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Histopathologic Effects of JP-4 Aviation Fuel on Fathead Minnows (*Pimephales promelas*)

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Military aircraft consume large volumes of petroleum derived JP-4 aviation fuel. The potential for an environmental mishap implies the need to assess its impact. Should a mishap such as a spill occur and the fuel gain access to the surface-water supply, the effects could be widespread. One method for evaluating the danger of such an incident to the ecology of a region is to determine the toxicity of the questionable substance on a member of the animal kingdom endogenous to the region. Teleosts are often targeted when such mishaps involve rivers, lakes, or oceans. At present, only one histopathologic study has been conducted using JP-4 and fish. The objective of this preliminary study using fathead minnows was to determine which tissues and organs are affected by acute exposure to the water soluble fraction (WSF) of JP-4.

METHODS AND MATERIALS

Fathead minnows (Pimephales promelas) ranging in weight from 0.75 to 1.25 grams, with no regard to sex, were divided into two control and two exposure groups of ten fish each. Each group of fish was placed in a 40 liter aquarium containing either control water or WSF/JP-4 water mixture. Two WSF JP-4 concentrations, one-fourth (5 mg/liter) and one-half (10 mg/liter) of the 96 hour median lethal concentration (unpublished data), were used for a 72 hour, unreplenished, static exposure. The 5 mg/liter (low dose) and 10 mg/liter (high dose) concentrations of the WSF JP-4 corresponded to 20 and 40 percent solutions of the saturated water/WSF JP-4 mixture.

The WSF JP-4 mixture was prepared by gently mixing a 95:5 ratio of water to JP-4 with a magnetic stirrer in a nine liter stoppered glass container for 24 hours. The mixture was allowed to stand for 24 to 48 hours prior to use. A Perkin Elmer 900 gas chromatograph and Hewlett Packard 7675A purge and trap sampler were used to quantitate the WSF JP-4. Concentrations of the WSF JP-4 were expressed unadjusted as milligrams of toluene, benzene, and ortho- and para-xylene per liter of water. These components were estimated to be about 87 percent of the WSF JP-4. Other environmental parameters including dissolved oxygen, pH, temperature, and conductivity of the water were monitored, and were 5.0-8.2ppm, 7.7-7.9, 20-21°C, and 400 umhos respectively.

With the exception of the 10 mg/liter group at the 72 hour time interval, at least one fish from each exposure group and a corresponding control was sacrificed at time intervals of 6, 12, 48, and 72 hours subsequent to initial exposure (Table 1). Additional sacrifices were performed as exposed fish became obviously moribund, and these fish were assigned to the nearest scheduled sacrifice period.

Table 1

Sacrifice Schedule and Number of Fish Examined

Time (hours)	Control Groups 1	2	Exposure Groups 5 mg/liter	10 mg/liter
6	1	5	1	5
12	2	3	2	3
24	3	1	3	1
48	2	1	2	1
72	2	-	2	-

Each fish was removed from the test aquarium, the abdomen incised along the ventral midline, and the entire body placed in Dietrich's fixative (YEVICH and BARSZCZ 1981) for at least 48 hours. Each fish was bissected mid-sagittally and then four transverse sections were prepared from the left half of the fish. The transverse sections were taken at the caudal edge of the external nares, the pupil of eye, midway between the caudal aspect of the pupil and the dorsal commissure of the operculum, and at the dorsal commissure of the operculum. Each transverse section was placed in a cassette with the caudal cut surface down. All sections, including the right sagittal half of the fish, were embedded in paraffin and sections 6 microns in thickness were cut and mounted. The tissues were stained with hematoxylin and eosin for histopathologic evaluation. The tissues examined included nasal mucosa, gill, pseudobranch, eye, liver, thymus, heart, kidney and hematopoietic tissue, brain, spinal cord, and integument.

RESULTS

The only lesions strongly implicated as being caused by exposure to WSF JP-4 were degeneration and necrosis of segments of the mucosa covering the olfactory rosette. Additionally, a low incidence of cellular alterations was found in the pseudobranch and gill of exposed fish suggesting possible toxicant related effect (Table 2).

Table 2

Lesions Found in Selected Organs of the Fathead Minnow (Pimephales promelas) Suspected of Being WSF JP-4 Induced

Organ/Tissue	Lesion	Control Group	5 mg/liter Group	10 mg/liter Group
Nasal Mucosa	Degeneration, Necrosis	0/20	0/10	5/10
Gill	Epithelial Hyperplasia	14/20[a]	2/10[b]	1/10[a]
Pseudobranch	Acidophilic cell Degeneration	0/15	2/7	2/8

a = focal distribution, mild to moderate severity
b = diffuse distribution, severe severity

Degeneration and necrosis of the nasal mucosa were absent in the control and low dose groups, but were present in 50 percent of the group exposed to 10 mg/liter WSF JP-4 and these lesions were seen in high-dose subjects as early as six hours after exposure. Affected segments of the mucosa (Figure 1) often appeared slightly thickened. Early degenerative changes usually began in the basilar portion of the mucosal stratified epithelium and progressively spread into more superficial regions. The outermost cells were least affected and occasionally the cilia from these cells were prominently displayed abutting the glycocalyx along the luminal surface of the mucosa. Shrunken, hyperchromatic, degenerate cells were scattered among more normal appearing cellular components in the mucosal epithelium. Pale staining, dilated intercellular spaces in the mucosa were present. Often associated with these pale areas, and clearly distinguishable from the larger degenerating hyperchromatic cells, were small uniformly rounded basophilic structures. These basophilic bodies were surrounded by a clear halo. Occasionally two or more of these structures were observed together surrounded by a confluent clear zone. These structures were always associated with the altered mucosal segments and were not observed in control fish.

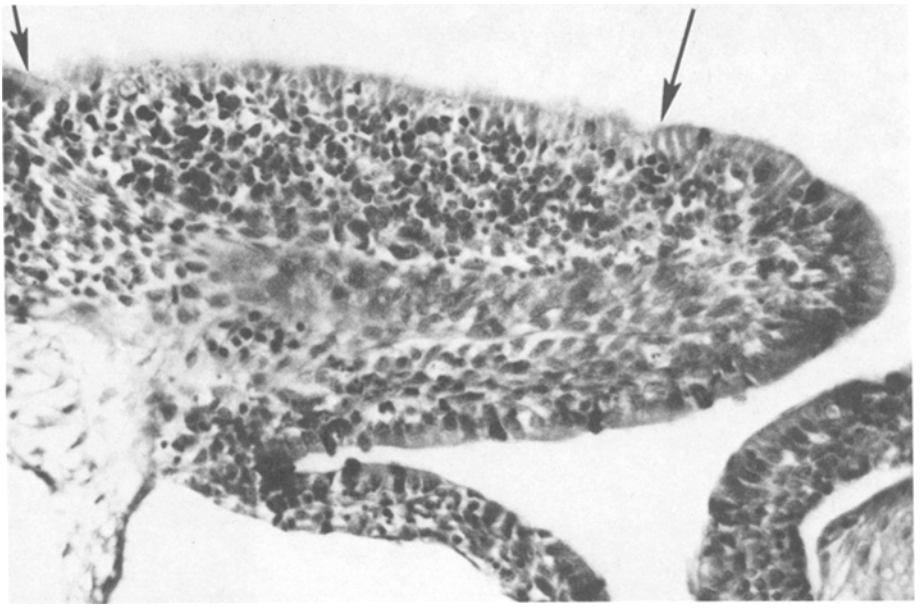

Figure 1. Lamellar fold of olfactory rosette, fathead minnow, WSF JP-4 exposed (10 mg/liter), 6.5 hr. Thickened mucosa (between arrows) with widened intercellular spaces, hyperchromatic degenerate cells, and small haloed basophilic structures are evident. H & E, x 580.

Focal cellular alterations were observed in acidophilic cells of the pseudobranch (Figure 2) in about one-fourth of the fish exposed to 5 and 10 mg/liter WSF JP-4. These alterations included acute cellular swelling, cytoplasmic vacuolization, and cytoplasmic inclusions. The acute cellular swelling was characterized by hypertrophied, acidophilic cells with clear cytoplasm and a few dispersed eosinophilic cytoplasmic granules. Likewise, the nuclei of some of these cells were swollen and exhibited clear centers and marginated chromatin. Occasionally, acidophilic cells in focal regions contained discrete, cytoplasmic vacuoles of various sizes that appeared to displace the eosinophilic cytoplasmic granules peripherally. The pseudobranch of one fish from the high dose group had several acidophilic cells in one area that contained multiple, intracytoplasmic inclusions of varying dimensions (Figures 2 and 3). These inclusions exhibited a homogenous, central matrix which was morphologically identical to unaffected regions of the cytoplasm.

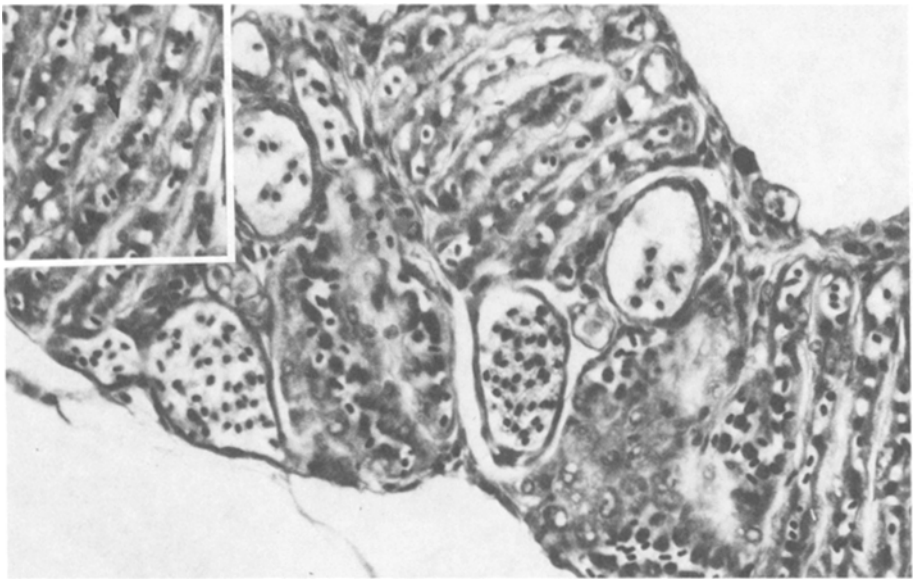

Figure 2. Pseudobranch, fathead minnow, WSF JP-4 exposed (10 mg/liter), 12 hr. Acidophilic cells in foci are swollen with vacuolated nuclei and cytoplasm. H & E x540.

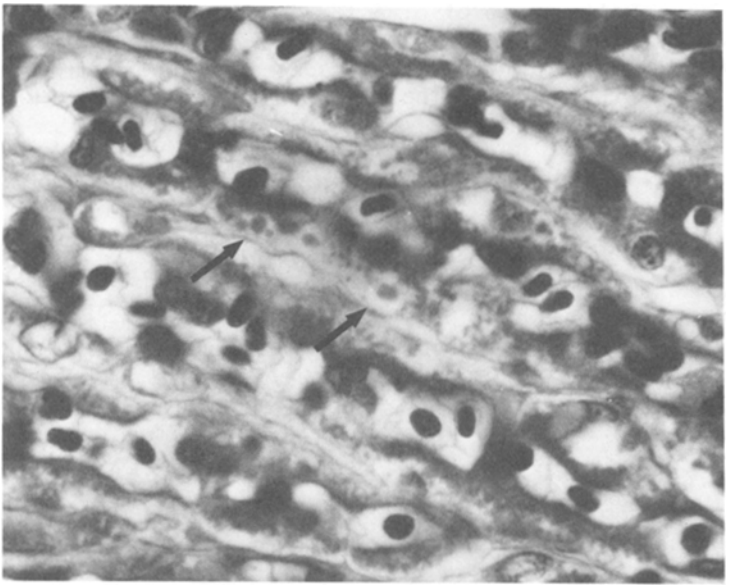

Figure 3. Excerpt of portion of pseudobranch in figure 2 (framed region). Cytoplasmic inclusions (arrows) in clear cytoplasm of degenerate acidophilic cells. H & E x1418.

The branchial or gill lesions included branchial hyperplasia, branchiitis, and trematodiasis. The only gill lesion suspected of being exposure related was branchial hyperplasia. Although focal, mild to moderate epithelial hyperplasia of gill lamellae was found in most control and some experimental fish (Table 2), the fish with the most diffuse and severe branchial hyperplasia were those in the low dose WSF JP-4 exposure group that were sacrificed after 72 hours; this was all of the low-dose fish exposed for 72 hours. All high-dose subjects were sacrificed because of their moribund condition before the 72 hour exposure time. The severe hyperplastic change was characterized by fusion of adjacent gill lamellae as a result of proliferation of lamellar epithelium that bridged the interlamellar spaces.

Other microscopic observations of low incidence in control and exposed fish that were not considered to be exposure related included the following: choroiditis of the eye, granulomatous thymitis, and hepatocellular cytoplasmic vacuolar changes. No significant lesions were observed in the other tissues examined microscopically.

DISCUSSION

The epithelium covering the folds of the olfactory rosette of some teleosts is reported to be composed of at least four cell types - receptor cells (bipolar neurons), supporting (sustentacular) cells, basal cells, and non-sensory ciliated cells (GRIZZLE and ROGERS 1976; GROMAN 1982). Although degeneration and necrosis of the olfactory mucosa usually appeared more severe in the deeper epithelial strata, the specific cell types affected were not discernible in this study. At the light microscopic level, it was equivocal as to whether the small, uniformly rounded basophilic structures present in the degenerative and necrotic nasal mucosa represented nuclear debris, cytoplasmic inclusions (in clear cytoplasm of swollen cells), or some type of migrating inflammatory cell. These structures were always seen associated with altered mucosal segments and were not observed in control fish. Electron microscopic studies might be of assistance in determining the nature of these structures as well as serving as an aid in identifying the specific kinds of rosette-cell most affected by WSF JP-4.

The nasal mucosa has been recognized by other investigators as a target organ for petroleum crude oil and oil WSF intoxication. Solangi and Overstreet (1982) found lesions in olfactory organs of Menidia beryllina and Trinectes maculatus exposed to 100 mg/liter WSF of a Louisiana crude oil. The damage was characterized by acute hyperplasia of sustentacular cells of the olfactory epithelium in M. beryllina after seven days of exposure, and necrosis of sustentacular and neurosensory cells by 20 to 30 days of exposure. T. maculatus exposed to 100 mg/liter oil WSF displayed increased mucosal cell proliferation early in exposure, and by 38 days, necrosis of both sustentacular and neurosensory epithelium had occurred. Gardner (1975) noted that epithelial olfactory tissue in M. Menidia exhibited metaplasia when exposed

to the WSF of Texas-Louisiana crude oil. Norton et al. (in press, 1982) found that basal and support cells in P. promelas were disrupted by 48 hours of exposure to 10 mg/liter WSF JP-4.

Although the functions of the teleost pseudobranch are not completely understood, this organ apparently serves as a baro/chemoreceptor, osmoregulator, and an endocrine gland, and supplies highly oxygenated blood to the optic choroid (LANGLER et al. 1962; ANDERSON and MITCHUM 1974; GARDNER 1975; and MATTEY et al. 1978). This organ has been found to be sensitive to hydrocarbon intoxication. Gardner (1975) and Norton et al., (in press, 1982) have reported lesions in the pseudobranch attributed to the toxic effects of hydrocarbon fuels. In our study, one-fourth of both exposure groups manifested histologic changes morphologically consistent with reversible cellular degeneration. These alterations probably represented early changes associated with exposure to WSF JP-4. However, because of the focal and mild nature of the lesions, coupled with small numbers of fish used and the absence of a clear dose response relationship, the association of these degenerative changes with JP-4 components is somewhat tenuous.

Hyperplasia of the gill epithelium is a non-specific response caused by a variety of physical insults and chemical agents (ELLER, 1975). In this study, epithelial hyperplasia of the gill was present in all groups including exposures and controls. The only fish with diffuse severe hyperplasia, however, were those in the 5 mg/liter WSF JP-4 concentration which were held for 72 hours prior to sacrifice. This suggests a relationship in regard to the distribution and severity of the gill lesion with exposure to the WSF JP-4. It is possible that some severe branchial hyperplasia would have resulted from exposure to the 10 mg/liter concentration of WSF JP-4, but no fish in the high dose group were available for evaluation at 72 hours.

The results of this preliminary study suggest that the olfactory rosette and possibly the pseudobranch and gill of the fathead minnow are injured by acute exposure to WSF JP-4. These observations are in the accordance with results other researchers have found studying the toxic effect of hydrocarbon fuels on teleosts. We believe that these selected tissues should be examined when utilizing fish for environmental toxicological research involving hydrocarbons.

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