

NEPHROTOXICITY OF HYDROCARBON PROPELLANTS TO
MALE, FISCHER-344 RATS

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INTRODUCTION

The Toxic Hazards Division of the Air Force Aerospace Medical Research Laboratory (AFAMRL), Wright-Patterson Air Force Base, Ohio, has sponsored extensive investigations to determine the toxicity and carcinogenicity of several hydrocarbon propellants of military and civilian interest. Most studies have been conducted in collaboration with the Toxicology Detachment, Naval Medical Research Institute and the Toxic Hazards Research Unit of the University of California (Irvine), both of which are located at Wright-Patterson AFB. Although results have been finalized for only about one-half of the hydrocarbon toxicity experiments originally scheduled, it has become clear that most of the test agents induce a toxic nephropathy in Fischer-344 rats which is exclusively restricted to male animals. This nephropathy has been reported by several other investigators (Alden et al., 1982; Carpenter et al., 1975 a and b, 1977; Gaworski et al., 1979; Parker et al., 1981; Phillips, 1982); however, no published reports have been found which address the histopathologic changes which occur in the kidneys of rats following long-term exposure to assorted hydrocarbon fuels. Therefore, the purpose of this report is to: (1) describe pathologic changes in the kidneys of male rats following both short and long-term exposure to hydrocarbon propellants, (2) outline agents which have produced renal disease in rats, and (3) discuss possible pathogenic mechanisms resulting in nephrotoxicity and carcinogenicity.

NEPHROPATHY INDUCED BY HYDROCARBON DISTILLATES

SHORT-TERM STUDIES

Hydrocarbon-induced nephropathy was first observed at AFAMRL in 1978 when a group of F-344 rats came to necropsy following a 90-day continuous inhalation exposure to petroleum-derived JP-5 jet fuel (P/JP-5). P/JP-5 is the U.S. Navy's principal jet fuel and is a petroleum distillate with straight-chain hydrocarbons between C₁₀ and C₁₅ making up the bulk of the mixture. Experimental design included two exposure groups of 150 and 750 mg/m³, with 75 male and 75 female rats assigned to each dose group. An equal number of rats of both sexes was held as controls. Immediately following exposure, one-third of the animals in each group was sacrificed for gross and histopathologic examination while the other two-thirds (100 rats per exposure group) were held for lifetime oncogenic evaluation.

When necropsy examinations were performed immediately following the 90-day inhalation period, the kidneys from exposed males exhibited slight, tan discoloration and minimal capsular mottling, but otherwise they were unremarkable. However, when renal specimens were examined histologically, even at subgross magnifications, dilated, cystic tubules were noted near the corticomedullary junction and were filled with a coarsely granular, eosinophilic debris (Figure 1). At higher magnifications, the tubules were focally dilated and the epithelial lining was markedly attenuated, probably due to compression by the intraluminal plugs of debris. These lesions were thought to occur where the pars recta of the proximal convoluted tubule enters the descending limb of the loop of Henle. In addition to lesions near the corticomedullary junction, there was a marked increase in cytoplasmic hyaline droplets in proximal tubular epithelial cells throughout the cortex, and with careful examination, one could discern that individual cells throughout the proximal tubules were dying, sloughing out, and were being replaced by the mitotic activity of adjacent epithelial cells (Figure 2). These findings were present in males of both exposure groups, but they were more severe in the high dose subjects, indicating a dose response relationship. Significant gross and microscopic lesions were absent in other organs, and kidney tissues in females and control males were essentially normal.

Because P/JP-5 was one of the first in a long series of hydrocarbon toxicity studies scheduled at AFAMRL, pathogenic mechanisms resulting in increased hyaline droplet formation and necrosis (or necrobiosis) of proximal tubular cells became a matter of intense interest. Especially troublesome in evaluating these unique expressions of nephrotoxicity, however, was the knowledge that cytoplasmic hyaline droplets have been frequently noted in tubular cells of untreated, adult, male rats where they have been regarded as resorbed protein undergoing lysosomal degradation

(Kretchmer et al., 1974; Logothetopoulos, 1955). Therefore, in P/JP-5 exposed males, mechanisms which might overburden or otherwise compromise tubular cell protein catabolism were considered to be likely etiologic factors. Correspondingly, the pathogenesis of tubular lesions was thought to include one, or a combination, of the following -- elevated plasma levels of filterable proteins, glomerular insult with filtration incompetence, inhibition or overload of tubular cell lysosomal functions or other cytotoxic actions directly affecting tubular cell protein degradation.

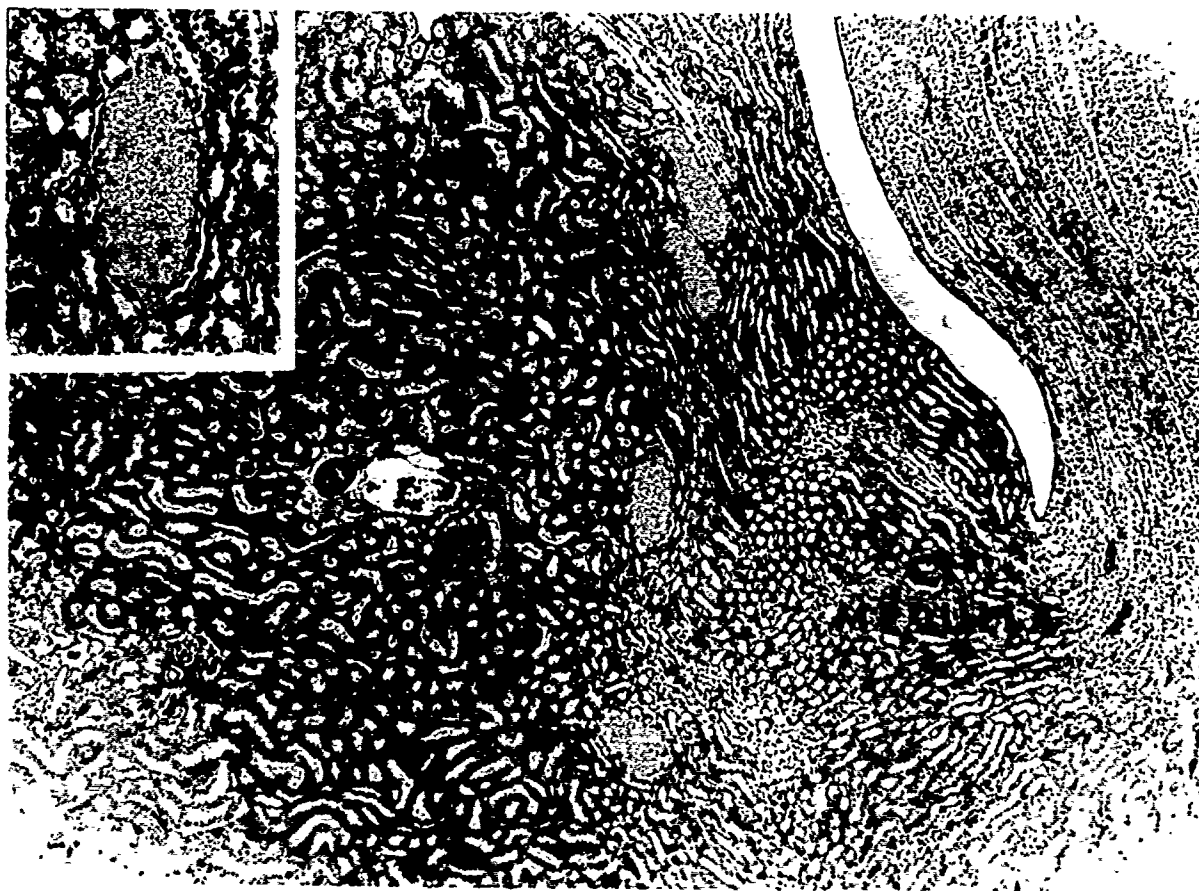


Figure 1. Dilated tubules (arrows) near the corticomedullary junction filled with coarse, proteinic debris. Mag 10X. (Inset - 25x mag of plugged tubule)

In evaluating these possibilities, the general absence of lesions in other organs and the failure of clinical chemistry tests to demonstrate elevated serum proteins suggested that expanded pathology studies should focus on the kidney alone. Therefore,

electron microscopic study of key renal structures was undertaken to search for ultrastructural lesions which might explain the origins of P/JP-5 induced tubular changes.

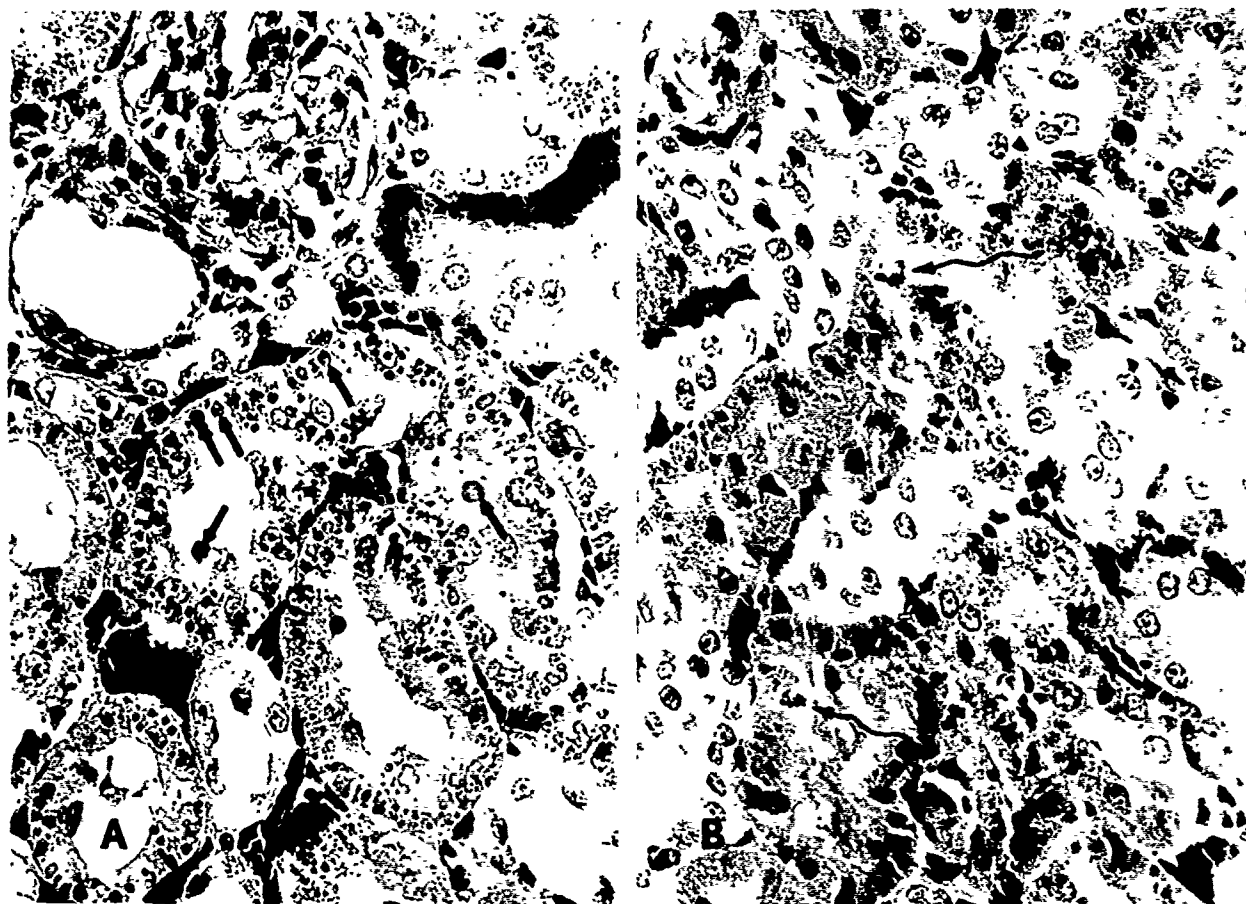


Figure 2. (A) Cytoplasmic hyaline droplets (arrows) in proximal convoluted tubular epithelium. (B) Mitotic activity (wavy arrows) by proximal tubular cells to maintain surface integrity. Mag 25X

Transmission electron microscopy revealed renal glomeruli to be morphologically unremarkable. It also confirmed the presence of abundant, membrane-bound, round to crystalline structures, compatible with densely packaged proteinic material, in the cytoplasm of proximal tubular epithelium. Most importantly, however, it disclosed that the amorphous, coarsely granular, tubular plugs near the corticomedullary junction consisted of degenerating cellular organelles and debris, and not products of hematogenous origin. These findings corroborated histopathologic interpretation with the light microscope, but failed to identify specific causes for increased hyaline droplet formation or accelerated tubular cell death.

LONG-TERM STUDIES

Approximately 20 months following the post-exposure sacrifice, rats held for lifetime oncogenic evaluation were euthanized. At necropsy, the kidneys of most exposed males and modest numbers of females and controls of both sexes were enlarged, pale and exhibited a rough external surface with numerous, subcapsular microcysts. Microscopically, most of these kidneys were found to have varying degrees of tubular dilatation, nephrosclerosis, and other degenerative changes entirely consistent with chronic progressive nephrosis (progressive renal disease) of old rats. These lesions, however, were clearly more prevalent and severe in male rats exposed to P/JP-5 fuel when compared with females or control males. Furthermore, two additional microscopic changes were present in exposed males which were not seen in controls or females.



Figure 3. Mineralized debris in medullary tubules (straight arrows). Papillary hyperplasia of pelvic urothelium over the surface of the renal papillus (wavy arrows). Mag 10X

These changes consisted of heavy deposits of mineralized debris in medullary tubules and mild to moderate, multifocal, papillary hyperplasia of pelvic urothelium along the surface of the renal papillus (Figure 3). No primary renal cell tumors were observed in animals assigned to this experiment; however, renal tissues are currently being re-evaluated to ensure the validity of this observation. Reasons for this are outlined in the discussion section.

Since the completion of pathology studies to establish the biohazardous potential of P/JP-5 fuel, AFAMRL, along with allied U.S. Navy and University of California scientists, has initiated comprehensive investigations to determine the toxicity and carcinogenicity of a modest number of distillate propellants. Although the final results have not been tabulated for all experiments, it has become abundantly clear that most hydrocarbon fuels induce renal changes in male rats. Table 1 lists hydrocarbon distillates of both petroleum and shale-oil origin which have been studied in 90-day inhalation experiments. In all cases where pathology has been completed, renal lesions in male rats have been virtually identical to changes in P/JP-5 exposed subjects. Additionally, significant numbers of primary renal neoplasms have not been documented in any group exposed continuously to distillates for 90 days. As with P/JP-5 long-term studies, however, these observations are being re-evaluated to ensure accuracy.

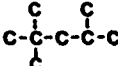
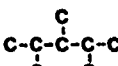
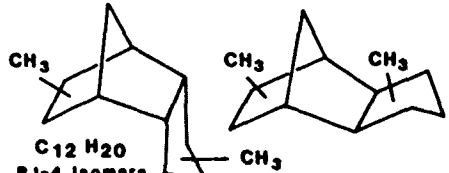
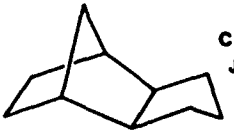
TABLE 1. CONTINUOUS 90-DAY INHALATION EXPOSURE OF MALE F-344 RATS TO DISTILLATE PROPELLANTS

<u>AGENT</u>	<u>DOSE</u>	<u>SUBCHRONIC RENAL PATHOLOGY</u>	<u>ONCOGENIC RENAL PATHOLOGY</u>
Pet.-JP-5	150/750 mg/m ³	Cytoplasmic Hyaline Droplets and Necrosis of Proximal Tubular Epithelium	Accentuated Tubular Degeneration
Shale -JP-5	150/750 mg/m ³		Medullary Mineralization
Pet.-DFM	50/300 mg/m ³	Dilated Tubules Impacted with Cellular Debris at Corticomedullary Junction	Increased Papillary Hyperplasia of Pelvic Urothelium
Shale -DFM	50/300 mg/m ³		
Pet.-JP-4	500/1000 mg/m ³		Oncogenic Pathology Incomplete

NEPHROPATHY INDUCED BY SPECIFIC HYDROCARBONS

In addition to experiments designed to establish the toxicity and carcinogenicity of distillate mixtures, a variety of studies have been initiated to determine the toxic/carcinogenic properties of select, "pure" hydrocarbon propellants. Most experiments with these specific chemical fuels fall into two categories -- short-term oral dosing and long-term inhalation exposures. Short-term oral dosing studies (Table 2) were conducted by the U.S. Navy to investigate the nephrotoxic effects of select agents administered by gavage over a 24-day period. Agents tested included, 2,2,4 and 2,3,4 trimethylpentane, RJ-4 fuel (perhydromethylcyclopentadiene), and JP-10 fuel (tricyclodecane). In most cases, kidneys of exposed males exhibited the same microscopic changes that were observed in male rats exposed to distillates, and lesions are increasingly more severe in high dose subjects.

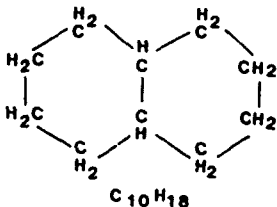
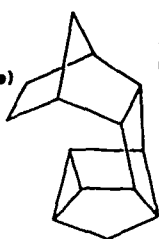
TABLE 2. SHORT TERM ORAL EXPOSURE OF MALE F-344 TO SPECIFIC (PURE) PROPELLANTS

AGENT		DOSE RANGE	DURATION	RENAL LESIONS
● 2,2,4 Trimethylpentane		.09,0.3, 1.0 ml/kg	8 doses over 24 days	Hyaline Droplets and Necrosis of Proximal Tubular Epithelium
● 2,3,4 Trimethylpentane		.09,0.3, 1.0 ml/kg	8 doses over 24 days	Impacted Cellular Detritus at the Corticomedullary Junction
● RJ-4 Fuel (Isomers of Perhydromethylcyclopentadiene)		.09,0.3, 1.0 ml/kg	8 doses over 24 days	
				
● JP-10 Fuel (tricyclodecane)		.09,0.3, 1.0 ml/kg	8 doses over 24 days	Hyaline Droplets in Proximal Tubular Epithelium
	$C_{10}H_{16}$ JP-10			

Long-term inhalation exposures to specific hydrocarbons have focused on three potential cruise missile fuels, decalin (decahydronaphthalene), JP-10, and RJ-5 [endo-endo-dihydrodi(norbornadiene)] (Table 3). Toxicology studies with decalin were patterned after the 90-day distillate inhalation experiments, and results were virtually identical. Experiments with JP-10 and RJ-5, however, proved far more interesting. Both studies included 1-year

intermittent inhalation exposures (6 hours/day for 5 days/week) and long-term, post-exposure holding. When kidneys from JP-10 exposed male rats were examined microscopically, the classic lesions of chronic, hydrocarbon-induced nephropathy were present. More importantly, however, four primary renal cell carcinomas were discovered in exposed males.* These findings were regarded as significant when compared with only one renal cell tumor in controls, and a background incidence of primary kidney tumors in male F-344 rats of 0.5% as tabulated in National Toxicology Program statistics (Chu et al., 1981). Furthermore, although histopathologic examination of RJ-5 exposed rats is incomplete, preliminary study of 26 "oncogenic group" males currently available for microscopic evaluation has identified 6 renal cell tumors.* These animals, also, have displayed non-neoplastic renal changes typical of previous hydrocarbon insult. Therefore, these findings incriminate both JP-10 and RJ-5 as both toxic and carcinogenic for kidney tissues in male rats exclusively.

TABLE 3. LONG-TERM INHALATION EXPOSURE OF MALE, F-344 RATS TO SPECIFIC (PURE) HYDROCARBONS

<u>AGENT</u>	<u>DOSE</u>	<u>LENGTH OF EXPOSURE</u>	<u>RENAL LESIONS</u>
DECALIN (decahydronaphthalene)  $C_{10}H_{18}$	5/50 ppm	90 days Continuous	Subchronic: Cytoplasmic Hyaline Droplets and Necrosis of proximal Tubular Epithelium. Impacted Tubules at Corticomedullary Junction. Oncogenic: Accentuated tubular degeneration, medullary mineralization and urothelial papillary hyperplasia
JP-10 (tricyclodecane)	100 ppm	1 year Industrial	No Post-Exposure Data. Oncogenic Subjects (24 mo.) Exhibited 3 renal cell carcinomas and 1 tumor of uncertain origin
RJ-5 endo-endo-dihydrodi (norbornadiene)  $C_{14}H_{20}$	30/150 mg/m ³	1 year Industrial	Study incomplete but increased renal tumors in exposed males is anticipated

* See Addendum 1

DISCUSSION

The observation of nephrotoxicity and carcinogenicity in male rats exposed to hydrocarbon propellants raises important questions regarding the biohazardous potential of numerous military and civilian fuels. Fundamental to any safety assessment, however, is the collateral observation that most test agents failed to induce significant toxic or neoplastic changes in other organs or in female rats. Furthermore, epidemiological evidence that hydrocarbon propellants are significant nephrotoxins or carcinogenic for man or other animals appears to be lacking. These findings raise the added question of, "What is physiologically unique about the male rat and its renal metabolism which predisposes it to hydrocarbon-induced nephropathy?" Certainly, answers to this complex question might exonerate numerous essential fuels as potential health hazards if metabolic pathways responsible for hydrocarbon nephrotoxicity in male rats were found to be absent in humans. In this connection, several physiologic parameters and possible pathogenic mechanisms targeted for research at AFAMRL deserve special comment.

Basic to understanding the early nephrotoxic events will be investigations to determine causes for accentuated hyaline droplet formation and accelerated death of proximal tubular cells. As mentioned in the introduction, several mechanisms may be contributing to these early toxic changes. One contributing mechanism might be the role of sex-dependent α 2u globulin (Irwin et al., 1971). This unique globulin is a low molecular weight (MW 26,400) protein which is produced in the liver of male rats at puberty and is readily filtered by the kidney. Reportedly, it is the major urinary protein in male rats and has not been documented in the liver of normal females or other species (Roy, 1973; Roy et al., 1966, 1967). Therefore, this protein is likely to be the major constituent of hyaline droplets, and factors resulting in its excessive accumulation in proximal tubular cells may be central to the pathogenesis of hydrocarbon nephropathy. In this regard, mechanisms leading to hyaline droplet formation might include excessive biosynthesis of α 2u by the liver or the inability of tubular cell lysosomal enzymes and ancillary catabolic pathways to degrade this special protein.

Once the pathogenesis of early renal changes is established, it will be necessary to link these events with long-term findings. Ostensibly, mineralized medullary deposits in exposed males held for oncogenic study represent mineralized casts of cellular detritus which were detected near the corticomedullary junction immediately following subacute or subchronic exposures. Their retention in medullary tubules could be attributed to the inability of rigid casts to negotiate the hairpin turn on the loop of Henle. In contrast, etiologic factors leading to papillary hyperplasia of the pelvic urothelium can only be theorized. Hyperplastic pelvic lining cells are not thought to be transformed. Instead, their pro

* See Addendum 2

liferation is believed to develop secondary to traumatic insult of medullary tubular cells by intratubular mineralized casts. The persistent destruction of tubular cells juxtaposed to surface urothelium probably results in the loss of inhibitory chalone (i.e. factors related to contact inhibition) and the subsequent "release" of nearby surface urothelium to proliferate extensively. This hypothesis must be evaluated with the understanding that additional factors such as intrapelvic friction associated with "old-rat nephropathy" and panrenal hypertrophy may be contributory to hyperplastic changes also.

Finally, one must account for accentuated tubular degeneration and neoplasia in long-term exposures. It is possible that both conditions relate to early tubular lesions. For example, accelerated death of proximal tubular epithelium following constipation with protein droplets would expose subjacent basal lamina to potentially noxious tubular contents. In turn, this could result in chronic insult to basement membrane structures with progressive thickening and tubular dysfunction -- a plausible mechanism in the pathogenesis of chronic progressive nephrosis of old rats (Gray et al., 1974; Gray, 1977). As an adjunct, increased repair activities to re-surface denuded tubular surfaces might allow for latent neoplastic tendencies to be expressed rather than through direct genotoxic actions of hydrocarbons. All of these mechanisms possibly relate to early hyaline droplet formation and the inability of the tubular cells to efficiently digest resorbed α 2u globulin following hydrocarbon exposure. Indeed, with the exception of minor quantitative differences in renal enzymes in male rats, the presence of α 2u globulin appears to be the major difference between males and unaffected females and other species. Accordingly, the role of this globulin in hydrocarbon-induced nephropathy of adult male rats must be thoroughly evaluated.

Of major interest in fuels toxicity/carcinogenicity experiments conducted at AFAMRL has been the absence of significant renal tumors in male rats exposed for 90 days or less to distillates and some pure agents. Conversely, increased kidney tumors have been noted with two 1-year exposures to high-density cruise missile fuels. In view of these findings, and recent information suggesting that hydrocarbon-induced renal tumors may be more frequent at the anterior/posterior poles of male rat kidneys, AFAMRL is thoroughly re-evaluating renal tissues to ensure that small, polar tumors did not go undetected (Halliwell, 1982). Renal tissues are being re-trimmed and additional sections are to be examined histopathologically.

SUMMARY

Male rats exposed subacutely or subchronically to a variety of hydrocarbon propellants developed renal lesions which were not observed in females, controls, or other experimental species.

Lesions consisted of greatly increased cytoplasmic hyaline droplets in proximal tubular epithelium followed by tubular cell necrosis and blockage of corticomedullary segments by plugs of necrotic cellular debris -- presumably at the juncture of the pars recta and Henle's descending limb. Following exposure, histopathologic examination of males held for long-term, oncogenic evaluation revealed abundant mineralized casts in medullary tubules, multifocal papillary hyperplasia of pelvic urothelium and accentuated tubular degeneration compatible with severe "old-rat nephropathy." Furthermore, increased renal cell tumors were documented in males exposed to several, high-density hydrocarbon fuels. Pathogenic mechanisms for most changes remain speculative, but may pivot upon the inability of renal tubular cells to efficiently degrade resorbed proteins. In this connection, the exclusive presence of sex-dependent α 2u globulin in male rats may explain why kidney disease is not observed in females or other species. Alpha 2_u globulin is a low molecular weight protein which is synthesized in the liver of male rats at puberty under the influence of testosterone and several other hormones. It is the major urinary protein in male rats, and therefore should be a major constituent of hyaline droplets. By deduction, its relation with all kidney lesions, including neoplasia, cannot be ignored.

ADDENDA

1. Expanded histopathologic examination of kidney tissues from rats assigned to both the JP-10 and RJ-5 one-year inhalation exposure studies have revealed a total of 9 primary renal cell tumors in male subjects assigned to the high dose groups of each experiment. Significant numbers of renal neoplasms have not been documented in controls of females of either study.
2. Recent studies have indicated that small amounts of α 2u globulin may be synthesized by the submaxillary gland ductular epithelium in males and females of all ages. Correspondingly, synthetic pathways in these salivary gland cells were considered to be different from the androgen-dependent pathways present in the hepatocytes of adult males. Further, the amount of α 2u globulin mRNA in submaxillary cells was estimated by dot blot analysis to be only one-sixteenth that present in hepatocytes. These findings suggest that α 2u protein may be produced by non-liver cells, but serum levels probably remain inconsequential (Antakly et al., 1982).

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