military personnel (Mattie et al., 1991). Beagle dogs, F344 rats, and C57BL/6 mice were selected as the test species to afford a comparison with the previously mentioned studies. Rodents were held for an additional 19–21 mo post-exposure to assess the oncogenic potential of JP-4.

MATERIALS AND METHODS

Test Material

JP-4 is a complex mixture of aliphatic and aromatic hydrocarbon compounds that is defined in terms of physical and chemical characteristics and includes various additives, all of which meet the requirements of Military Specification MIL-J-5624E. Upper limits for some of the constituents are detailed in the military specifications listed:

Sulfur, 0.4% (by weight)
Mercaptan sulfur, 0.001% (by weight)
Aromatics, 25.0% (by volume)
Olefins, 5.0% (by volume)
Various butyl phenol antioxidants, 24 mg/L
Aliphatic diamine metal deactivators, 5.8 mg/L

These constituents represent only a fraction of the total content of JP-4 jet fuel. The remainder consists of unspecified hydrocarbon compounds in the kerosene boiling range.

Test Material Quality Control

Twelve barrels of JP-4 were received from the U.S. Air Force fuels depot for use in these studies. The JP-4 samples were removed from fuel storage tanks being used by the Air Force. Before initiating the animal inhalation exposures, the JP-4 was subjected to quality control procedures. Samples of fuel were taken from all 12 barrels and subjected to a gas chromatographic (GC) fingerprint analysis. A typical gas chromatographic tracing of JP-4 was published previously (Bruner et al., 1993). The percent of total area under the chromatogram was calculated for each of the 10 major peaks to ensure that all barrels were supplied from the same production batch. All barrels were found to have similar component composition. Headspace samples were taken from three drums for GC analysis of benzene. Benzene concentrations ranged between 0.41 and 1.10% with a mean of 0.78%. Prior to beginning animal exposures the benzene concentration of the highest target concentration (1000 mg JP-4/m^3) was determined to be 0.51% (mean of 3 samples) or approximately 1.5 ppm benzene.

Animals

Male and female F344 rats (225 each sex) were purchased from Charles River Breeding Labs, Kingston, NY; 450 female C57BL/6 mice were purchased from Jackson Laboratories, Bar Harbor, ME; and 9 male and 9 female purpose-bred beagle dogs were purchased from a commercial breeder. The rats were 9 wk of age, the mice 10 wk of age, and the dogs ranged between 8 and 18 mo of age at exposure initiation. Quality control

assessments were conducted routinely in dogs and indicated they were in acceptable health. All rodents were subjected to an extensive health evaluation as previously described in Kinkead et al. (1991b) prior to use in this study.

The animals were group housed in stainless steel cages in conformance with Institute of Laboratory Animal Resources (ILAR) standards (National Institute of Health Publication 85-23, 1985) for laboratory animal care. Automatic water and feed were available ad libitum except when food was removed for 12 h prior to necropsy. During the exposure phase the animal food was changed daily and no attempt was made to quantitate the amount of test material that might have been ingested with the daily ration. Ambient temperatures were maintained at 21–25°C.

Contaminant and Vapor Generation System

The contaminant introduction system for JP-4 was similar to the systems described for previous fuel studies (Gaworski et al., 1984; Bruner et al., 1993). Vaporization procedures were designed to produce chamber atmospheres similar to those encountered in field conditions. Evaporator columns were regulated so that generated vapor consisted of hydrocarbon mixtures similar to those encountered during routine refueling operations or accidental spills. The liquid material was pumped under low pressure from a 55-gallon supply drum and then passed through flow meters to glass evaporator columns heated to an air temperature not greater than 48°C (120°F). The airstream flowing through the evaporator carried the vapor into the main air supply for the chambers. Excess fuel that was not vaporized in the evaporator was drained into the receiving tank, where it was collected for disposal. Thermocouples were placed at the top and bottom of the glass evaporator to sense any hazardous increase in temperature and to activate both an alarm and a solenoid valve system that would cut off the fuel supply. The fuel supply and waste drums were maintained within ventilated, grounded safety cabinets.

Contaminant Analytical System

Continuous analysis of the chamber concentrations was done by pumping air samples from each chamber into a total hydrocarbon analyzer. During previous testing of fuels by this laboratory, it was found that propane gave the same hydrocarbon detector sensitivity as the fuels. Therefore, known propane concentrations were used as the calibration standards.

Output of the vapor by the generation system was a function of the fuel flow rate, airflow rate, and temperature. Under defined operating conditions, the output was stable. Therefore, hourly checks were made to assure that the predetermined settings were maintained. A Royco (model 225, Royco Instruments, Menlo Park, CA) particle counter equipped with a 508 digital monitor was used to measure the possible formation of vapor condensate aerosol within the exposure chamber.

Exposure Regimen

Dogs, rats, and mice were placed in Thomas dome chambers (Thomas, 1965) and exposed continuously (except for a short period each day to service animals) to either 500 or 1000 mg JP-4 vapor/m^3 for 90 days. Each chamber housed 3 male and 3 female beagle dogs, 150 female mice, and 75 male and 75 female rats. A control group of equal numbers of animals was housed in a nearby, fully accredited animal facility in bioclean laminar flow rooms. Upon termination of the 90-day exposure, all dogs and one-third of the rodents from each treatment group were euthanized. The rodents not euthanized following exposure were held for either 19 or 21 mo (when the number of surviving animals reached 10% of the original group number) to evaluate postexposure tumorigenesis. During this time, body weights for rats were obtained monthly.

The concentrations selected, 500 and 1000 mg JP-4/m^3, were chosen after analysis of the benzene concentration of this lot of JP-4 fuel. These concentrations ensured that the level of benzene in the chambers would not exceed that equivalent to a 6-h time-weighted average of 10 ppm (ACGIH, 1993).

Animal Response Assessments

Animals were carefully observed throughout the study. The rats were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Dogs were individually weighed at biweekly intervals during the exposure. Mice were weighed by sex and treatment groups, and mean weights were recorded. Rats that died or were in moribund condition were necropsied and subjected to a complete histopathologic examination.

Blood samples were taken from the dogs via the cephalic vein biweekly for the following hematologic and clinical chemistry tests: hematocrit, hemoglobin, red blood cell (RBC) count, white blood cell (WBC) count, WBC differential, alanine aminotransaminase (ALT), bilirubin, alkaline phosphatase, aspartate aminotransaminase (AST), blood urea nitrogen (BUN), calcium, creatinine, globulin, potassium, and sodium. Monthly blood samples were taken for red blood cell fragility determinations. Blood was taken via the portal vein from the rats at the conclusion of the exposure period for the same clinical determinations mentioned previously. Liver, spleen, and kidney weights were obtained from the dogs and rats at necropsy. Clinical chemistry analyses were performed with a DuPont automated clinical analyzer (E. I. DuPont de Nemours & Co., Inc., Wilmington, DE). A Coulter blood analyzer (Coulter Instruments, Hialeah, FL) was used to obtain hematologic values. Absolute leukocyte differentials were determined according to established procedures. All animals that died or were euthanatized during the study were necropsied, and tissues were collected for histopathologic examination. Euthanasia was via halothane inhalation overdose. Tissues for histopathologic examination were fixed in 10% neu-

tral buffered formalin, trimmed, and further processed via routine methods for hematoxylin-eosin-stained, paraffin-embedded sections (Luna, 1968).

Also at necropsy following the 90-day inhalation exposure, male rat kidney tissue was collected and fixed by vascular perfusion for transmission electron microscopy (TEM) examination. The kidney slices were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 *M* cacodylate buffer at pH 7.4. Sections 1 μm in thickness were prepared in plastic sections. Thin sections cut from representative regions seen in the 1-μm sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (model 100B, JOEL, USA, Inc., Peabody, MA).

Statistics

Data from routine animal weighing, hematology, blood chemistry, and organ weighings were analyzed for statistical significance using an analysis of variance with Scheffe multiple comparisons (Barcikowski, 1983; Dixon, 1990). Pathologic lesion incidence was analyzed using the chi-square test for equality of proportion (Fleiss, 1981). Morphometric analysis was performed by a two-factorial analysis of variance with multivariate comparisons (Barcikowski, 1983). A probability of .05 or less implied a significant change from controls.

RESULTS

Clinical Findings and Mortality

Clinical signs of toxic stress were not apparent during or following the 90-day inhalation exposure regimen. Mortality during the exposure period was insignificant and independent of JP-4 concentration. Mean body weights for male and female rat groups are provided in Figures 1 and 2. Mean body weights of both sexes were significantly depressed at many of the weighing periods throughout the 90-day study. The mean body weights of the JP-4-exposed female rats recovered during the postexposure observation period, while the depression in mean body weight continued through much of the postexposure period in the JP-4-exposed male rats. Mouse mean weights revealed no exposure-related effects. The mean body weights of dogs exposed to JP-4 vapor continuously for 90 days were unaffected by exposure.

Hematology and Clinical Chemistry

Mean hematology and blood chemistry values for male and female rats following the 90-day exposure showed no treatment-related effects. Likewise, no treatment-related differences in these parameters were noted in dogs at any of the sampling times. All clinical measurements made on dog blood samples were within normal species variation, and no treatment-

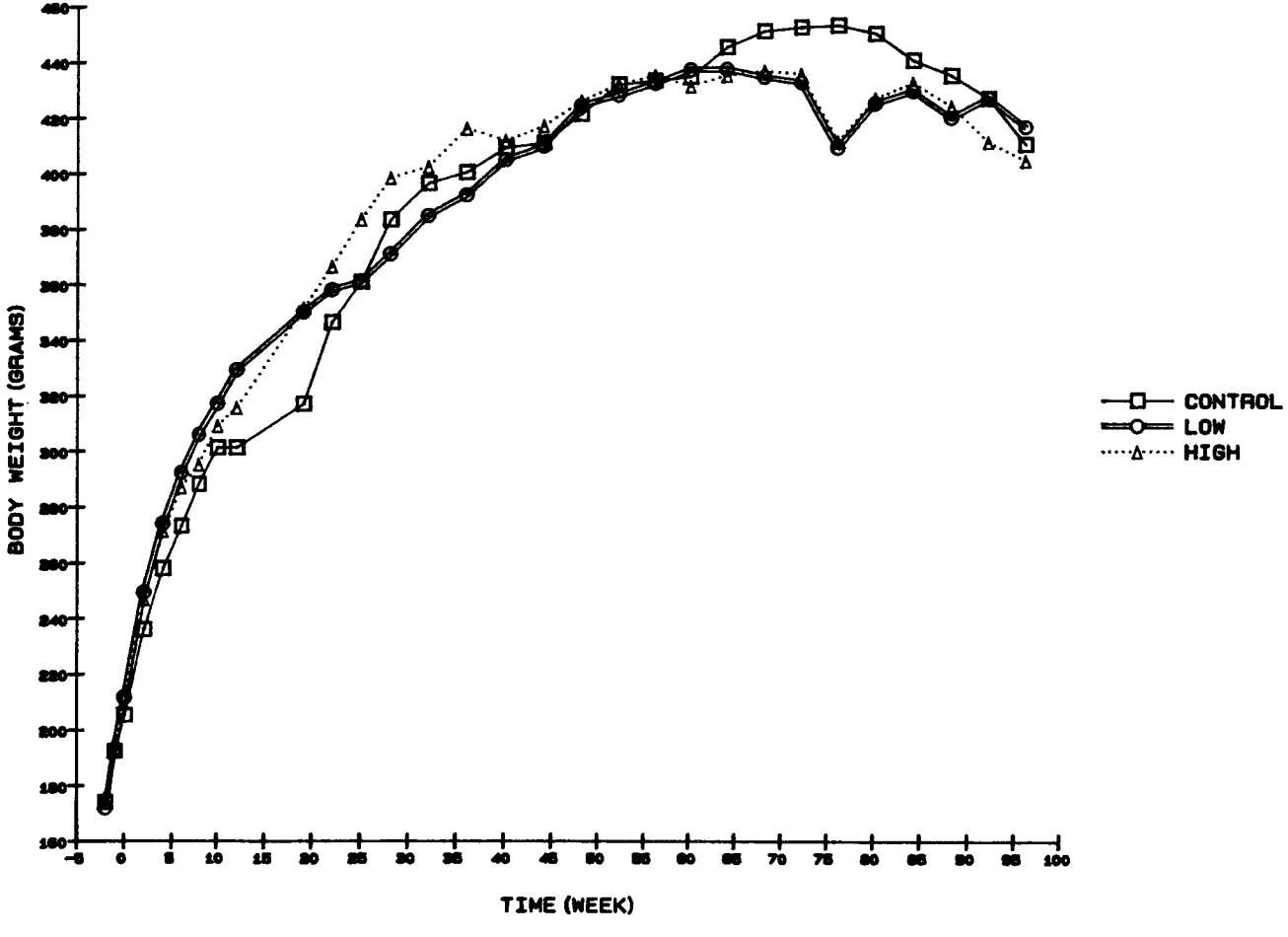

FIGURE 1. Body weight means for male F344 rats exposed by inhalation to JP-4 jet fuel.

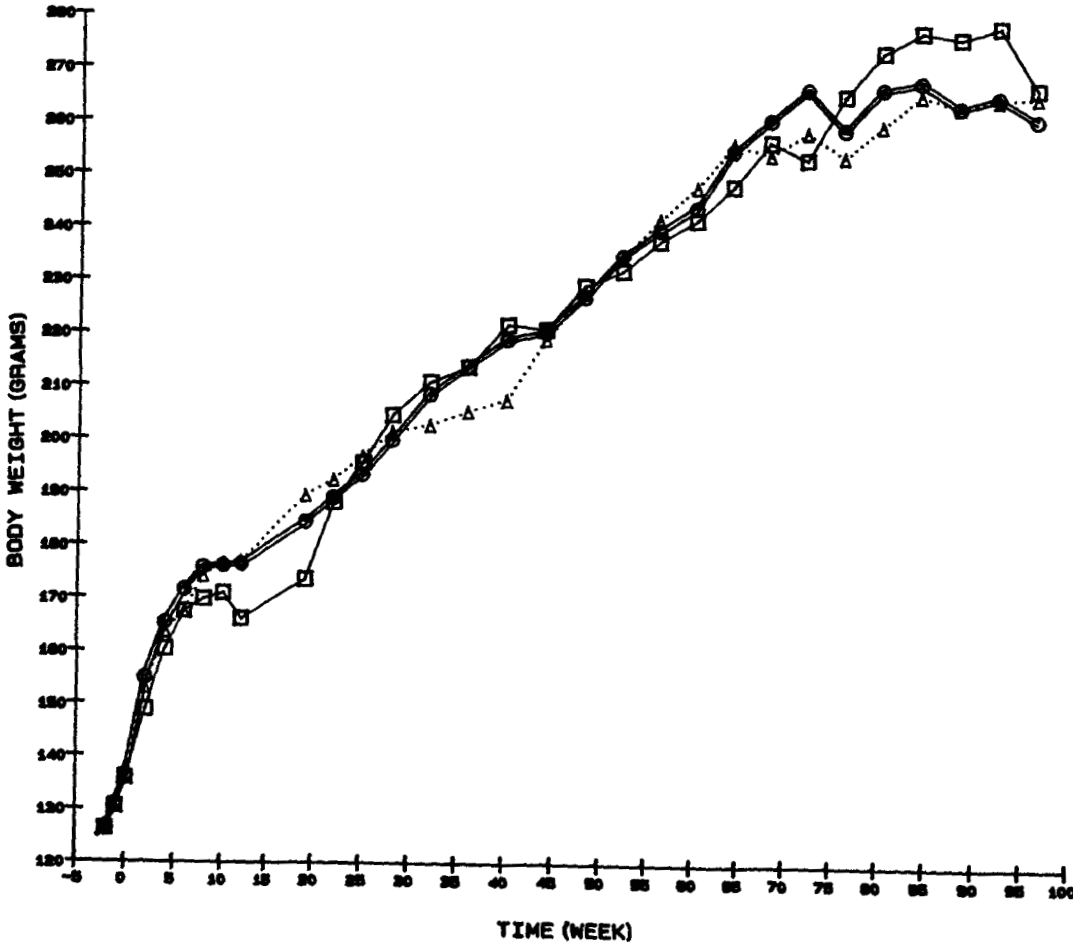

FIGURE 2. Body weight means for female F344 rats exposed by inhalation to JP-4 jet fuel.

related changes in osmotic fragility were noted (data not shown). Blood chemistry evaluations performed on male and female rats postexposure also showed no treatment-related effects (data not shown).

Organ Weights

A treatment-related difference in absolute and relative kidney weights was observed in the male rat groups euthanized following the 90-day exposure period (Table 1). Relative spleen weights were also slightly increased in the treated male rat groups. Additionally, absolute liver weights were decreased in the low-concentration male rats. This difference was not a factor when corrected for the difference in whole body weight. No significant concentration-related differences in mean absolute or relative organ weights were observed in the female rat groups. No treatment-related differences were noted in dog organ weights. No differences occurred in organ weights of either male or female rats measured following the postexposure period.

Dog Pathology

Gross examination of dogs, all euthanized immediately postexposure, revealed roundworm infection in 10 of 12 test dogs and 1 of 6 control dogs. All microscopic lesions observed in dogs were regarded as sponta-

TABLE 1. Organ Weights (g)[a] and Organ-to-Body Weight Ratios[b] of F344 Rats Following 90-Day Continuous Exposure to JP-4 Vapor

	Control	500 mg/m³	1000 mg/m³
		Males	
Liver	8.30 ± 0.13	7.57 ± 0.14[c]	8.08 ± 0.18
Ratio	2.56 ± 0.02	2.47 ± 0.04	2.61 ± 0.04
Spleen	0.55 ± 0.01	0.57 ± 0.01	0.58 ± 0.02
Ratio	0.17 ± <0.01	0.19 ± <0.01[c]	0.19 ± <0.01[c]
Kidneys	2.04 ± 0.03	2.11 ± 0.04	2.31 ± 0.04[c]
Ratio	0.63 ± 0.01	0.69 ± 0.01[c]	0.75 ± 0.01[c]
Whole body	324.3 ± 3.6	306.0 ± 3.3[c]	309.6 ± 4.2[c]
		Females	
Liver	4.30 ± 0.11	4.10 ± 0.08	4.24 ± 0.05
Ratio	2.50 ± 0.04	2.46 ± 0.02	2.57 ± 0.03
Spleen	0.37 ± 0.01	0.34 ± 0.01	0.37 ± 0.02
Ratio	0.21 ± <0.01	0.21 ± <0.01	0.22 ± <0.01
Kidneys	1.24 ± 0.03	1.22 ± 0.03	1.24 ± 0.02
Ratio	0.72 ± 0.01	0.73 ± 0.01	0.75 ± 0.01
Whole body	172.1 ± 2.6	166.8 ± 1.8	164.8 ± 1.5[d]

[a]Mean ± SEM, males $n = 25$; females $n = 24$.
[b]Organ weight/body weight × 100.
[c]Significantly different from control, $p < .01$.
[d]Significantly different from control, $p < .05$.

neous changes or natural diseases in laboratory beagles. In all groups the most significant finding was mild to moderate inflammatory changes in the lungs and associated lymphoid tissues. These changes were not treatment related, and, in almost all cases, were entirely compatible with infection by canine lungworms, *Filaroides* sp.

Rat Pathology

Histopathologic findings in rats following the 90-day inhalation exposure were confined to the kidneys of male rats. Hyaline droplet formation was present in 100% of the 1000 mg JP-4/m^3 group and 96% of the 500 mg JP-4/m^3 group, as opposed to only 7% of the control group (Table 2). This lesion consisted of the formation of hyaline crystalloid intracytoplasmic inclusions. Additionally, granular casts were present in 100% of the high concentration group and 96% of the low concentration group. Casts were not observed in any of the rats from the control group. A slight increase in severity of these two lesions was present in the high concentration group.

Mouse Pathology

The most significant finding in mice following the 90-day inhalation exposure was hepatocellular fatty change. This lesion was found in a high percentage of the low-concentration group (89%) and the high-concentration group (90%) as opposed to control mice (4%). This finding was most prominent in the centrolobular region of the liver. The lesion consisted of multiple, discrete vacuoles of varying sizes within the cytoplasm of hepatocytes.

TABLE 2. Incidence Summary of Selected Microscopic Renal Lesions in F344 Rats Examined Immediately Following 90 Days of Continuous Exposure to JP-4 Vapor

	Male			Female		
	Control	500 mg/m^3	1000 mg/m^3	Control	500 mg/m^3	1000 mg/m^3
Number examined	27	27	27	25	25	25
Nephropathy	0	1	0	0	1	0
(Severity)	(0.0)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)
Tubular mineralization	0	1	1	0	3	0
(Severity)	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)
Granular protein casts	0	25[a]	27[a]	0	0	1
(Severity)	(0.0)	(1.6)	(2.3)	(0.0)	(0.0)	(0.1)
Hyaline droplet accumulation	2	26[a]	27[a]	0	0	1
(Severity)	(0.1)	(1.0)[a]	(1.9)[a]	(0.0)	(0.0)	(0.1)
Pelvic urothelium hyperplasia	0	0	0	0	0	0
(Severity)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

Note. Mean severity score based on 0 = normal; 1 = minimal; 2 = mild; 3 = moderate; and 4 = severe. Score is the mean score of the affected animals.

[a]Significantly different from control $p < .01$.

Acute inflammatory changes consisting of infiltrates of eosinophils in the submucosa of the gallbladder were seen with slightly greater frequency in the JP-4-exposed mice (22 and 33%, high- and low-exposed group) when compared with controls (9%). Frequently, but not exclusively, this change was accompanied by hyaline degeneration changes of the mucosal epithelium.

Renal tubular dilation was noted with slightly greater frequency in the low concentration mice (29%) when compared with the controls (7%) and high concentration (10%) mice. This lesion consisted of slightly dilated tubules near the corticomedullary junction, filled with pink, homogeneous fluid.

Ultrastructural Examination

Examination of kidney ultrastructure of JP-4-exposed male rats revealed hyaline, crystalloid intracytoplasmic inclusions in the proximal tubule cells. The severity of the inclusions was greater in the high-dose group, although occasional proximal tubule cells from the low-concentration group contained crystalloid inclusions equal in size and number to the high-concentration proximal tubules. Mitochondria and endoplasmic reticulum were not affected until proximal tubules became engorged with inclusions, exhibited excessive dilation of the abluminal cell membrane, or began to form a cast. The appearance of the mitochondria then became more irregular in shape and the cristae appeared more dilated than in the control group. The casts were composed of necrotic, exfoliated tubular epithelial cells, which formed prominent tubular plugs near the corticomedullary junction and resulted in focal tubular dilation. Distal tubules appeared normal in both low- and high-dose groups. Glomeruli were unaffected by JP-4 exposure.

Histopathology—Postexposure Results

Histopathologic findings at 19 and 21 mo postexposure were limited to male rats. Protein casts and hyaline droplets were no longer significant findings in the kidneys of treated animals (Tables 3 and 4). Instead, increased incidence of medullary tubular mineralization and hyperplasia of pelvic urothelium was noted.

No JP-4-related lesions were noted in the mice examined postexposure. Incidence of lesions noted in the JP-4-exposed mouse groups did not differ significantly from those of the control mouse group.

DISCUSSION

Pathophysiological changes noted in dogs, mice, and rats following 90 days of continuous exposure to JP-4 vapor indicated no significant respiratory toxicity. Except for a depression in mean body weight gains of rats, all species tolerated the exposures without adverse clinical signs or increase in morbidity

TABLE 3. Incidence Summary of Selected Microscopic Renal Lesions in F344 Rats 19 Mo after a 90-Day Continuous Exposure to JP-4 Vapor

	Male			Female		
	Control	500 mg/m³	1000 mg/m³	Control	500 mg/m³	1000 mg/m³
Number examined	31	26	27	31	25	22
Nephropathy	29	24	25	7	11	11
(Severity)	(1.7)	(1.8)	(2.2)	(0.2)	(0.5)	(0.5)
Tubular mineralization	2	25[a]	26[a]	1	0	1
(Severity)	(0.1)	(1.9)[a]	(2.1)[a]	(0.0)	(0.0)	(0.0)
Granular protein casts	0	0	1	6	0	2
(Severity)	(0.0)	(0.0)	(0.0)	(0.2)	(0.0)	(0.1)
Hyaline droplet accumulation	0	0	0	1	0	0
(Severity)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0 0)
Pelvic urothelium hyperplasia	1	7	16[a]	0	0	0
(Severity)	(0.0)	(0.3)	(1.1)[a]	(0.0)	(0.0)	(0.0)

Note. Mean severity score based on 0 = normal; 1 = minimal; 2 = mild; 3 = moderate; and 4 = severe. Score is the mean score of the affected animals.

[a]Significantly different from control, $p < .01$.

TABLE 4. Incidence Summary of Selected Microscopic Renal Lesions in F344 Rats 21 Mo after a 90-Day Continuous Exposure to JP-4 Vapor

	Male			Female		
	Control	500 mg/m³	1000 mg/m³	Control	500 mg/m³	1000 mg/m³
Number examined	16	21	20	15	20	18
Nephropathy	16	21	20	8	17	10
(Severity)	(1.9)	(2.5)[a]	(2.5)[a]	(0.7)	(1.0)	(0.7)
Tubular mineralization	0	21[b]	19[b]	0	1	0
(Severity)	(0.0)	(1.7)[b]	(2.0)[b]	(0.0)	(0.1)	(0.0)
Granular protein casts	0	0	0	2	1	5
(Severity)	(0.0)	(0.0)	(0.0)	(0.1)	(0.1)	(0.3)
Hyaline droplet accumulation	0	0	0	0	0	0
(Severity)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Pelvic urothelium hyperplasia	1	17[b]	17[b]	0	0	0
(Severity)	(0.1)	(2.0)[b]	(1.9)[b]	(0.0)	(0.0)	(0.0)

Note. Mean severity score based on 0 = normal; 1 = minimal; 2 = mild; 3 = moderate; and 4 = severe. Score is the mean score of the affected animals.

[a]Significantly different from control $p < .05$.

[b]Significantly different from control $p < .01$.

or mortality. Hematologic and clinical chemistry evaluations conducted on rats immediately following exposure, and again at 19 mo postexposure, did not identify changes considered to be of toxicological significance.

Significant microscopic lesions following the 90-day continuous exposure were primarily restricted to renal lesions found in male rats. The renal lesions were constant with those characterized in previous studies (Kinkead et al., 1991a; Carpenter et al., 1975a, 1975b; Gaworski et al., 1984; Mattie et al., 1991). The hyaline droplets formed in proximal tubular epithelial cells have been shown to consist of $\alpha_{2\mu}$-globulin (Swenberg et al., 1989). This protein is synthesized in extraordinary amounts in the liver of male rats exposed to hydrocarbons and is under the control of androgens. Alpha$_{2\mu}$-globulin is filtered by the glomerulus and resorbed by the epithelial cells of the proximal convoluted tubules forming the hyaline droplets. The hyaline crystalloid inclusions found in the ultrastructural examination of the kidneys are now known to represent this protein within phagolysosomes (Mattie et al., 1991). The granular protein casts observed in this and other studies represent entrapped cellular debris, most likely from the proximal convoluted tubular epithelium, although concurrent necrosis was not observed. Ultrastructurally these casts were located near the junctions of the pars recta and the descending limb of the loop of Henle.

By 19 mo postexposure, hyaline droplets and protein casts had resolved and the principal lesions were linear concretions of the inner medulla, thought to be mineralized necrotic debris trapped in the hairpin turn of the loops of Henle and, in the high exposure group, hyperplasia of the renal pelvic urothelium. Hyperplasia of the pelvic urothelium is often associated with mineralization that extends into the renal pelvis and may be due to mineral-induced rigidity of the renal papilla, which results in friction/stimulation of the overlying pelvic urothelium. These changes were also the lesions of significance noted at the final necropsy (21 mo postexposure). Chronic progressive nephrosis is a background lesion affecting control and exposed animals of both sexes. Although the incidence of this lesion was similar for all groups, the severity was greatest for those males exposed to JP-4 vapor, suggesting some interaction between $\alpha_{2\mu}$-globulin nephropathy and chronic, progressive nephrosis.

Although several neoplasms were encountered in rats, there were no statistically significant differences in incidence between treated and control groups. The numbers and types of tumors are within the expected historical normal range for aging F344 rats (Haseman et al., 1990). Only one renal tumor was noted in this study, a male control rat examined 21 mo postexposure. Bruner et al. (1993) observed that renal tumor incidence was increased in male rats that had been exposed for at least 1 yr to hydrocarbons that induce $\alpha_{2\mu}$-globulin nephropathy. Renal tumors were not observed in similar hydrocarbon studies in which the animals were held up

to 21 mo postexposure following 90 days of continuous exposure. Bruner et al. (1993) concluded that increased renal neoplasia may be expected when male rats are exposed for 1 yr or longer to hydrocarbons that promote tubular cell death (and proliferation) following perturbations in the renal handling of $\alpha_{2\mu}$-globulin.

The hepatocellular fatty change noted in mice examined immediately postexposure is considered to be a degenerative process and is reversible if there is no further damage to the cell. As expected, following 19 and 21 mo exposure this lesion had resolved with only 2 control and 1 low-concentration mouse showing fatty change. Gallbladder lesions, although higher in the JP-4-exposed mice following exposure, were not dose related and were not noted in any of the mice examined 19 or 21 mo postexposure.

Renal tubular dilation found in mice following the 90-day exposure is produced by a fluid that is thought to originate from an incompetent glomerular filtration mechanism secondary to mild membranous glomerulonephritis. Glomerulonephritis is a lesion common in aging C57BL/6 mice (Frith et al., 1983) and was equally distributed between treated and control mouse groups at the 19 and 21 mo examination.

CONCLUSIONS

The most significant finding in this study is the increased incidence of renal disease in the male rats. No renal neoplasms were found in the JP-4-exposed male rats. Many organic chemicals, including military aviation fuels, can produce hyaline droplet nephropathy in the male rat (Bruner, 1984; Bruner et al., 1993; Kinkead et al., 1974, 1991a; Gaworski et al., 1984, 1985; Mattie et al., 1991). The mechanism associated with this syndrome is specific for the male rats and involves the production and accumulation of $\alpha_{2\mu}$-globulin. Renal hyperplasia and neoplasia develop subsequently (Alden et al., 1984; Ridder et al., 1990; Borghaff et al., 1991). Because $\alpha_{2\mu}$-globulin is nonexistent in humans, chemicals producing nephropathy and/or renal tumors via this mechanism are not considered to produce similar renal effects in humans.

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