Bull. Environ. Contam. Toxicol. (1995) 54:297–301

Environmental Contamination and Toxicology

Histopathology in Winter Flounder, *Pleuronectes americanus*, Following Chronic Exposure to Crude Oil

R. A. Khan

Department of Biology and Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7

Received: 21 July 1993/Accepted: 2 June 1994

Chronic exposure to crude oil has a profound affect on living organisms. These effects vary from reduced feed intake, behavioral, physiological, reproductive and pathological changes. Fish, which submerge themselves in sediment when not foraging for food are more likely to be affected than others that live in the water column. Flounders (Pleuronectidae), sediment-inhabiting fish, have been shown to be affected after exposure to oil-contaminated sediment (McCain et al. 1978; Haensly et al. 1982). Two previous studies reported the effects of various concentrations of oil-contaminated sediment on winter flounder, Pleuronectes (= Pseudopleuronectes) americanus, following chronic exposure (Dey et al. 1983; Khan 1991). The present study reports the histopathological changes in juvenile and adult winter flounder that survived following chronic exposure to crude oil.

MATERIALS AND METHODS

Juvenile (weight 57-74 g, length 10-15 cm) and adult (weight 80-120 g, length 25-40 cm) winter flounder were obtained by SCUBA divers from Conception Bay, Newfoundland, at depths of 5-15m in September-October of each year (1985-1986). Fish were held in aquaria 1-3 mo prior to exposure to crude oil-contaminated sediment in 300-L aquaria through which ambient seawater (1-6 °C) flowed. Only fish free of external lesions and parasites were used. Beach sand was obtained from an uncontaminated site, (Conception Bay; 47° 39'N, 52° 55'W) washed three times before it was mixed with crude oil obtained during exploratory drilling on the Grand Banks. Various concentrations were prepared by a method reported previously and total hydrocarbon concentration (PHC) estimated (Khan 1991). Oil-contaminated sediment was placed in 300-L aquaria, about 5 cm deep, through which seawater (0 °C) flowed (5L/min). Each trial involved about 20 fish/group, comprising of five or six groups, viz., controls, 100, 300, 600, 1000 and 2200 (adults only) μg/g PHCs. The fish were not fed as they do not feed normally during winter. After 8 wk, surviving fish were killed and tissues, which included gill, liver, spleen, kidney, heart, stomach and intestine were excised and fixed in Bouin's fluid, processed by conventional histological methods and stained with hematoxylin and eosin. Cross sections of

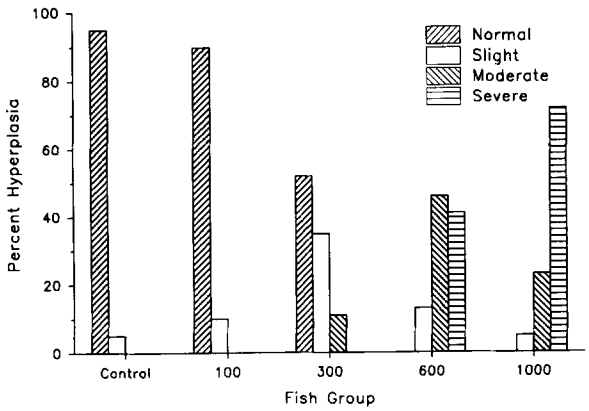

Figure 1. Prevalence (%) of hyperplasia (slight, moderate and severe) in the secondary lamellae of juvenile winter flounder exposed 8 wk to various concentrations of PHCs (μg/g). (n= 25/group).

spleen were also stained with Perl's Prussian blue, a diagnostic stain to detect hemosiderin in tissues. Hemosiderin concentration was estimated as a percent of the area scanned (Khan and Nag 1993). A 1-way ANOVA, with Duncan's multiple group test, was used for comparison of the data from different groups of fish. Prevalence between groups was compared by the G-test. Differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Histological changes in the tissues of juvenile flounder varied according to the level of hydrocarbon concentration. Secondary gill lamellae extending from the proximal to distal extremities were normal in control juvenile flounder (Fig. 1). Slight hyperplasia of branchial epithelium was observed in the distal one-third of the secondary filaments of flounder exposed to sediment with 100-300 mg/g PHCs. Flounder exposed to 600 μg/g PHCs displayed moderate to severe hyperplasia, again in the distal one-third of the secondary filaments. This was accompanied by some increase (> one-third) in thickness of the basal interlamellar troughs. The most severe and extensive lesions were observed in juveniles exposed to the highest concentration of PHCs (~ 1mg/g). Hyperplasia of the epithelium culminating in fusion of adjacent filaments in about 50% of each primary lamellae was apparent. An increase in thickness of the interlamellar bases was also observed. There was excessive mucus secretion especially in the distal extremity of each primary lamella. In the proximal extremity of each primary filament, moderate to extensive hyperplasia of the secondary lamellae also occurred. Telangiectasis fusion of adjacent secondary lamellae, epithelial lifting or necrosis were not observed.

Observations on the gills of adult flounder showed similar histological changes

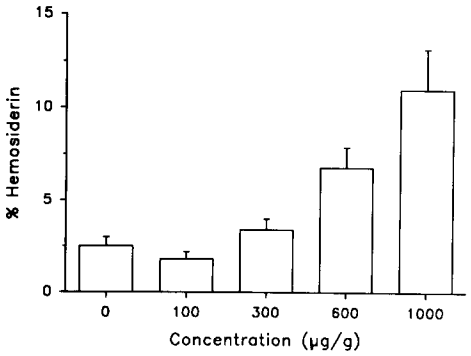

Figure 2. Prevalence (%) of hemosiderin in the spleen of juvenile winter flounder exposed 8 wk to various concentrations of PHCs (n = 20/group).

after exposure for 8 wk. There was evidence of slight hyperplasia of the epithelium of the secondary lamellae extending less than 20% from the distal extremity in control fish. Changes in the gills of fish exposed to 100-300 µg/g PHCs were minimal with slight to moderate hyperplasia that occurred mostly in the distal third of each secondary filament. However, at a higher concentration (> 600 µg/g) PHCs, moderate to severe hyperplasia occurred more often. Severe hyperplasia of adjacent secondary lamellae and an increase in thickness of the interlamellar troughs (< one-third), was also observed. Except for excessive mucus secretion at the tips of the primary lamellae no other changes were observed.

Adult winter flounder were also exposed to higher concentrations (2.2mg/g PHCs) for 24 wk. Hyperplasia was observed in both control and oil-treated groups and differences based on increased thickness of the secondary lamellae were not apparent. However, telangiectasis (10%) and fusion of adjacent secondary lamellae (25%) occurred only in the group (20) exposed to oil-contaminated sediment.

Hemosiderosis, occurring as pigmented melanomacrophage centers, was the main lesion observed in the spleen and less often in the liver and kidney of flounder exposed to oil-contaminated sediment. These pigmented areas of variable shapes and sizes in the spleen as determined by image analysis were significantly ($P < 0.05$) more abundant in juvenile flounder at 600-1,000 µg/g PHCs than in controls or at lower concentrations (Fig. 2). Similarly, hemosiderin concentration in adult flounder was significantly ($P < 0.05$) greater at levels > 300 µg/g PHCs than in controls but differences between the oil-treated groups did not differ ($P > 0.05$) from each other (300 to 2,200 µg/g PHCs).

Lesions in other tissues were rare or absent. Bile duct hyperplasia areas observed

in the liver of 25% of 20 adult flounder exposed to 2.2 mg/g PHCs for 24 wk but not at similar or lower concentrations after 8 wk exposure. Additionally, depletion of energy reserves (lipid and glycogen), characterized by dense-staining rather than vacuolated hepatocytes, was observed in 30% of 20 adult winter flounder exposed to 2.2 mg/g PHCs for 24 wk but not at similar or lower concentrations for shorter periods as fish in these trials were not fed.

The predominant lesions in winter flounder following exposure to oil-contaminated sediment, in the present study, were observed in the gills and spleen. These varied according to the concentration of PHCs and were more pronounced at the higher than lower levels. Extensive epithelial hyperplasia of the secondary gill filaments and excessive mucus secretion most likely impaired oxygen uptake. Haensly et al. (1982) reported lesions of a similar nature in plaice (Pleuronectes platessa L.) examined at intervals (9 to 27 mon) after the Amoco Cadiz spill in 1978 off the coast of Brittany, France. However, additional lesions which included subepithelial inflammatory reactions and epithelial sloughing were not observed in flounder in the present study. Differences between these two studies might be attributed to the longer exposure period of plaice to PHCs than the winter flounder.

Organ lesions in winter flounder were restricted to an increase of melanomacrophage centers especially at the higher concentrations. These centers contain hemosiderin, a degraded bi-product of hemoglobin. Although hemoglobin and hematocrit values of flounder exposed to oil-contaminated sediment (2.2 mg/g) were not significantly lower than controls (see Khan 1991), the substantial increase of the pigmented centers in oil-treated fish suggests that blood loss did occur. Although the centers are believed to increase during starvation (Agius 1979), this had no influence on the oil-treated and control flounder as none of the fish were fed during the period of exposure. Moreover, the absence of additional tissue damage (such as gastric gland degeneration, dilation of Bowman's space in the kidney and muscle fiber degeneration) might be associated with absence of feed intake since the latter represents a major route of hydrocarbon entry. Additionally, hepatic lesions and neoplasms reported in English sole (Parophrys vetulus) inhabiting waterways and embankments of Puget Sound, Washington, where high levels polycyclic aromatic hydrocarbons occur, were not apparent in the present study (Myers et al. 1991). It is probable that the progressive sequence of lesions culminating in neoplasms, as reported by Myers et al. (1991) and Schiewe et al. (1984) only occurs after continuous exposure of fish for periods and PHC concentrations that exceed those used in the present study.

Acknowledgments. I am grateful to Natural Sciences and Engineering Council of Canada for financial support.

REFERENCES

Day AC, Kiceniuk JW, Williams UP, Khan RA, Payne JF (1983) Long term exposure of marine fish to crude petroleum. 1. Studies on liver lipids and fatty acids in cod (Gadus morhua) and winter flounder (Pseudopleuronectes americanus). Comp Biochem Physiol C 75: 93-101

Haensly WE, Neff JM Sharp JR, Morris AC, Bedgood ME, Beom PD (1982) Histopathology of Pleuronectes platessa L. from Aber Wrac'h and Aber Benoit, Brittany. France: long-term effects of the Amoco Cadiz crude oil spill. J Fish Dis 5: 365-391

Khan, RA (1991) Influence of concurrent exposure to crude oil and infection with Trypanosoma murmanensis (Protozoa: Mastigophora) on mortality in winter flounder, Pseudopleuronectes americanus. Can J Zool 69: 876-880

Khan RA, Nag K (1993) Estimation of hemosiderosis in seabirds and fish exposed to petroleum. Bull Environ Contam Toxicol 50: 125-131

McCain BB, Hodgins HO, Gronlund WD, Hawkes JW, Brown DW, Meyers MS, Vandermeulen JH (1978) Bioavailability of crude oil from experimentally oiled sediments to English sole (Parophyrs vetulus) and pathological consequences. J. Fish Res Board Can 35: 657-664

Myers MS, Landahl JT, Krahn MM, McCain BB (1991). Relationships between hepatic neoplasms and related lesions and exposure to toxic chemicals in marine fish from the U.S. West Coast. Environ Health Perspect 90: 7-15

Schiewe MH, Weber DD, Myers MS, Jacques FJ, Reichert WL, Krone CA, Malins DC, McCain BB, Chan S-L, Varanasi U (1991) Induction of foci of cellular alteration and other hepatic lesions in English sole (Parophyrs vetulus) exposed to an extract of an urban marine sediment. Can J Fish Aquat Sci 48: 1750-1760