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THE EXPERIMENTAL DETERMINATION OF SAFE ATMOSPHERIC EXPOSURE CONCENTRATIONS OF JP-10 JET FUEL

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

BRUCE O. STUART, PhD
Director Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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Four animal species were exposed for 1 year to 100 ppm (556 mg/m^3) JP-10 to determine its long-term toxic and oncogenic effects. Rats, mice, and hamsters were maintained for 1 year postexposure, while dogs were held for 5 years postexposure. Mean body weights of exposed hamsters and male rats were lower than controls during exposure. Weight recovery occurred in hamsters, but not in male rats during postexposure phase of the study. Significant JP-10 exposure related effects were renal tubular nephrosis together with an increase in benign and malignant renal cell tumors in male rats.

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PREFACE

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). This document constitutes a final technical report on The Experimental Determination of Safe Atmospheric Exposure Concentrations of JP-10 Jet Fuel. The research covered in this report began in June 1978 and was completed in June 1984, and was performed in part under Air Force Contract Nos. F33615-76-C-5005 and F33615-80-C-0512. K. C. Back, Ph.D. and M. K. Pinkerton served as contract technical monitors for the Air Force Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D. served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor, Department of Community and Environmental Medicine. Acknowledgement is made to J. D. Diaz, G. L. Fogle, J. C. Welch, R. K. Blasingame, and J. L. Monroe for their significant contributions and assistance in the preparation of this report.

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INTRODUCTION

JP-10 is used as a jet fuel either alone or as a major constituent of JP-9 fuel because of its high density and other desirable properties. It is also used as a missile propellant in air breathing turbojet engines. JP-10 is a synthetic saturated polycyclic hydrocarbon identified as tricyclo ($5.2.1.0^{2,6}$) decane. It is the exo isomer of tetrahydrodicyclopentadiene. Gas chromatographic analysis of this fuel indicated that it has a 98% purity with the endo isomer of tetrahydrodicyclopentadiene as the major impurity (about 2%). This information was presented in a detailed review of the chemistry and the use of JP-10 at the Thirteenth Conference on Environmental Toxicology (1982) by Inman.

The acute toxicity of JP-10 was reported by Kinkead et al. (1979). The oral LD_{50} values were unobtainable for male or female Fischer 344 rats and for Golden Syrian hamsters since the maximum usable volume dose of 20 mL/kg caused only partial mortality in either species. MacEwen and Vernot (1979) reported an LD_{50} for female C57BL/6 mice of 3.9 mL/kg. Deaths occurred within 48 hours of treatment with convulsions preceding death. The ip LD_{50} values for rodents were 1.2 mL/kg and 1.6 mL/kg, respectively, for male and female Fischer 344 rats; 1.1 mL/kg for female C57BL/6 mice and 1.4 mL/kg for male Golden Syrian hamsters. JP-10 caused no irritation to eyes or skin of New Zealand White rabbits but was found to produce mild dermal sensitization in Hartley strain guinea pigs. MacEwen and Vernot also reported that the 4-hour LC_{50} for inhaled JP-10 was 1221 ppm for male rats and 1194 ppm for female rats. The ALC_{50} for female mice was given as 930 ppm. No mortality was seen in hamsters exposed for 6 hours to saturated vapor pressure concentrations. JP-10 produced no deaths in a group of New Zealand White rabbits following dermal applications of 20 mg/kg (MacEwen and Vernot, 1980).

Emergency exposure limit studies of inhaled JP-10 vapors were reported by Kinkead et al. (1979). After single short high level exposures of beagle dogs, rats, and mice they found slight CNS responses at very high concentrations. They recommended concentrations of 1000, 600, and 150 ppm for 10, 30, and 60 minutes, respectively, as short-term exposure limits.

JP-10 was not embryotoxic for pregnant ICR mice treated with doses of this fuel up to 0.8 mg/kg during organogenesis (Lyng,

1981). In studies reported by Keller (1983), pregnant rats were treated with doses of JP-10 up to 1000 mg/kg on gestation days 6 through 15. Fetal weights, numbers, and types of malformation and resorptions in treated litters were not different from controls. Inhalation exposures of pregnant rats to 600 ppm for 6 hours/day during this same gestation period caused some convulsions in the dams but no measureable changes in the fetuses or pups from resulting litters.

Short-term bioassays were described by Arthur D. Little Co. (1982) in which only a marginal clastogenic effect was reported for the CHO/chromosome aberration assay. Negative or inconclusive responses were reported for the Ames Salmonella/mammalian microsomal mutagenicity assay, the CHO/HGPRT gene mutation assay, the CHO/sister chromatid assay, and the BALB/C-3T3 neoplastic transformation assay.

The tissue distribution of JP-10 after intraperitoneal injection of radiolabelled fuel was reported by Inman (1982) who also identified the major urinary metabolite as 5-hydroxy exo-tetrahydrodicyclopentadiene excreted as the glucuronide conjugate.

Because the use of JP-10 in operational missiles has been expanded, the number of fuel handlers exposed to this material has increased and there is a need to develop data for hazard evaluation and to establish safe exposure limits.

Preliminary acute inhalation experiments had shown that mice were the most sensitive species to JP-10 when 6 animals exposed to 1000 ppm died within 4 hours during exposure. To aid in selection of a concentration of JP-10 suitable for use in a year-long, 6 hours/day, 5 days/week exposure regimen, groups of 5 female rats and 5 female mice were exposed to 250 ppm for five 6-hour exposure days. The coordination of the mice appeared slightly affected on the first day of exposure. Respiration rates of both rats and mice were more rapid than normal during the second day of exposure. One mouse had a slight convulsion early on the second exposure day, but recovered and appeared normal thereafter. For the rest of the exposure, no further signs of toxic stress were noted in either species. Mean body weights of the mice did not increase during the week following termination of exposure.

As a result of the toxic effects shown in mice in the short-term inhalation tests, a concentration of 100 ppm (556 mg/m^3) JP-10 was selected for chronic inhalation studies with animals to determine safe exposure limits.

MATERIALS AND METHODS

Test Agent

The JP-10 used for these animal exposures was obtained by the Air Force from Suntech, Inc., Marcus Hook, Pennsylvania.

The known physical properties of JP-10 are shown below:

Molecular weight:	136
Boiling point:	182°C (360°F)
Density, 70°F:	0.940
Viscosity, 70°F:	3.5
Flash point:	57.2°C (135°F)
Saturated vapor concentration:	~1500 ppm

Animals

Purebred beagle dogs were selected from a baseline group on the basis of examination and general observation of good health and several preexposure clinical chemistry determinations. Fischer 344 rats and Golden Syrian hamsters were obtained from the Charles River Breeding Laboratories, Wilmington, Massachusetts. C57BL/6 mice were purchased from the Jackson Laboratory, Bar Harbor, Maine and beagle dogs from Ridgelan Farms Inc., Mt. Horeb, Wisconsin.

Dogs and rats were housed in one exposure chamber and mice and hamsters in a companion chamber. The numbers of animals in each chamber and cage were compatible with ILAR standards for animal care. The numbers of rodents permitted a statistically valid number of each species to reach the required age for tumor induction with natural and toxicologic attrition.

Exposure Conditions

Animal exposures to JP-10 were conducted for 1 year, using an industrial work week schedule of 6 hours/day, 5 days/week, with holidays and weekends off to simulate a human exposure regimen. The Thomas Dome exposure chambers (Thomas, 1965) were operated with nominal airflows of 30 cfm at a slightly reduced pressure, 725 mm Hg, to avoid leakage of JP-10 vapor into the laboratory environment. Distribution of the animal groups and other pertinent information is shown in Table 1.

TABLE 1. SPECIES, SEX, STRAIN, AND NUMBER OF ANIMALS EXPOSED TO JP-10 VAPORS FOR 12 MONTHS

Species	Sex	Strain	100 ppm (556 mg/m^3) JP-10 Chamber 1	100 ppm (556 mg/m^3) JP-10 Chamber 2	Unexposed Controls
Rats	M	Fischer 344	---	50	50
Rats	F	Fischer 344	---	50	50
Mice	F	C57BL/6	200	--	200
Hamsters	M	Golden Syrian	100	--	100
Dogs	M	Beagle	---	4	4
Dogs	F	Beagle	---	4	4

Food was provided to the animals during nonexposure times, and the chambers were cleaned daily following the completion of the 6-hour exposure and minimum 30-minute air purge. Analysis of chamber concentration was used to verify the adequacy of the purge time. In approximately 20 minutes the JP-10 vapors were reduced to less than 10 ppm.

Measurements and Pathology

All animals were observed hourly during the exposure phase of the study. Daily observations were conducted during the post-exposure phase until termination of the experiment. Rats, hamsters, and dogs were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in groups on a monthly basis throughout the experimental period.

Blood samples were drawn from all dogs at biweekly intervals for the following tests:

HCT	Potassium
HGB	Calcium
RBC	Albumin/Globulin
WBC	Total Protein
Differential Cell Counts	Glucose
MCV	Alkaline Phosphatase
MCH	SGPT
MCHC	SGOT
Sodium	Bilirubin
BUN	Creatinine

Following the 1-year exposure period, 20 mice/group and 10 hamsters/group were necropsied to determine chronic exposure

effects, while the remaining rodents were held for a year of postexposure observation. The dogs were held for postexposure observation for 5 years, during which time they received quarterly physical examinations and semi-annual blood analysis.

Gross and histopathologic examinations were conducted on all animals that died or were sacrificed during and at the completion of the study. The necropsy protocol followed NCI methods for oncogenic screening (Sontag et al., 1976) and included a complete external and internal examination with the collection of 33 standard tissues and all gross lesions for microscopic evaluation. Autolysis or cannibalization prevented partial or complete examination in some cases. Tables of selected tumor and non-tumor incidence were compiled and statistical analysis using the Fisher Exact Test was performed by the UCI staff.

Vapor Generation and Analysis

A Buchler Polystaltic® Pump was used to deliver the liquid JP-10 from a storage drum into a spiral evaporator where it was vaporized and introduced into the chamber air supply system through a 1/4" stainless steel line. The exposure dome concentrations were monitored using a Beckman Model 400 Hydrocarbon Analyzer. Sequential sampling was conducted on the pair of chambers. The supply of JP-10 in use was analyzed once per month during the study, and each time a sample drum was changed. This was done using a Varian Model 3700 gas chromatograph with a 1/8" x 10' stainless steel column containing 10% SE 30 on Chromosorb W and helium carrier gas at 30 mL/min. The column was temperature programmed from 90°C to 200°C, at 10° per minute. The detector and injector temperatures were 220°C and the injection volume was one microliter.

RESULTS

Chamber Concentration Analysis

The desired concentration of 100 ppm JP-10 was maintained in both chambers with little variation between the chambers throughout the 12-month exposure phase of the experiment (June 5, 1978 through June 4, 1979). Monthly mean chamber concentrations are listed in Table 2. Overall mean concentrations for Chambers 1 and 2, respectively, were only 0.4% and 0.2% lower than the 100 ppm desired concentration.

TABLE 2. MONTHLY JP-10 CONCENTRATIONS (PPM) THROUGH THE 12-MONTH EXPOSURE PERIOD (MEAN ± STANDARD ERROR)

Month	Chamber 1	Chamber 2
June, 1978	99.2 ± 0.28	100.0 ± 0.08
July, 1978	99.4 ± 0.20	99.5 ± 0.30
August, 1978	100.8 ± 0.10	98.9 ± 0.14
September, 1978	101.4 ± 0.08	101.1 ± 0.10
October, 1978	100.3 ± 0.12	100.3 ± 0.10
November, 1978	99.3 ± 0.07	100.0 ± 0.09
December, 1978	99.4 ± 0.08	99.7 ± 0.06
January, 1979	99.8 ± 0.08	100.5 ± 0.08
February, 1979	99.6 ± 0.05	99.7 ± 0.09
March, 1979	100.4 ± 0.06	99.9 ± 0.04
April, 1979	99.9 ± 0.12	100.4 ± 0.11
May, 1979	100.2 ± 0.11	99.7 ± 0.08
June, 1979	99.7 ± 0.23	100.0 ± 0.25
Overall Mean	99.6	99.8

Mortality

The mortality ratios at the end of the 12 months of exposure and at 12 months postexposure immediately prior to the sacrifice of all surviving rodents are shown in Table 3. This table does not include the animals killed at the end of exposure and submitted for necropsy to determine if tissue changes were present at that time. There was no effect of JP-10 exposure on rodent mortality. One female control dog died 25 months postexposure. This animal had demonstrated epileptic seizures several times.

TABLE 3. MORTALITY RATIOS FOR GROUPS OF JP-10 EXPOSED AND CONTROL ANIMALS AT EXPOSURE CONCLUSION AND AT 12 MONTHS POSTEXPOSURE

		Unexposed Controls		100 ppm JP-10 Exposed	
Species	Sex	Exposure Conclusion	12-Months Postexposure	Exposure Conclusion	12-Months Postexposure
Mice	F	30/200	[a]128/180	20/200	[a]123/180
Rats	M	0/50	16/50	0/50	8/50
Rats	F	4/50	21/50	0/50	20/50
Hamsters	M	5/100	[a]37/90	9/100	[a]33/90
Dogs	M	0/4	0/4	0/4	0/4
Dogs	F	0/4	0/4	0/4	0/4

[a] Censored for 10% of initial group sacrifices at exposure conclusion.

Animal Weights

Mean body weights for groups of exposed and control male rats, female rats, and male hamsters, obtained on a biweekly schedule throughout 12 months of exposure and monthly through 12 months postexposure, are shown in Figure 1. Weights of male rats and hamsters showed depression relative to controls as a result of JP-10 exposure. Values for male rats were statistically different from control values at all times during exposure and postexposure. Values for exposed hamsters were also statistically different from controls at all weighing periods during exposure but not during the postexposure phase of the experiment. Exposed female rat weights were not significantly different from controls at any phase of the study. An examination of all mean weights of dogs and mice taken during and after exposure revealed no effect of JP-10 exposure.

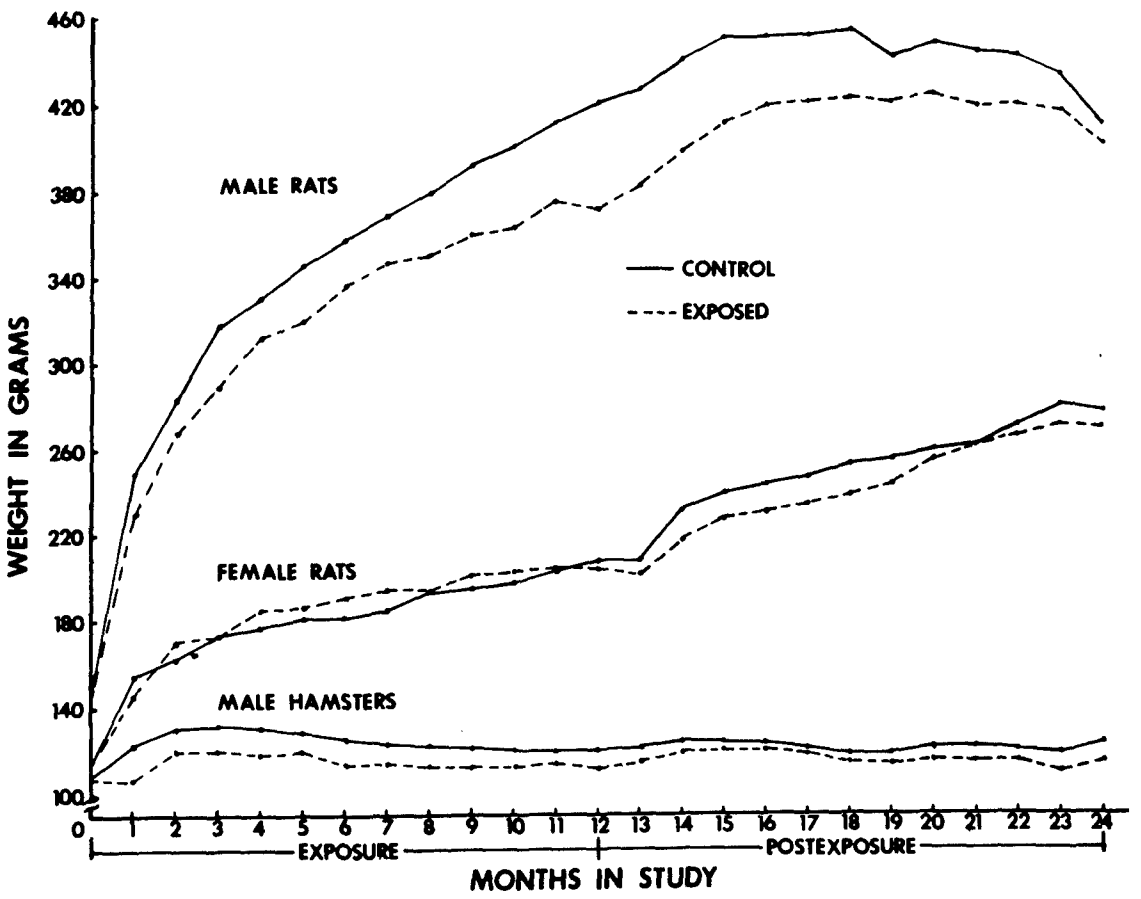

Figure 1. Mean body weight of rats and hamsters exposed intermittently to 100 ppm JP-10 for 1 year.

Hematology

An examination of the hematology and clinical chemistry values from the battery of tests conducted biweekly on dogs throughout the 52 weeks of exposure revealed nothing noteworthy except for total protein and globulin results. Albumin values for exposed dogs were stable and comparable with controls throughout the exposure. Slightly elevated protein values, therefore, reflected slight elevations in the calculated globulin fraction from weeks 2 through 52. Statistical differences from control values were seen in 9 of 16 measurements from exposure week 22 to exposure conclusion, but the albumin/globulin ratios for the exposed dogs were well within normal limits for this species. No toxicologic significance is attached to this finding. The results of postexposure, quarterly physical examinations, and semi-annual clinical chemistry measurements indicated that exposed and control dogs were in good health. The 15 surviving dogs were maintained until June 1984.

Pathology

Dogs - As mentioned previously, one female control dog died approximately 3 years after the start of the experiment. This animal had a record of epileptic seizures, but microscopic examination of tissues failed to reveal the cause of death. CNS lesions were not observed grossly or microscopically.

Histopathologic findings in the 15 dogs that were sacrificed 5 years postexposure were considered to be common changes typically seen in aging dogs. Two exposed males had patchy, mild testicular atrophy characterized by loss of spermatogonia. In that evidence of long-term testicular damage was lacking, the possibility that this lesion was exposure related was slight. Three tumors were seen in the dogs used in this study. One control male had an adematous polyp of the anal mucosa while an adrenocortical adenoma was seen in another male control. An adrenal pheochromocytoma was observed in an exposed female dog and was the only neoplasm recorded in exposed canine subjects.

Mice - Significant non-neoplastic lesions observed in mice are listed in Table 4. Lesions noted in mice that died or were killed during or immediately following the 1-year exposure are tabled under the heading "Through 1 Year of Exposure." Correspondingly, non-tumor changes recorded in mice that died or were killed incidental to the 1-year postexposure holding period or final sacrifice are listed under "Through 1 Year Postexposure." Several unusual trends in the incidence of non-neoplastic lesions are evident in the table; however, most were regarded as spontaneous changes and probably unrelated to JP-10 exposure.

TABLE 4. SIGNIFICANT NON-NEOPLASTIC LESIONS[a] IN FEMALE C57BL/6 MICE EXPOSED TO JP-10

Lesion Description	Through 1 Year of Exposure		Through 1 Year Postexposure	
	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Skin				
Inflammation (Ulcerative Dermatitis)	16(33)	0(0)[b]	21(14)	29(19)
Nose				
Hyaline Degeneration Crystals	21(46)	2(7)[b]	85(58)	66(43)[b]
Lung				
Hyaline Degeneration Crystals	0(0)	0(0)	18(12)	39(25)[b]
Alveolar Macrophages	1(2)	1(4)	16(11)	20(13)
Perivascular Cuffing	2(4)	4(14)	17(12)	19(12)
Bone Marrow				
Granulocytic Hyperplasia	22(49)	9(31)	55(40)	81(57)[b]
Spleen				
Granulocytic Hyperplasia	15(34)	4(15)	29(20)	30(20)
Liver				
Fatty Change	31(67)	21(75)	70(47)	95(61)[b]
Gallbladder				
Hyaline Degeneration	5(13)	5(21)	11(9)	29(24)[b]
Kidney				
Glomerulonephritis	0(0)	3(11)	18(12)	21(14)
Uterus				
Cysts	0(0)	0(0)	1(1)	21(14)[b]
Ovary				
Cysts	0(0)	1(4)	23(18)	40(29)[c]
Thyroid				
Follicle, Papillary Hyperplasia	0(0)	1(4)	100(73)	86(60)[c]

[a] Number of lesions (% incidence).
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

Severe ulcerative dermatitis was observed in 33% of the control mice, whereas no skin lesions were seen in exposed mice through one year of exposure. This is a common finding in C57BL/6 mice, but the high incidence in the controls suggests that exposure to JP-10 may have provided some protective effect for ulcerative skin disease. In this connection, it is possible that JP-10 vapors may have inhibited olfactory senses and reduced skin insult associated with trichophagia and cannibalism. Regardless, the high incidence of ulcerative dermatitis was considered to be a significant factor for increased mortality in control mice during the exposure phase of the study. Following exposure termination, the frequency of ulcerative dermatitis was nearly equal for exposed and control mice during the postexposure holding period. It should be emphasized that ulcerative skin lesions were considered to be a major contributing factor for splenic/bone marrow granulocytic hyperplasia and hepatocellular fatty change noted in most groups of mice. Hyaline degeneration/ crystals of respiratory and gallbladder epithelia is a common aging change in mice. However, these changes were slightly, but statistically, increased in the lungs and gallbladder of exposed subjects during the postexposure holding period. Etiologic factors for hyaline degeneration remain obscure, but may be related to common diseases altering the immune status of the animal such as malignant lymphoma and chronic skin disease. Although statistically increased in exposed mice held for long-term observation, hyaline change was not believed to be a direct effect of JP-10 exposure.

Fatty livers were found in both the exposed and control mice. The incidences of these lesions were comparable immediately postexposure, but were significantly higher in exposed mice at the termination of the experiment. As with hyaline change, the relationship of hepatocellular fatty change with JP-10 exposure remains unclear. However, in many instances fatty livers were associated with chronic, debilitating diseases thought to be unrelated to JP-10 exposure.

Ovarian and uterine endometrial cysts are extremely common in aged female mice, but statistically significant increases of both lesions are seen for exposed mice through 1-year postexposure. Uterine cysts, particularly, showed a twenty fold increase in the exposed group over controls at the end of the postexposure period. No explanation was apparent for this finding.

The incidences of neoplastic lesions in exposed and control mice were similar. The majority of tumors occurred with equally low frequency in both groups. There was a large number of pituitary adenomas and lymphomas of multiple organs in all mice

through the 1-year postexposure period, and these common tumors were thought to be responsible for many secondary non-neoplastic changes such as uterine endometrial cysts, hepatocellular fatty change, and hyaline degeneration in several organs. Statistical calculations reveal no significant differences in the incidence of these tumors between exposed and control animals.

Hamsters - Non-neoplastic lesions found in hamsters are shown in Table 5. During exposure, the only lesion more prevalent in exposed animals was congestion of the lungs. This is a common finding, however, and may have resulted from non-exposure related events, such as postmortem hemostasis. In exposed hamsters maintained through 1 year exposure, there was a statistical increase of hyperplastic lesions of the adrenal cortex. This adrenal hyperplasia was most often seen in the zona glomerulosa (outer zone) as opposed to the other zones of the cortex. Additional findings in exposed hamsters included slight but significantly increased incidences of testicular and pancreatic atrophy. An interesting finding, but without statistical support, was the increased incidence of fatty livers in the exposed hamsters compared with controls. The only neoplastic lesions of interest noted in hamsters are listed in Table 6. Adrenocortical tumors were found in both the exposed and control hamsters through 1-year postexposure. The combined incidence of cortical adenomas and carcinomas was 27% in controls and 28% in exposed subjects. Of this group, 11 of the exposed animals exhibited adenomas of the zona glomerulosa, whereas only 3 of the control hamsters had tumors of this, the outer zone. In considering the prominent numbers of adrenocortical neoplasms observed in this study, it is important to recognize that tumors of this tissue are very common in aging hamsters. In a review of spontaneous and non-viral induced neoplasms in hamsters by Kirkman and Algard (1968), the combined incidence of adenomas and carcinomas found in 4,575 untreated control hamsters was 35.5% for the second year of life and the vast majority of these lesions were found in the zona glomerulosa. These background incidence figures suggest that adrenocortical neoplasms recorded in this study were well within the range of normal variation for the hamster.

Rats - Table 7 lists non-neoplastic lesions seen in female and male rats following 1 year of exposure to JP-10 and 1 year of postexposure holding. Various lesions indicative of accentuated renal tubular nephrosis in exposed male rats are in strong evidence. Accentuated renal tubular degeneration, medullary mineralization, and papillary hyperplasia of the pelvic transitional epithelium were observed with significantly increased frequency or severity in exposed males. These lesions were entirely

TABLE 5. SIGNIFICANT NON-NEOPLASTIC LESIONS[a] IN MALE GOLDEN SYRIAN HAMSTERS EXPOSED TO JP-10

Lesion Description	Through 1 Year of Exposure		Through 1 Year Postexposure	
	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Lung Congestion	6(40)	12(71)	9(11)	6(8)
Liver Fatty Change	0(0)	0(0)	39(46)	44(56)
Pancreas Atrophy	1(10)	0(0)	0(0)	6(9)[b]
Testis Atrophy	0(0)	0(0)	12(14)	28(35)[c]
Prostate Atrophy	0(0)	0(0)	6(8)	8(12)
Seminal Vesicle[d] Atrophy	----	----	2(3)	6(8)
Adrenal Cortex Hyperplasia	2(13)	3(18)	38(45)	67(86)[c]

[a] Number of lesions (% incidence).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.
[d] Not obtained through 1 year of exposure.

consistent with histopathologic findings in male rats exposed subchronically (90-day continuous exposure) to other nephrotoxic hydrocarbons and held for long-term evaluations (MacEwen and Vernot, 1978, 1979, 1980, 1981, and 1982). Marked medullary mineral deposits were present in all exposed males but were entirely absent from controls. These deposits were regarded as mineralized cell debris which originated from toxic necrosis of proximal tubular epithelium. Papillary hyperplasia of the renal pelvis was noted in 53% of the exposed and only 4% of the controls. The pathogenesis of this lesion remains obscure but may be related to the abrasive action of mineralized concretions shed

TABLE 6. NEOPLASTIC LESIONS[a] IN THE ADRENAL CORTEX OF MALE GOLDEN SYRIAN HAMSTERS EXPOSED TO JP-10

	Through 1 Year of Exposure		Through 1 Year Postexposure	
Tumor Type	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Adrenal Cortex	N = 15	N = 17	N = 85	N = 78
Adenoma	0(0)	1(6)	12(14)	15(19)
Carcinoma	0(0)	0(0)	11(13)	7(9)
Total	0(0)	1(6)	23(27)	22(28)

N = Number examined.
() = Percent incidence.
[a] Number of lesions.

from renal tubules, or to increased α-globulins in the final filtrate. More frequent and more severe findings of tubular degeneration were documented in exposed males where 88% exhibited minimal to moderate tubular degeneration as compared with 65% of the controls where changes were graded as only minimal to mild. On a severity scale of 0 to 4, where 0 = no effect and 4 most severe, tubular degeneration was graded as 1.8 in the exposed male rats and 1.3 in the controls. Since many of the morphologic features of accentuated tubular degeneration were entirely compatible with spontaneous, chronic progressive nephropathy of old rats, frequency and severity data are important in establishing a relationship with JP-10 exposure. Bruner has described in detail the pathology of nephrotoxicity of hydrocarbons in male rats in a number of experiments conducted in our laboratory in a presentation at the Thirteenth Conference on Environmental Toxicology (Bruner and Pitts, 1982). In this regard, it is becoming increasingly apparent that "hydrocarbon nephrotoxicity" in male rats caused by a large variety of hydrocarbon chemicals may be directly associated with a special class of α-globulin serum proteins which are uniquely restricted to male rats. Accordingly, the presence of hydrocarbon-induced nephropathy in male rats may have poor predictive value in toxicologic extrapolations to humans.

TABLE 7. SIGNIFICANT NON-NEOPLASTIC LESIONS[a] IN FEMALE AND MALE FISCHER 344 RATS FOLLOWING EXPOSURE TO JP-10

	Females		Males	
Lesion Description	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Spleen				
Hemosiderosis	13(28)	25(51)[b]	6(12)	11(22)
Heart				
Myocardium Fibrosis	5(10)	7(14)	26(52)	32(64)
Blood Vessels				
Pulmonary Artery Mineralization	12(24)	20(40)	24(48)	17(34)
Liver				
Fatty Change	7(14)	10(20)	1(2)	4(8)
Focal Cellular Change	26(53)	27(55)	37(76)	40(80)
Bile Duct Hyperplasia	18(37)	22(45)	45(92)	49(98)
Kidney				
Tubular Pigmentation	4(8)	17(34)[c]	4(8)	5(10)
Medullary Mineralization	0(0)	0(0)	0(0)	49(100)[c]
Pelvic Papillary Hyperplasia	0(0)	0(0)	2(4)	26(53)[c]
Tubular Degeneration	1(2)	2(4)	32(65)	43(88)[c]
Adrenal				
Focal Cellular Change	18(37)	13(27)	13(26)	17(34)

[a] Number of lesions (% incidence).
[b] Different from control, $p < 0.05$.
[c] Different from control, $p < 0.01$.

There were statistically significant differences in the incidences of spleen and kidney pigments in exposed female rats compared with controls. Minimal to mild splenic hemosiderosis was present in 51% of the exposed females, while 28% of the controls showed increased hemosiderin deposits. Similarly, renal

tubular cytoplasmic pigmentation was observed in 34% of the exposed animals and in 8% of the control group. In most cases, the increased frequency of these pigment deposits in female rats was thought to be secondary to the increased incidence of mononuclear cell leukemia (large granular lymphocyte leukemia) recorded in this group.

Neoplasms seen in male and female exposed and control rats following the 1-year postexposure period are shown in Table 8.

TABLE 8. NEOPLASTIC LESIONS[a] IN FEMALE AND MALE FISCHER 344 RATS FOLLOWING EXPOSURE TO JP-10

	Females		Males	
Tumor Type	Unexposed Controls	100 ppm Exposed	Unexposed Controls	100 ppm Exposed
Kidney				
Carcinoma	0	0	1(2)	4(8)
Adenoma	0	0	0	5(10)[c]
Testes				
Interstitial Cell Tumor	----	----	44(88)	47(96)
Pituitary				
Adenoma	15(32)	15(33)	15(33)	11(23)
Pancreas				
Islet Cell Adenoma	0	3(6)	1(2)	0
Multiple Organs				
Mononuclear Cell Leukemia	2(4)	11(22)[b]	6(12)	5(10)

[a] Number of lesions (% incidence).
[b] Different from control, $p < 0.01$.
[c] Different from control, $p < 0.05$.

As indicated, mononuclear cell leukemia was present in 22% of the exposed females and in 4% of the controls. Although the increase in exposed females is significant at the 0.01 level, mononuclear cell leukemia is very common in aging Fischer 344 rats. More importantly, 4 renal cell carcinomas and 5 renal cell adenomas

were found in the kidneys of exposed male rats as compared with 1 found in controls. These findings strongly suggest that chronic exposure to JP-10 vapors causes both malignant and benign renal cell tumors in male rats. However, it should be emphasized that pathogenic mechanisms resulting in "hydrocarbon nephropathy" in male rats include a markedly accelerated death and exfoliation of proximal tubular epithelial cells. Correspondingly, repair efforts include increased mitotic activity and proliferative events which would enhance the expression of any latent neoplastic tendency for renal tubular epithelium. Therefore, increased renal neoplasia in exposed male rats may be directly related to chronic tubular insult and accelerated repair activities rather than a direct genotoxic effect of JP-10. Since "hydrocarbon nephropathy" is unique for male rats and associated with pathophysiologic events which are specific for the male rat, it is probable that JP-10 would not cause increased renal cell neoplasia in humans. More study is needed to explain this phenomenon.

CONCLUSIONS

The outstanding effects of repeated exposure to 100 ppm JP-10 were renal tubular nephrosis together with a significant increase in benign and malignant renal cell tumors in male rats. The significance of renal carcinoma and increased renal nephropathy in the male rat is thought to be related to the presence of a special serum α-globulin exclusively found in male rats. It is thought that this globulin (termed $\alpha_{2\mu}$ globulin) easily complexes with JP-10, or its metabolite, rendering a protein-hydrocarbon complex which is readily filtered by the kidneys; subsequently, the complex is reabsorbed by the proximal tubular cells but cannot be easily digested by lysosomal enzymes, and ultimately results in constipation and death of the tubular cell. As a result, tubular nephrosis and neoplasia are increased in male rats exposed to many hydrocarbons. This finding has not been seen in the female rat nor in mice, hamsters or dogs. To date there has been no evidence of renal effects in man from exposure to hydrocarbon fuels.

The results of this study provide evidence that 100 ppm JP-10 may not be a safe exposure level for man. Based on the information developed in this study, a TWA interium exposure limit of 25 ppm JP-10 has been recommended (McNaughton, 1981; McNaughton et al., 1984).

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