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Pathology of Brown Bullhead, *Ictalurus nebulosus*, from Highly Contaminated and Relatively Clean Sections of the Hudson River

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A review of the literature regarding the PCB contamination of the Hudson River noted that although much is known about the transport and fate of PCB in the River, little effort has been directed towards the identification of toxic effects in aquatic life (Limburg *et al.* 1985). Use of pathobiological surveys to elucidate the effects of aquatic contamination or other environmental factors may be limited by the lack of suitable control groups. Such appears to be the case with regard to the etiology of hepatocellular neoplasms found in a large proportion of Atlantic tomcod (*Microgadus tomcod*) from the Hudson River estuary (Smith *et al.* 1979; Dey *et al.* 1986).

A portion of the upper Hudson River provides an unusual model to compare the health status of fish exposed to varied levels of xenobiotics. Barriers to upstream movement of fish, including a waterfall and a dam separate a relatively uncontaminated reach of river from a downstream reach grossly contaminated by PCB. Downstream movement of fish presumably assures no genetic distinction between fish from contaminated and uncontaminated sections. A decade after the elimination of the PCB discharge, river sediments resupply the ecosystem with PCB to levels which greatly exceed human health-based standards for PCB in fish and water (Brown *et al.* 1985). Although PCB contamination has been most notable, sediment concentrations of lead, chromium, and cadmium are also elevated due to a former industrial discharge near the sites of the PCB discharge (Limburg *et al.* 1985). Polychlorinated dibenzofurans and polychlorinated dibenzodioxins are also present in sediments at concentrations approximately 10^{-6} those of PCB (Brown *et al.* 1988).

This report summarizes gross and microscopic observations of adult brown bullhead (*Ictalurus nebulosus*) collected from contaminated and uncontaminated sections of the Hudson River.

MATERIALS AND METHODS

During June 16-20, 1986 brown bullhead were collected from the Hudson River by electroshocking. In the highly contaminated section

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45 bullhead were collected near Griffin Island (rivermile 190) and 17 were collected in Stillwater (rivermile 175). This group is hereafter referred to as the experimental fish. Upstream of the PCB discharge sites, 45 bullhead were collected near Corinth (approx. rivermile 200). These fish are referred to as the control fish.

The necropsy procedure included external examination for the presence of tumors, trauma and other abnormalities. Internal organs such as liver, spleen, kidney, intestine and other grossly affected tissues were collected immediately after death and fixed in 10% neutral-buffered formalin. Blocks of fixed tissues were embedded in paraffin, cut at 6 um and stained with hematoxylin and eosin.

Tissues for transmission electron microscopy were fixed in a formaldehyde-gluteraldehyde fixative (McDowell and Trump 1976). Specimens were postfixed in osmium tetroxide and embedded in epoxy. Ultrathin sections were cut with diamond knives on a LKB Ultramicrotome III, stained with uranyl acetate and lead citrate, placed on 300-mesh copper grids, and examined with a Phillips 301 electron microscope at 80 kV.

PCB concentrations in fillets of 21 control-site fish and 20 experimental-site fish selected to represent the spectrum of ages within each sample, were determined by Hazleton Laboratories America Inc., Madison, Wisconsin. Weighed portions of ground tissue were dried with anhydrous sodium sulfate and extracted with three portions of petroleum ether. The combined extracts were transfered following partial evaporation to a Florisil column. PCBs were eluted from the column using hexane which was further concentrated prior to injection to a gas chromatogrph (GC) with an electron capture dectector and a column packed with 3% OV-1. The GC was standardized using Aroclors 1221, 1242 and 1254 which were quantitated using multiple representative peak areas.

Age determinations were made using the pectoral spine sectioning method (Sinnott and Ringler 1987) by staff of the State University of New York, College of Environmental Science and Forestry, Syracuse, New York.

Standard parametric statistical methods were used with the 95% confidence level to interpret data. Thus all stated significant differences are at $p<0.05$.

RESULTS AND DISCUSSION

Some pertinent characteristics of control and experimental fish samples are summarized in Table 1. Average weight but not average length differed significantly between control and experimental samples. No site-by-sex interaction was detected. The mean condition factor of experimental fish was significantly lower than that of the control fish. There were no differences in mean condition factors between sexes at a collection site. The respective mean ages of control and experimental fish were 2.6 and 3.2 years. There was a significant difference in age distribution between control and experimental groups ($X^2=13$;

df=4). The PCB concentrations in skeletal muscle reflect the substantially greater exposure of fish in the experimental group to PCB than the control group. Respective mean PCB concentrations for control and experimental groups were 0.61 and 38 ug/g. The highest observed PCB concentration in the control group was 1.4 ug/g. The lowest PCB concentration in the experimental group was 16.3 ug/g.

There were no significant differences in the prevalance of gross abnormalities between the two groups. Some of the most common lesions were oral and mandibular papilloma (control/experimental - 9%/26%), melanosis (7%/11%), petechial hemorrhages (29%/27%) and skin laceration and erosion on the pectoral fin (2%/3%). Other minor lesions were swollen nostrils (4%/8%), hematoma on the dorsal fin (2%/3%), caudal peduncle erosion (4%/6%), yellowish liver (7%/10%), head trauma (2%/2%) and congested liver (2%/3%). The grossly visible petechial hemorrhages (minute hemorrhagic spots in the skin) appear related to electrocution and handling since histopathologic examination failed to reveal bacteria or other established viremia, although viral isolation was not attempted. Some of the skin laceration and erosion may be related to the lamprey, which are present in the river (Smith 1985). Swollen nostrils, head injuries and swollen urogenital papilla may relate to conditions of capture, transport, and handling prior to necropsy.

Table 1. Experimental and control sample characteristics.

Collection site	Experimental	Control
Number of fish	62	45
Mean length (mm)	311 ± 35.0[a]	306 ± 26.9
Mean weight (g)	378 ± 96.0	485 ± 123
Mean condition factor[b]	1.30 ± 0.150	1.50 ± 0.146
Age (yr) mean	3.2	2.6
median	4	3
range	1-6	1-5
Sex ratio (M/F)	0.25	1.2
Muscle PCB (ug/g) mean	38.3 ± 22.2	0.61 ± 0.27
range	16.3-102	0.38-1.4
number analyzed	20	21

[a] Mean ± standard deviation.
[b] 0.01 X weight (g)/length (mm)^3

The prevalence of various histopathologic observations, summarized in Table 2, differ from gross observations with the former considered definitive. The significant histopathologic differences between control and experimental fish were splenic hemosiderosis (pigmentation from hemoglobin degradation), renal hemosiderosis, swim bladder trematode and bile-duct hyperplasia (proliferation and thickening). Dermal muscular atrophy, nodular and mucous-cell hyperplasia, suppurative acute dermatitis, verminous granulomatous gastritis (parasitic inflamation of the stomach membrane), cestode infestation in the bile duct, non-specific brachitis and protozoa in the gills were observed in both groups at comparable frequencies ($p>0.05$).

Bile-duct hyperplasia accompanied by hepatic and renal hemosiderosis occurred more frequently among brown bullhead caught in the contaminated section of the river than in the uncontaminated area. Varying degrees of bile-duct hyperplasia occurred among experimental bullhead indicating more florid and assymetrical proliferation of the septal and interlobular bile-ducts. Advanced stages of damaged bile-ducts exhibited dense infiltrates of mononuclear cells; the ductal epithelial cells were invariably hyperplastic and hypertrophied with fibrosis. Fibrous septae extended beyond the triads to form portal bridges that were probably enlarged due to structural changes. Numerous hemosiderin-like pigmental materials were dispersed throughout the hepatobiliary system.

Although sex ratios in the control and experimental groups were different, within each group there was no significant difference in the extent of bile-duct hyperplasia between males and females ($p > 0.05$).

Table 2. Relative frequencies of histopathologic observations among Hudson River brown bullhead (percent).

Histopathologic Findings	Control (n=45)	Experimental (n=62)
Oral and mandibular papilloma or benign growth	11	13
Melanosis	7	6
Nodular and mucous-cell hyperplasia - skin	4	21
Suppurative acute dermatitis	7	24
Subacute nonspecific enteritis	2	3
Egg-yolk peritonitis	2	0
Verminous granulomatous gastritis	27	31
Swim-bladder trematode	2	65[a]
Glycogen-loaded liver	13	16
Dermal hemorrhages	36	6
Ulcerative dermatitis	9	13
Intestinal hemosiderosis	2	5
Bile-duct hyperplasia	13	78[a]
Splenic hemosiderosis	12	79[a]
Renal hemosiderosis	3	77[a]
Nonspecific steatitis	0	3
Dermal muscular atrophy	4	11
Bile-duct cestode	0	15
Nonspecific brachitis	2	11
Gill protozoa	0	3

[a] Significant at $p<0.05$ (Fisher exact test)

Thus it is highly unlikely that the observed differences between the control and experimental groups are due to the differences in sex ratios. A record-keeping problem precluded analysis of age-effects. However, considering the overlap in age distributions and the large

difference in the relative frequency of bile-duct hyperplasia between samples, it would appear that age could only account for a small portion of the variability of this pathology at best.

A moderate to severe hemosiderosis occurred universally in spleen and kidney of bullheads collected from the experimental site.

Electron-microscope examination of a control brown bullhead liver revealed typical cuboidal or low columnar epithelia on dome-shaped apical cytoplasmic protrusions as described in other fish species (Sideon and Young 1983); the walls of bile canaliculi were composed of specialized plasma membranes in which the apical portion contained numerous microvilli. The cytoplasm contained amorphous material, mitochondria, lysosomes, intracytoplasmic cisterna and a Golgi apparatus. In contrast, hepatobiliary ducts of experimental fish revealed a marked endoplasmic-recticulum reduplication with few organelles and dark electron-dense granular pigments. Endoplasmic recticulum and associated changes accounted for more than 80 percent of the cytoplasm. The organelle consists of continuous networks of smooth surfaced tubules and a flattened envelope coated on the cytoplasmic surface by polyribosomes. Electron-dense pigmental bodies were dispersed throughout apparently without any host reaction. Excessive hemosiderin and other melanin-like pigments also occured in the liver and spleen suggesting the breakdown of red blood cells.

No significant neoplasms were seen among Hudson River brown bullhead with the exception of oral and mandibular papillomas. These lesions are known to occur spontaneously without any apparent relationship to chemical pollution (Pileher and Fryer 1978). Dermal mucous-cell hyperplasia has been reported to occur more frequently among brown bullhead in contaminated waters (Zuchelkowski *et al.* 1981).

The presence of excessive hemosiderin in liver, spleen, and kidneys suggests the excessive breakdown of red blood cells. The toxic mechanism underlying such hemolyses are largely unknown, although metals such as cadmium, lead, and mercury are suspected causative agents. Heavy metals are known to contaminate the Hudson River as a result of industrial discharge and urban runoff (Limburg *et al.* 1985) and are known to be at high levels in sediment downstream of the control site (Brown *et al.* 1988). Cadmium toxicity in fish (*Punctius conchonius*), exposed to 0.63 or 0.84 mg/L cadmium chloride for 90 days produced morphologically obvious hemolytic effects and thrombocytopenia (Gill and Pant 1985).

The most significant finding was the high frequency of bile-duct hyperplasia, accompanied by hepatic and renal hemosiderosis among brown bullhead from the contaminated section of the Hudson River. Since the hepatobiliary system plays a major role in removing toxic materials from the blood after absorption from the gastrointestinal tract and is the site where biotransformation and excretion of xenobiotics is taking place, the observed bile-duct abnormalities could serve as an indicator of chemical contamination of an aquatic environment.

This is the first documented report of bile-duct hyperplasia occurring in feral freshwater fish as an apparent consequence of aquatic contamination. Biliary hyperplasia, however, has been documented in marine species (Long 1984). Gas chromatographic and mass spectrometric analyses revealed the presence of hexachlorobenzene and octachlorostyrene in addition to PCB in several samples of renal tissue from experimental site fish. Although the results do not implicate any single contaminant, bile-duct hyperplasia has been a notable response of rats to dietary PCB (Norback and Weltman 1985). The conclusion that the observed bile-duct hyperplasia is a response to exposure to organic contaminants is supported by the marked reduplication of enzyme-synthesizing organelles in fish from the experimental site. Levels of liver microsomal cytochrome P-450 assayed using warfarin as a substrate (Murphy *et al.* 1980) were substantially higher in two composite samples of experimental fish than a composite sample of control fish liver. Induction of hepatic cytochrome P-450 is a notable response of fish exposed to xenobiotics.

Hepatic neoplasms and hyperplasia are inducible in mammals by many chemicals, including carbazole and derivatives, DDT, dieldrin, nitrosoamine, and phenobarbital (Mallus *et al.* 1984; Reynolds 1977). A variety of elements, including arsenic, chromium, silver, indium, iron, molybdenum, selenium, tellurium, and thallium also possess hepatotoxic properties and can cause bile-duct hyperplasia during hepatocarcinogenic processes (Mallus *et al.* 1984; Reynolds 1977; Black *et al.* 1984). In fish, hepatocellular and cholangiocellular neoplasm can be produced by exposure to methanol acetate, benzene, N-2-fluoroenylacetamide, o-toluene, diethyl nitrosomine, dimethyl nitrosamine, N-methyl-N-nitrosoguanidine, and 7,12-dimethylben-zoanthracene (Mallus *et al.* 1984; Reynolds 1977; Hawkins *et al.* 1984). All of these may be able to produce bile-duct hyperplasia but the specific chemicals or metabolites responsible for the observed bile-duct hyperplasia remain to be investigated.

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