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14. ABSTRACT

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PCBs, Liver Lesions, and Biomarker Responses in Adult Walleye (*Stizostedium vitreum vitreum*) Collected from Green Bay, Wisconsin

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ABSTRACT. *Adult walleye were collected from several locations in the Lower Fox River and Green Bay, Wisconsin (the assessment area) and two relatively uncontaminated reference locations (Lake Winnebago and Patten Lake, Wisconsin) between July and October in 1996 and 1997. Whole body and liver samples collected in 1996 were analyzed for total PCBs, PCB congeners, and liver histological lesions. Follow-up sampling in 1997 included examination of liver histopathology, PCBs in liver samples, measurement of ethoxyresorufin-O-deethylase (EROD) activity, immunological evaluation of kidney and blood samples, measurement of plasma vitellogenin, and examination of tissues for parasites as well as bacterial and viral infections. Mean PCB concentrations in whole body and liver samples were elevated in assessment area walleye (4.6 to 8.6 and 3.6 to 6.4 mg/kg wet weight, respectively) compared to PCB concentrations in reference areas (0.04 mg/kg in walleye fillets from Lake Winnebago). A significant*

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($p < 0.01$) elevation was observed in the prevalence (26%) of hepatic preneoplastic foci of cellular alteration (FCA) and neoplasms in 5 to 8 year old walleye collected from the assessment area, compared to reference area fish (6% prevalence). Walleye from the assessment area also contained multiple FCA and hepatic tumors per liver sample, whereas no tumors and a reduced prevalence of FCA were observed in reference area walleye. Both tumors and FCA were more prevalent in female fish than in male fish within the 5 to 8 year age classes. There were no remarkable effects on immunological parameters in assessment area walleye, although hematocrit was elevated and blood monocyte counts were 40% lower than those of reference area fish. The data did not show any clear distinctions in the prevalence of disease between reference and assessment area walleye. EROD activity was similar in assessment area and reference area walleye. Plasma vitellogenin was elevated in female walleye from eastern Green Bay, but was not detected in male fish from this location. The results of this investigation demonstrate significant elevation in hepatic preneoplastic lesions and hepatocellular adenomas and carcinomas in assessment area walleye exposed to elevated concentrations of PCBs. These histopathological lesions are consistent with long-term exposure to tumor promoters such as PCBs, although quantitative association between tumors and PCBs was not observed at the level of the individual fish. Additional research would be needed to elucidate the causal mechanisms underlying tumorigenesis.

INDEX WORDS: *PCBs, walleye, cancer, histopathology, biomarkers, tumors, Green Bay.*

INTRODUCTION

The Lower Fox River/Green Bay (Wisconsin, U.S.A.) ecosystem (the assessment area) is contaminated with polychlorinated biphenyls (PCBs). PCBs were released into the assessment area from Fox River paper company facilities that processed PCB-containing carbonless copy paper waste (Wisconsin DNR 1998). Estimates of the amount of PCBs discharged into the Fox River from paper company facilities range from 420,000 to 825,000 pounds from 1954 to 1998 (Wisconsin DNR 1998). An extensive study of PCB fate and transport in the assessment area system demonstrated that PCBs move into the river and bay where they enter the aquatic food chain (DePinto *et al.* 1994). A mass balance study estimated that over 90% of the PCBs entering Green Bay in 1989 were from the Fox River (DePinto *et al.* 1994). Elevated PCB concentrations in assessment area fish have been documented since the 1970s by the Wisconsin Department of Natural Resources (Jensen *et al.* 1982, Sullivan *et al.* 1983, Wisconsin DNR 1995). These PCB concentrations tend to be highest in predatory fish in the Green Bay system, such as walleye, salmon, and trout (Wisconsin DNR 1996). Numerous advisories on the consumption of assessment area fish have been issued by state agencies as a result of the PCB contamination of the fish (U.S. FWS 1998).

PCB exposure can cause a variety of adverse effects in animals, including cancer, physiological malfunctions, and physical deformities (Safe 1994). PCB exposure in fish is associated with hormone and enzyme modulation, histopathological lesions, and reproductive and developmental impairments (Niimi 1996). PCBs also may modulate immunological and/or stress responses in fish (Vijayan *et al.* 1997). Environmental PCB contamination has been associated with increased tumor frequencies and other histological lesions in feral fish, including ovarian atresia and hepatocellular lesions (Niimi 1996). For example, Teh *et al.* (1997) reported severe lipidosis and vacuolated and basophilic foci of cellular alteration (FCA) in largemouth bass collected from a PCB contaminated reservoir, whereas reference area fish did not have these lesions.

Relative to PCBs, other contaminants in fish are less elevated in the assessment area than in reference areas. For example, inspection of the Wisconsin Department of Natural Resources fish contaminant database (Wisconsin DNR 1996) and the National Study of Chemical Residues in Fish (U.S. EPA 1992) shows that most chlorinated pesticides are present at 10-fold lower concentrations than PCBs and show limited if any elevation above reference areas such as Lake Winnebago, Wisconsin. Contaminants other than PCBs that are elevated in Green Bay fish relative to reference areas include 2,3,7,8-TCDD, specific chlorinated pesticides (DDT and metabolites), and mercury. Polycyclic aromatic hydrocarbons (PAHs) can be elevated in sediments in proximity to industrial areas, but bioaccumulation of the larger carcinogenic PAHs is generally limited in fish (Varanasi and Stein 1991). Although not specifically evaluated in this study, only limited trophic transfer of PAHs is expected in pelagic fish such as walleye (Varanasi and Stein 1991). The objective of this study was to evaluate the association between PCB contamination and a

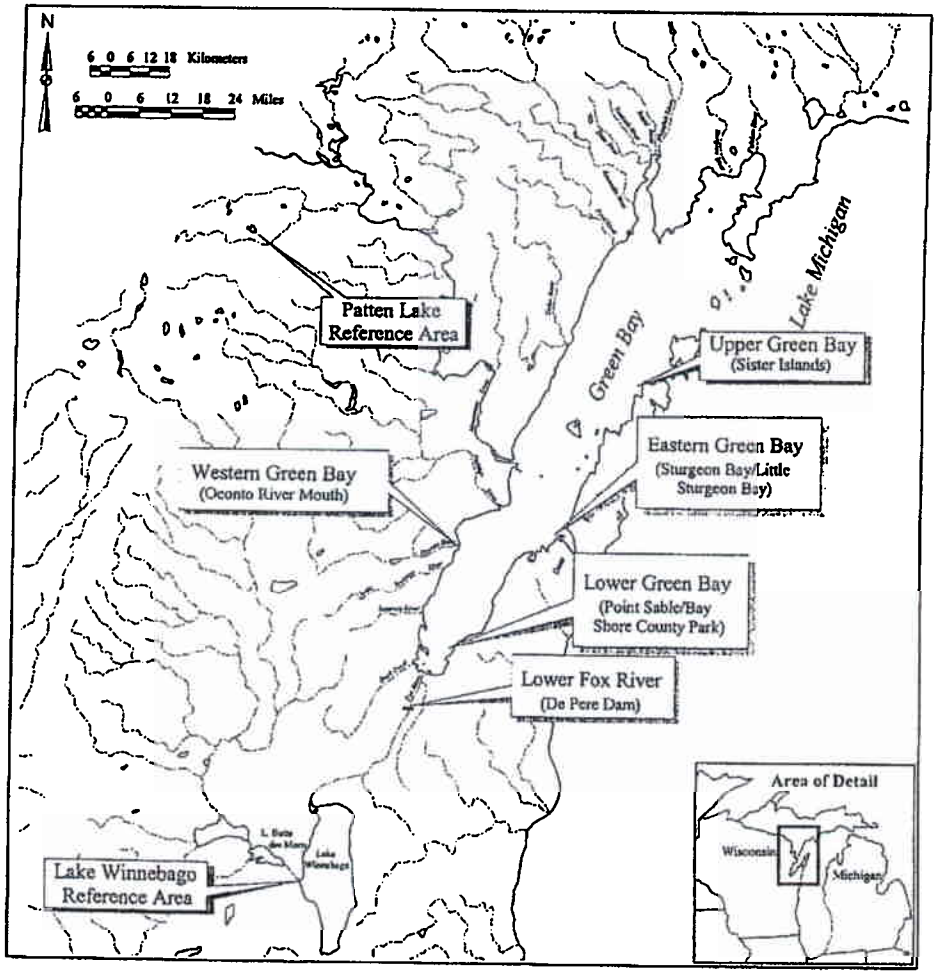

FIG. 1. Study area and walleye collection locations.

suite of physiological and biomarker responses of adult walleye collected from several locations in the assessment area and two reference sites (Lake Winnebago and Patten Lake, Wisconsin). The intent of the study was to broadly evaluate biomarker responses that were known to be responsive to PCBs, rather than comprehensively evaluate individual biomarkers. Sampling areas and PCB analytical methods were similar to those used in the Green Bay Mass Balance study (Connolly *et al.* 1992) to aid in the evaluation of temporal trends in PCB residues in walleye. Walleye were assessed for parasites, bacterial and viral infections, liver histopathological lesions, hepatic EROD activity, plasma vitellogenin levels, kidney and blood immunological parameters, and whole body and liver concentrations of PCBs. The results of this study provide a foundation for further research on the relationship between contamination and hepatic neoplasia and biomarker responses of Green Bay walleye.

MATERIALS AND METHODS

Study Design and Sampling Locations

Walleye sampling locations in the assessment area and two reference areas are shown in Figure 1. Assessment area locations included the Lower Fox River below De Pere dam, lower Green Bay (near Point Sable/Bay Shore County Park), eastern Green Bay (near Sturgeon Bay/Little Sturgeon Bay), western Green Bay (near the Oconto River mouth), and upper Green Bay (near the Sister Islands). Assessment area locations were selected to correspond to

areas sampled in the Green Bay Mass Balance study by Connolly *et al.* (1992). Reference areas were in Lake Winnebago (central Wisconsin) and Patten Lake (northern Wisconsin). Reference areas were selected because of the proximity to the assessment area, the ability to collect similar age and size walleye as fish collected in the assessment area, and low concentrations of PCBs in fish. Additional sample and analysis information can be found on the Internet at http://www.fws.gov/r3pac/nrda/walleye.pdf.

Walleye were collected from all assessment area locations for PCB analysis and preliminary histopathological evaluations in 1996. In 1997, walleye were collected from the two reference locations and all assessment area locations excluding upper Green Bay. Upper Green Bay was not sampled in 1997 because of limited capture success and high effort required in 1996. In 1997, walleye were assessed for parasites and bacterial and viral infections, liver histopathological lesions (including tumors), hepatic EROD activity, plasma vitellogenin levels, and blood and kidney immunological responses. Liver samples were collected in 1997 for PCB analysis . The Patten Lake location was chosen as a reference area after efforts to collect walleye in Lake Superior were not successful. Table 1 summarizes sample sizes, location, and relevant PCB and health measurements performed during the 2 years of sampling.

Fish Collections

Adult walleye were collected between July and October, before the period of gonadal maturation (which may affect vitellogenin, EROD, and possibly other biomarkers) for this species (walleye typically spawn in March and April). In 1996, fish were sampled by electroshocking or gill nets. In 1997, all fish captured by electroshocking, dip netted, and immediately place into a 120 L holding tank. Following the recommendation of Rice *et al.* (1996), the holding tank contained a sublethal concentration of buffered tricaine methane sulfonate (MS222) at 25 mg/L to minimize sampling stress (Rice *et al.* 1996). Fish were typically processed for blood and tissues within two hours of capture. Processing and tissue collection from each fish ranged between 15 and 20 minutes. Fish were anesthetized with lethal levels of sodium bicarbonate-buffered MS222 before tissue processing. To minimize age-related variance in measurements, adult fish were targeted for capture. Initially, fish ranging from 37 to 60 cm were targeted in 1996. Based on 1996 size data, fish greater than 45 cm were targeted in 1997 to ensure that adult fish were captured.

Weight, Length, and Age Determination, and Tissue Sampling

Fish were weighed to the nearest 0.01 kg and total length was measured to the nearest 0.1 cm. Scales were removed from above the lateral line below the posterior insertion of the dorsal fin, and age was determined from the number of annuli. Decontaminated and pre-cleaned dissecting instruments and pre-cleaned and certified glassware were used in tissue sampling. Written protocols and standardized procedures were used at all collection locations.

Blood was removed for immunological and vitellogenin assays from the caudal vein or dorsal aorta using a heparinized syringe. Livers, spleens, and gonads were removed and weighed to the nearest 0.01 g. A 1 cm section was removed from the center for histopathological analysis. The remaining liver was split for EROD and contaminant analyses. Gonads were removed for sex determination. Gills, intestine, spleen, and trunk kidney were removed for health screening (see below). The head kidney was removed for immunological assays. Samples were shipped on wet ice (blood, spleen, head kidney, trunk kidney) or dry ice (plasma, gill, liver, intestine), or in buffered formalin (liver, gonad; see below) under strict chain of custody procedures to the processing or analytical laboratory. Any samples that were unlabeled, in broken containers, or did not meet sample holding times or holding temperature criteria (thawed tissue samples) were not analyzed.

Analytical Chemistry

Walleye were analyzed for concentrations of total PCBs in 1996 and 1997 liver samples and PCB congeners in composites of 1996 whole body samples. Briefly, total PCBs in liver were quantified by gas chromatography/electron capture detection (GC/ECD) using a single capillary column (DB-5). Total PCBs were quantified as the individual Aroclors or 1:1 mixture of individual Aroclors they most closely resembled. Detection limits were approximately 0.002 mg/kg for total PCBs.

Composites of three to six whole fish were analyzed for 106 congeners using GC/ECD. Samples were quantified using a DB-5 capillary column, and

TABLE 1. Summary of collection locations, sample type, and number.[1]

Sample Location	Measurement	Tissue	Sample Size by Year 1996 (7/29 to 10/8)	1997 (8/6 to 9/16)
		Assessment Area		
Lower Fox River	PCBs[2]	whole[3]	7	—
		liver	1*	19
	Health Exam	mult[4]	—[5]	20
	Histopathology	liver	4	20
	Immunology	mult	—	10
	EROD	liver	—	20
	Vitellogenin	plasma	—	20
Lower Green Bay	PCBs	whole	6	—
		liver	4	12
	Health Exam	mult	—	12
	Histopathology	liver	4	12
	EROD	liver	—	12
Eastern Green Bay	PCBs	whole	11	—
		liver	4	17
	Health Exam	mult	—	17
	Histopathology	liver	4	17
	Immunology	mult	—	12
	EROD	liver	—	17
	Vitellogenin	plasma	—	13
Western Green Bay	PCBs	whole	4	—
		liver	4	14
	Histopathology	liver	4	14
	EROD	liver	—	10
Upper Green Bay	PCBs	whole	3	—
		liver	4	—
	Histopathology	liver	4	—
		Reference Areas		
Lake Winnebago	PCBs	liver	—	12
	Health Exam	mult	—	12
	Histopathology	liver	—	21
	Immunology	mult	—	13
	EROD	liver	—	12
Patten Lake	PCBs	liver	—	13
	Health Exam	mult	—	13
	Histopathology	liver	—	13
	EROD	liver	—	13

[1]Only includes analyzed samples.
[2]PCB analysis. Liver: total PCBs by Aroclor method; Whole: sum of congener method.
[3]Whole body composite of three to six fish; some samples contained no or partial livers. n is the number of separate sample analyses.
[4]Mult: multiple tissues analyzed; see Tables 6 and 7.
[5]No data.
* Composite of four livers.

data were acquired simultaneously from a second, DB-17 column (but not used for quantification). Calibration solutions containing all 106 target congeners and internal standards were used. Approximately 30% of the samples were also run on GC/mass spectrometry to provide confirmation of peak identification. One whole body composite sample from each location was also analyzed for nonortho-substituted PCB congeners (coplanar PCB 37, 77, 81, 126, 169). Coplanar congeners were isolated using carbon column isolation (according to Draft EPA Method 1668) and analyzed by GC/ECD. Detection limits were approximately 0.00002 to 0.00015 mg/kg for individual congeners.

Percent moisture and lipid content were determined on all samples. PCB concentration results are expressed on both a wet weight and lipid-normalized basis. Total PCBs in whole body samples were determined by summing the concentrations of the measured congeners, excluding PCB 85 because of analytical interference (coelution with other contaminants). Quality assurance and quality control procedures included use of procedural blank, blank spike, instrument blank, certified reference material, and duplicate samples. All PCB analytical data were validated by an independent data validator.

Histopathological Examination

Walleye collected in 1996 and 1997 from the Lower Fox River and lower, eastern, western, and upper Green Bay (1996 only), and from both reference areas (1997 only), were assessed for liver histopathological lesions and inspection of gonads for sex determination. Liver sections and gonads were fixed in 10% neutral buffered formalin, and then embedded in paraffin. Paraffin blocks were sectioned at 5 to 7 µm, mounted on glass slides, and then stained with hematoxylin and eosin. Stained gonads were screened for gender determination, and liver sections were screened for lesions and subjected to detailed, semiquantitative histopathologic analyses. Data were reported as histologic scores (0 to 3 depending on lesion severity). Because of the importance of numbers of foci of cellular alteration (FCA) and hepatic tumors (HT) in the progression of fish hepatocarcinogenesis, these lesions were counted rather than scored for severity. FCA and HT data were reported both as a prevalence value (frequency of fish with the lesion) and as the number of lesions per liver sample. Greater than 95% of all lesion scores were either identical or within a value of one determined by the primary pathologist and confirmed by a second pathologist.

Health Screening

Walleye collected in 1997 from the Lower Fox River, lower and eastern Green Bay, and both reference areas were assessed for the prevalence and severity of viral, bacterial, and parasitic infections.

Virology

An approximately 3 to 5 mm^2 portion of kidney and spleen tissues was placed into HBSS (Hank's Balanced Salt Solution) and transported on ice to the U.S. Fish and Wildlife Service La Crosse Fish Health Center (LFHC). Samples from five fish were pooled into one sample for virology and were processed within 72 hours of collection according to the methods in Thoesen (1994). Samples were decontaminated by adding antibiotic (Gentamicin) and antifungal (Nystatin) agents to buffer solutions, followed by centrifugation and filtration (0.45 µm) of the supernatant. Supernatant samples were inoculated with bluegill fry (BF-2) and chinook salmon embryo (CHSE-214) cells and incubated for 14 days at 20°C and 15°C, respectively. Tissue samples were presumed negative for viruses if cell pathological effect was not observed after the 14 day incubation period.

Bacteriology

A sterile loop was inserted into the posterior hind kidney and inoculated onto a brain heart infusion agar (BHIA) slant and incubated for 7 days at 22°C. A second loop sample was inoculated onto a cytophaga slant and incubated for 7 days at 15°C. Cultures were then streaked for colony isolation onto plates of the appropriate media. Biochemical tests performed on cells isolated from each individual colony included (1) cytochrome oxidase, (2) gram stain, (3) triple sugar iron, (4) motility by hanging drop, and (5) peroxidase (Thoesen 1994, Lasee 1995). Bacterial cultures with identical test results and indistinguishable colony and cell morphologies were presumed to be the same organisms. Two to three isolates of the same organism were then identified using the Minitek™ Numerical Identification System (BBL Microbiology Systems, Becton Dickinson and Company). Yeast cultures were identified by the following characteristics: (1) unicellular, (2) colonies resembling those of the bacteria, and (3) absence of demonstrable hyphae

(Cano and Colome 1986). Molds were identified by the following characteristics: (1) nucleated organisms, (2) filamentous hyphae, and (3) exhibition of swirling dry growth patterns on BHIA plates.

Parasitology

Gills and the intestinal tract of each walleye were removed and preserved using the quick freezing technique of Bush and Holmes (1986). These organs were placed into a labeled sealable plastic bag and instantly frozen using 95% ethanol that had been cooled to –70°C by dry ice. The samples were then transported to the LFHC on dry ice and stored frozen at –80°C until analyzed. Organs were thawed and then examined for parasites with a stereo microscope. Gastrointestinal tracts were opened longitudinally and the contents scraped from the mucosal surface into a petri dish containing isopropyl alcohol. Gill arches were separated and examined individually. All parasites were removed, sorted, identified, counted, and stored in 70% isopropyl. Cestode numbers were based on recovery of scolices; free segments without scolices were ignored. Conventional whole mount permanent preparations (Dailey 1996) were made of all common helminths. Prevalence and mean intensity were calculated as defined by Margolis *et al.* (1982).

EROD Assays

Walleye collected in 1997 from the Lower Fox River; lower, eastern, and western Green Bay; and both reference areas were assessed for hepatic EROD activity. Liver samples were harvested and wrapped in aluminum foil, placed in individual sealable plastic bags, then frozen on dry ice. Samples were shipped on dry ice and then stored frozen at –80°C until analyzed. Microsomes were prepared from the frozen liver samples. EROD activity was determined at 25°C in microtiter plates on the same day as microsome preparation. Microsomal EROD activity was measured by the direct method (Pohl and Fouts 1980, Ankley *et al.* 1989) as modified for microtiter plate procedures (Tysklind *et al.* 1994). The relative fluorescence intensity derived from the sample plates was compared to a linear fit of a seven-point resorufin standard curve (six replicates/concentration), and the relative intensity units were converted to pmol resorufin. Resorufin in each well was plotted against time to observe any deviations from linearity of the reaction. A linear regression was then performed on the data from each well to determine an EROD rate (pmol/min). The amount of protein in each well, determined using bovine serum albumin standards, was used to normalize the EROD activity for that well. A positive control (liver from channel catfish administered benzo(a)pyrene) was used with each sample batch. Twenty percent of the liver samples were split into triplicate samples before microsomal preparations to assess methodological precision.

Immunological Assays

Walleye collected in 1997 from the Lower Fox River, eastern Green Bay, and the Lake Winnebago reference area were assessed for a immune alterations using a standardized suite of immune assays similar to those described by Zelikoff (1993) and Zelikoff *et al.* (1997). Preliminary studies were conducted to optimize assay conditions with walleye tissues and to determine the effects of fish handling procedures and holding times. Blood indices assessed were hematocrit (red blood cell volume), leucocrit (white blood cell volume), and blood differential counts (percentage of white blood cell types). Kidney cells from walleye were used to measure phagocytosis of fish-serum opsonized latex particles, unstimulated and concavalin A stimulated T-cell lymphoproliferation, and unstimulated and phorbol myristate acetate (PMA) stimulated intracellular superoxide anion (SOA) production. Method validation studies showed that all parameter measurements were reproducible and were stable within the holding period encompassing shipment from the field to the assay laboratory.

Vitellogenin Assays

Walleye collected in 1997 were assessed for plasma levels of vitellogenin (Vtg) to screen for estrogenic effects in a limited number of male and female fish. Only two locations were sampled for Vtg analyses (Lower Fox River and eastern Green Bay). Vtg was quantified using methods described by Folmar *et al.* (1996). In brief, a monoclonal fish antisera was produced by injecting purified striped bass Vtg into a mouse. This antisera has been demonstrated to be cross reactive, sensitive, and specific to walleye Vtg (Folmar and Denslow unpublished data). The antisera was used in a quantitative capture ELISA by coating microtiter plates with antibody and colorimetrically determining the Vtg captured (Folmar *et al.* 1996).

Statistical Analyses

For statistical comparisons, data for assessment area fish were pooled and compared to the pooled values of those from the reference area. Sample sizes were not adequate to statistically determine the independent influence of fish size and age on biomarker responses. Regional differences in histological observations that were expressed as counts of cellular features (FCA and HT lesions), other biomarker responses (EROD activity; liver:body weight ratio; condition factor; immumological parameters), and tissue concentrations of PCBs were assessed with the Mann Whitney test (Conover 1980). Prevalence of histological abnormalities was defined as the proportion of individual fish that were affected, and regional and sex differences in prevalence were assessed with the t-test for proportions (Snedecor and Cochran 1980). Regional comparisons of histological features that were expressed as categorical or semi-quantitative categories (lesion histologic scores) were assessed as contingency tables using Fisher's exact test (Conover 1980). All comparisons of histological features were based on pooled records from 1996 and 1997 collections and were restricted to fish aged 5 to 8 years because these ages were present in both the assessment and reference areas. All other comparisons (condition factor, liver:body weight ratio, EROD activity, immunological parameters) were based on 1997 data (only year of data collection) for all ages and sexes combined. Statistical calculations were performed using S-PLUS (Mathsoft, Inc.).

RESULTS

PCBs in Walleye Tissues

Mean concentrations of PCBs in whole body samples were elevated at all assessment area locations, ranging from 4.6 (western Green Bay) to 8.6 mg/kg ww (eastern Green Bay) (Table 2). Mean concentrations of PCBs (wet weight) in liver samples from Green Bay fish were similar to whole body concentrations and ranged from 3.6 mg/kg (eastern Green Bay) to 6.4 mg/kg (Lower Fox River). Mean PCB concentrations in livers of reference area walleye were 0.02 mg/kg in Patten Lake and 0.94 mg/kg in Lake Winnebago (Table 2). Liver PCB concentrations in assessment area walleye were significantly ($p < 0.01$) elevated above liver PCBs in reference area fish on both a wet weight and lipid weight basis. Congener patterns in walleye collected from all five assessment area locations were generally similar (Fig. 2: eastern Green Bay; other bay locations not shown), with the exception of the Lower Fox River (Fig. 3). The congener pattern in walleye from the Lower Fox River exhibited a greater representation of lower chlorinated congeners. Coplanar PCBs, including PCB 126 and 77, were detected in fish from all assessment area collection locations (Table 2).

Liver Histopathology

FCA, which are preneoplastic lesions that may develop into tumors, and hepatocellular neoplasms were the most remarkable liver lesions observed in walleye collected from the assessment area in both 1996 and 1997 (Table 3; Fig. 4). In 1996, the prevalence of FCA was 0% for the Lower Fox River, upper Green Bay, and western Green Bay; 25% for eastern Green Bay; and 50% for lower Green Bay (Table 3). Prevalence of hepatocellular neoplasms was 0% for Lower Fox River and eastern Green Bay, 25% for western and lower Green Bay, and 50% for upper Green Bay (Table 3). Several walleye had multiple hepatic neoplasms: one fish from lower Green Bay had three hepatocellular adenomas and another from western Green Bay had four tumors. Thus, the prevalence of either lesions or preneoplastic foci of alteration was 0% for Lower Fox River, 50% for lower Green Bay, 25% for eastern and western Green Bay, and 50% for upper Green Bay. The results of the 1996 sampling indicated that assessment area walleye appeared to have elevated incidences of FCA and HT lesions, although sample sizes were small and no collections were made from reference areas.

Sampling in 1997 included larger sample sizes and collection of fish from reference locations. In 1997, the prevalence of FCA in reference areas was 0% for Patten Lake (0/13 fish) and 10% for Lake Winnebago (2/21 fish) (Table 3). The prevalence of hepatocellular neoplasms in reference areas was 0% for both Patten Lake and Lake Winnebago. The prevalence of FCA in assessment area fish was 10% for Lower Fox River (2/20 fish), 17% for lower Green Bay (2/12 fish), 24% for eastern Green Bay (4/17 fish), and 43% for western Green Bay (6/14 fish). The prevalence of hepatocellular neoplasms in assessment area fish was 5% for Lower Fox River (1/20 fish), 0% for lower Green Bay (0/12 fish), 6% for eastern Green Bay (1/17 fish), and 7% for western Green Bay (1/14 fish). Thus, in 1997 the prevalence of either tumors or preneoplastic lesions was 22% across all Green Bay locations and

TABLE 2. Mean and standard deviation (SD) of concentrations of total PCBs and nonortho PCB congeners in walleye collected in 1996 and 1997.

Sample Location	Tissue	n	Total PCBs mg/kg ww	Total PCBs µg/g Lipid	PCB Congener[3] (µg/kg ww) 77	81	126	169
Assessment Area								
Lower Fox River	whole[1]	7	6.0 (2.2)	47.5 (13.7)	8.1	0.9	0.7	0.1
	liver[2]	20	6.4 (1.7)	61.8 (22.8)	—[4]	—	—	—
Lower Green Bay	whole[1]	6	5.7 (2.9)	34.0 (15.0)	11.2	1.4	1.1	0.1
	liver	16	4.3 (3.4)	44.3 (37.6)	—	—	—	—
Eastern Green Bay	whole[1]	11	8.6 (3.6)	52.9 (21.8)	4.8	ND[4]	0.6	0.1
	liver	21	3.6 (2.7)	36.4 (17.9)	—	—	—	—
Western Green Bay	whole[1]	4	4.6 (0.6)	30.8 (6.6)	7.5	0.5	0.8	0.1
	liver	18	4.0 (2.0)	33.3 (10.2)	—	—	—	—
Upper Green Bay	whole[1]	3	5.8 (1.3)	33.3 (6.6)	2.1	ND	0.3	ND
	liver	4	4.4 (1.4)	32.4 (9.5)	—	—	—	—
Reference Area								
Lake Winnebago	liver	12	0.94 (0.55)	27.0 (18.0)	—	—	—	—
Patten Lake	liver	13	0.02 (0.07)	0.47 (1.7)	—	—	—	—

[1]Size weighted whole body composites of 3 to 6 fish. n is the number of separate sample analyses.
[2]Composite of whole livers from 4 fish. n is the number of separate sample analyses.
[3]n = 1 (one whole body composite sample analyzed); determined from carbon column isolation and GC/ECD analysis. Data for PCB 37 not shown.
[4]— : not measured; ND: not detected.

6% in reference areas. In addition to a higher prevalence, the average number of hepatic FCA and neoplasms in assessment area fish was also substantially elevated above that in reference area fish (Table 3). For example, assessment area fish exhibited multiple FCAs, with two fish exhibiting more than 10 FCAs. One fish from the Lower Fox River and another from western Green Bay had two hepatocellular adenomas each.

Because the prevalence and number of neoplasms may be related to fish age or sex, the prevalence of FCA and hepatic tumors was compared for each age class of walleye sampled (Fig. 5) and, within common age classes, for males and females (Table 4). Figure 5 shows that the fish collected from reference areas ranged from 5 to 8 years old, with age 6 and 7 year old fish most prevalent. Fish collected from the assessment area ranged from 4 to 11 years old, with 6 and 7 year old fish also most prevalent.

Limiting the comparison to 5 to 8 year old walleye (excluding those ages of fish from the assessment area that were not obtained from the reference areas) and combining 1996 and 1997 shows a 26% prevalence of preneoplastic lesions and tumors in

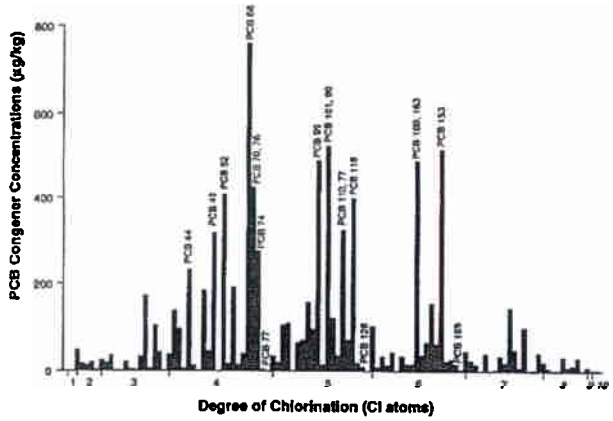

FIG. 2. PCB congener pattern in walleye (whole body composite) collected from eastern Green Bay.

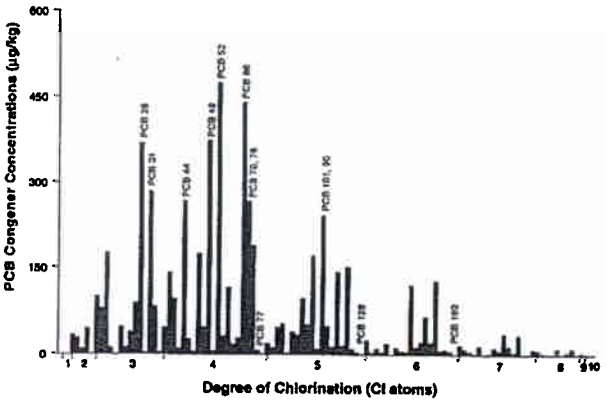

FIG. 3. PCB congener pattern in walleye (whole body composite) collected from the Lower Fox River.

assessment area fish compared with the 5.9% prevalence in reference area fish (Table 4). Table 4 also shows FCA and HTs for male and female walleye (5 to 8 years old). The prevalence of FCAs and HTs was significantly elevated in assessment area walleye (5 to 8 years old) compared to reference area fish (both sexes combined; $p = 0.004$) and in female fish ($p = 0.003$).

Other hepatic lesions observed in walleye included hepatic glycogen depletion, pericholangial and perivascular leukocytes, megalocytosis/karyomegaly, lipidosis, granulomas, macrophage aggregates, focal parenchyme leukocytes, and single cell necrosis (Table 5). Lesions that were significantly lower in assessment area fish compared to reference area fish (5 to 8 year old walleye; sexes combined) included glycogen depletion ($p < 0.001$), and megalocytosis/karyomegaly ($p = 0.001$).

Other Biomarker Responses

Other biomarker responses evaluated in this study included immunological parameters, disease prevalence, EROD, and Vtg. The intent was to screen for a suite of biomarker responses rather than perform a comprehensive evaluation of a few parameters. For statistical comparisons, data for assessment area fish were pooled and compared to the pooled values of reference area fish. Samples sizes were not adequate to statistically determine the influence of fish size, age, and specific collection location.

An immunological assessment was conducted in fish collected during 1997 to identify the potential for immune function impairment in walleye collected from the Lower Fox River and eastern Green Bay relative to fish collected from the Lake Winnebago reference area (Table 6). Results of the study showed that assessment area walleye (all ages and sexes combined) exhibited: (1) a significant ($p = 0.007$) elevation of hematocrit, (2) a significant ($p = 0.002$) reduction in monocyte counts, (3) a significant elevation in stimulated lymphoproliferation of kidney T-cells (Table 6: S, $p = 0.04$; S – U, $p = 0.02$), and (4) no significant ($p > 0.05$) differences in other immunological parameters between assessment area and reference area walleye (Table 6).

Walleye (all ages and sexes combined) were subjected to virology, bacteriological (kidney), and parasitology (gills and intestinal tract) evaluations (Table 7). There were no detectable viruses in any samples or any lesions indicative of typical viruses of walleye. Three gram-negative and five gram-positive bacteria were isolated from the walleye kidney samples from assessment area and reference areas; however, none of the walleye had overt clinical signs suggestive of bacteremia. A large number of isolates of an unidentified yeast and one identified mold were recovered from walleye from the Lower Fox River, lower Green Bay, and eastern Green Bay, but not from reference area locations. Seven parasites were recovered from walleye from assessment and reference areas (Table 7). The prevalence of the gill parasite *E. luciopercarum* was very high in assessment area fish.

TABLE 3. Mean weight (standard deviation), and % prevalence of lesions and mean number of lesions per liver (foci of cellular alteration and hepatic tumors) in walleye collected from assessment and reference areas in 1996 and 1997.[1]

	1996						1997					
		Fish Weight	% Prevalence		Lesions per Liver[2]			Fish Weight	% Prevalence		Lesions per Liver[2]	
Sampling Location	n	(kg)	FCA	HT	FCA	HT	n	(kg)	FCA	HT	FCA	HT
Lower Fox River	4	1.08 (0.35)	0	0	—[3]	—	20	1.09 (0.27)	10	5.0	2.5	2.0
Lower Green Bay	4	1.70 (0.42)	50	25	1.0	3.0	12	1.56 (0.41)	17	0	3	—
Eastern Green Bay	4	2.16 (0.51)	25	0	1.0	—	17	1.90 (1.17)	24	5.9	2.3	1.0
Western Green Bay	4	1.80 (0.26)	0	25	—	4.0	14	2.00 (0.29)	43	7.1	> 6.8	2.0
Upper Green Bay	4	2.75 (0.53)	0	50	—	1.0	NS[3]	NS	NS	NS	NS	NS
Assessment Area Average	20	1.90 (0.68)	15	20	1.0	2.3	63	1.60 (0.75)	22	4.8	>4.4	1.7
Lake Winnebago	NS	NS	NS	NS	NS	NS	21	0.90 (0.14)	9.5	0	1.0	—
Patten Lake	NS	NS	NS	NS	NS	NS	13	0.80 (0.11)	0	0	—	—
Reference Area Average	—	—	—	—	—	—	34	0.85 (0.13)	5.9	0	1.0	—

[1]FCA: foci of cellular alteration; HT: hepatocellular tumor.
[2]Average FCA or HTs per liver sample of fish containing these lesions.
[3]— : Not applicable (0% prevalence); NS: not sampled.

Hepatic microsomal EROD activity was generally similar between assessment area and reference area walleye (Table 8; all ages and sexes combined; $p = 0.3$). EROD activity ranged from 2 to 73 pmol/min/mg protein in assessment area fish and from 12 to 56 pmol/min/mg in reference area fish.

Vtg was assayed in the plasma of male and female adult walleye collected from the Lower Fox River and eastern Green Bay; reference area fish were not sampled (Table 9). Vtg ranged from 0.25 to 8.4 mg/mL in female walleye from the eastern Green Bay, but was not detected (< 0.001 mg/mL) in any of the fish collected from the Lower Fox River.

Liver weight as a percentage of body weight was significantly ($p = 0.003$; all ages and sexes combined) higher in assessment area walleye than in reference area fish (Table 10). Condition factor in walleye collected from the reference areas was significantly ($p < 0.001$; all ages and sexes combined) lower than for walleye from the assessment area (Table 11).

DISCUSSION

PCBs in Walleye Tissues

Mean concentrations of PCBs (whole body, wet weight) measured in assessment area walleye ranged from approximately 4 to 9 mg/kg. Figure 6 shows PCB concentrations (as mg/kg wet weight and µg/g lipid) in walleye samples collected from the assessment area between 1975 and 1996. PCB concentrations in whole body samples ranged from

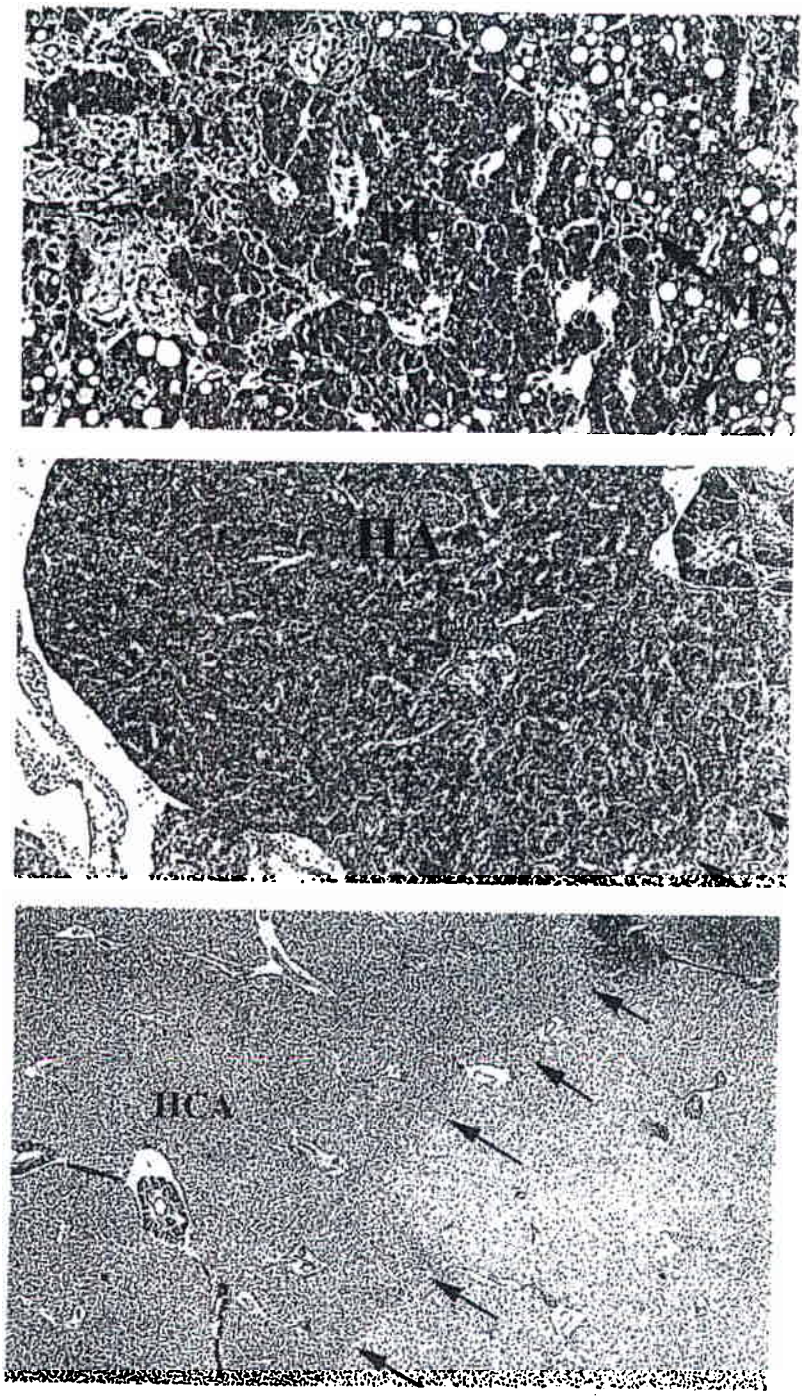

FIG. 4. ***Foci of cellular alteration (basophilic focus, BF; top panel), benign tumor (hepatocellular adenoma, HA; middle panel), and malignant tumor (hepatocellular carcinoma, HCA; bottom panel) in assessment area walleye.***

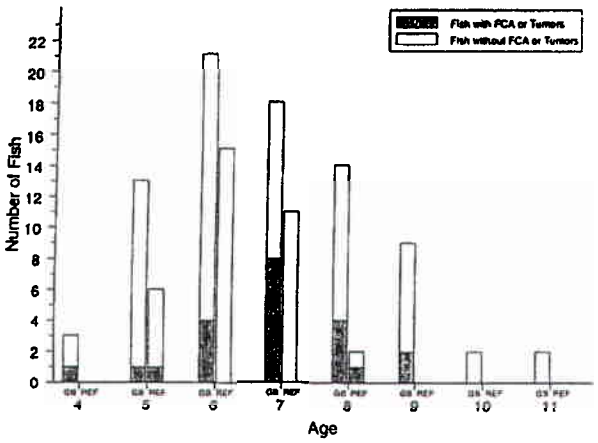

FIG. 5. Prevalence of hepatic FCA and neoplasms in walleye from the assessment area (GB: left-hand bars; data for 1996 and 1997; all locations combined) and reference areas (REF: right-hand bars; data for Patten Lake and Lake Winnebago combined) by age class.

0.5 to 18 mg/kg wet weight, and from 10 to 100 µg/g lipid in whole body and fillet samples. In contrast to the assessment area, reference area walleye contain substantially lower PCBs. For example, fillets from walleye collected from Lake Winnebago had 0.04 mg/kg PCB wet weight (Wisconsin DNR 1996). Model simulations with the Green Bay Mass Balance Model, a bioenergetics-based food web bioaccumulation model, indicate that adult walleye in the assessment area accumulate the majority of PCBs from their prey, including alewife, rainbow smelt, and gizzard shad (Connolly *et al.* 1992).

PCB concentrations in walleye whole body and liver samples collected in 1996 were within tenfold of each other at all assessment area collection locations. Mean concentrations of PCBs (wet weight) in liver samples from Green Bay walleye (3.6 to 6.4 mg/kg) were significantly elevated ($p < 0.01$) above mean PCB concentration in the livers of reference area fish (0.02 and 0.94 mg/kg). Liver PCB concentrations in assessment area walleye were significantly ($p < 0.01$) elevated above liver PCBs in reference area fish on both a wet weight and lipid weight basis. Congener patterns in walleye were generally similar across the assessment area. The congener pattern in walleye from the Lower Fox River exhibited a greater representation of lower chlorinated congeners, consistent with Aroclor 1242 releases into the Lower Fox River and a general loss of lower-chlorinated congeners as the PCBs move into the bay and are subject to environmental weathering processes (Farley *et al.* 1994, Gevao *et al.* 1997). However, these fish were also generally smaller and younger than fish from other collection areas. The dioxin-like coplanar PCBs 77 and 126 were observed in walleye collected from all assessment area locations.

TABLE 4. Foci of cellular alteration and hepatic tumors in male and female walleye (5 to 8 years old; 1996 and 1997 data combined).

Location	Sex	Average Weight (kg)[1]	n	Prevalence: Number of Fish with Lesions (%) FCA and/or HT	FCA	HT	No FCA and/or HT	Mean Lesions per Liver Sample[2] FCA	HT
Assessment Area	Male	1.29	23	3 (13%)	3 (13%)	0 (0%)	20 (87%)	2	—
	Female	1.62	41	14 (34%)[3]	11 (27%)[3]	7 (17%)[3]	27 (66%)	> 4.6	2
	Combined	1.48	66[4]	17 (26%)[3]	14 (21%)[3]	7 (11%)[3]	49 (74%)	> 4.1	2
Reference Area	Male	0.81	5	0 (0%)	0 (0%)	0 (0%)	5 (100%)	—[5]	—
	Female	0.86	29	2 (6.9%)	2 (6.9%)	0 (0%)	27 (93%)	1	—
	Combined	0.85	34	2 (5.9%)	2 (5.9%)	0 (0%)	32 (94%)	1	—

[1]Computed using available data (weights not recorded on all fish).
[2]Only includes fish with an FCA or HT.
[3]Significantly different prevalence compared to reference area fish ($p < 0.05$; t-test)
[4]Includes fish of undetermined sex.
[5]Not applicable (no FCA or HT).

TABLE 5. Liver histologic scores of walleye (5 to 8 years old) collected from Green Bay and reference areas in 1996 and 1997.

Lesion Descriptor (p-value in parentheses)[1]	Green Bay Number of Fish (%)	Reference Area Number of Fish (%)
Glycogen Depletion ($p < 0.001$)		
None	35 (53)	4 (12)
Mild depletion	19 (29)	6 (18)
Moderate depletion	8 (12)	14 (41)
Severe depletion	4 (6.1)	10 (29)
Liver Macrophage Aggregates (LMA; $p = 0.06$)		
No LMA	11 (17)	1 (2.9)
< 3 LMA	44 (67)	26 (77)
3 to 6 LMA	7 (11)	7 (21)
> 6 LMA	4 (6.1)	0 (0)
Lipidosis ($p = 0.35$)		
No hepatocytes with lipid vacuoles	41 (62)	26 (77)
< 10% of hepatocytes	15 (23)	7 (21)
10 to 50% of hepatocytes	8 (12)	1 (2.9)
> 50% of hepatocytes	2 (3.1)	0 (0)
Perivascular and/or Pericholangial Leukocytes ($p = 0.88$)		
1 or 2 lymphocytes per vessel	12 (18)	8 (24)
> 2 lymphocytes, not in surrounding parenchyma or muscular tunics	43 (65)	21 (62)
Lymphocytes extend into parenchyma	7 (11)	4 (12)
Severe lymphocyte lesion	4 (6.1)	1 (2.9)
Granuloma, Foreign Body ($p = 0.77$)		
No granulomas	61 (92)	33 (97)
Total granuloma area >10% of section area	4 (6.1)	1 (2.9)
Severe granuloma	1 (1.5)	0 (0)
Focal/Multifocal Parenchymal Leukocytes or Lymphocytes (FPL; $p = 0.38$)		
No FPL per section	32 (49)	20 (59)
1 to 3 FPL per section	24 (36)	13 (38)
3 to 5 FPL per section	8 (12)	1 (2.9)
Severe FPL	2 (3.1)	0 (0)
Megalocytosis/Karyomegaly (MEG; $p = 0.001$)		
No MEG	64 (97)	25 (74)
1 to 2 MEG	2 (3.1)	6 (18)
3 to 5 MEG	0 (0)	2 (5.9)
> 5 MEG	0 (0)	1 (2.9)
Single Cell Necrosis (SCN; $p = 0.15$)		
No necrosis	54 (82)	23 (68)
1 to 3 SCN	11 (17)	11 (32)
4 to 5 SCN	1 (1.5)	0 (0)

[1]p value determined with Fisher's exact test.

Preneoplastic and Neoplastic Lesions in Walleye Livers

The most notable liver lesions observed in walleye from the assessment area were FCA, hepatocellular adenomas, and hepatocellular carcinomas. The prevalence of preneoplastic and neoplastic lesions was significantly ($p = 0.004$; both sexes combined) elevated in 5 to 8 year old walleye from the assessment area (26% prevalence) compared to reference area fish (6% prevalence). The prevalence in FCA

TABLE 6. Mean immunological responses (SD) of walleye from assessment area and reference areas collected in 1997.

Sample Location	n[1]	HC[2] (%)	LC[3] (%)	Blood Counts (%)[4] M	S	L	P	B	G	SOA[5] U	S	S-U	LP[6] U	S	S-U	Phagocytic Activity I[7]	C3[8]	C4[9]
Lower Fox River	10	47.3 (5.7)	0.21 (0.07)	3.30 (2.67)	32.5 (10.7)	41.6 (10.7)	8.10 (2.33)	13.90 (11.50)	0.70 (0.95)	0.04 (0.07)	1.33 (1.10)	1.29 (1.05)	0.13 (0.05)	0.45 (0.10)	0.33 (0.09)	43.0 (2.7)	83.3 (3.9)	16.3 (4.0)
Eastern Green Bay	12	43.2‡ (13.1)	0.38‡ (0.12)	2.55‡ (1.37)	23.5‡ (7.6)	50.9‡ (9.7)	13.18‡ (4.64)	9.36‡ (5.73)	1.0‡ (0.63)	0.19 (0.42)	0.67 (0.37)	0.48 (0.49)	0.40§ (0.18)	1.02§ (0.12)	0.62§ (0.17)	45.1 (11.2)	78.6 (7.3)	20.3 (6.9)
Assessment Area Average	22	45.1 (10.2)[9]	0.30 (0.13)	2.90 (2.07)[9]	27.8 (10.1)	46.5 (11.0)	10.76 (4.47)	11.52 (9.02)	0.86 (0.79)	0.12 (0.31)	0.97 (0.84)	0.85 (0.87)	0.20 (0.16)	0.62 (0.28)[9]	0.41 (0.18)[9]	44.2 (8.4)	80.7 (6.4)	18.5 (6.0)
Lake Winnebago	13	27.3 (7.5)	0.58† (0.78)	4.77 (1.30)	31.2 (8.5)	45.7 (9.2)	10.78 (3.87)	7.39 (4.48)	1.5 (1.20)	0.03 (0.06)	2.22 (2.96)	2.18 (2.97)	0.14 (0.08)	0.41 (0.06)	0.27 (0.08)	39.9 (2.5)	77.8 (6.1)	22.0 (6.4)

[1]Number of fish sampled for immunological analyses.
[2]Blood hematocrit.
[3]Blood leucocrit.
[4]Differential blood counts. M: monocytes; S: small lymphocytes; L: large lymphocytes; P: polymorphonuclear leukocytes; B: blast cells; G: granulocytes.
[5]Intracellular superoxide anion production in head kidney (optical density units). U: unstimulated; S: stimulated with phorbol myristate acetate. Optical Density × 15.87.
[6]Lymphoproliferation of kidney T cells (optical density units). U: unstimulated; S: stimulated with 50 µg/mL concavalin A; S-U: difference between stimulated and unstimulated responses.
[7]I: phagocytic index [(total number of cells with particles/total number of cells counted) × 100].
[8]Phagocytic capacity. C3: % phagocytically active cells containing 1 to 3 particles; C4: % cells containing ≥ 4 particles.
[9]Significantly different from reference area (Lake Winnebago) response ($p < 0.05$; Mann Whitney test).
†n = 10 (two samples rejected because of physiologically unrealistic values).
‡n = 11 (one blood sample damage).
§n = 6 (6 samples not analyzed).

TABLE 7. Health assessment of walleye collected from assessment and reference areas in 1997.

Sample Location	n	% with Kidney Bacterial Growth	Gill Parasites			Intestinal Tract Parasites			
			Species[1]	% Prevalence	Intensity (range)	Species[1]	% Prevalence	Intensity (range)	Viruses[2]
Lower Fox River	20	50%	E[3]	40.0%	29.8 (1-132)	B	60	9.4 (2–26)	ND
Lower Green Bay	12	100%	E[3]	58.3%	26.3 (26-37)	B	58.3	6.3 (3–13)	ND
Eastern Green Bay	17	53%	E[3]	94.1%	19.1 (2-79)	B	17.6	4.7 (3–10)	ND
						N	11.8	8.0 (2–16)	
						L	5.9	2.0 (2–2)	
Lake Winnebago	12	33%	M	8.3%	1.0 (1-1)	B	58.3	9.2 (1-22)	ND
						P	8.3	1.0 (1-1)	ND
Patten Lake	13	100%	M	15.4%	1.0 (1-1)	B	58.3	16.3 (1-91)	ND
						C	7.7	1.0 (1-1)	

[1]E: *Ergasilus luciopercarum;* M: *Monogenea* sp; B: *Bothriocephalus cuspidatus*; N: *Neoechinorhynchus cylindratum*; L: *Leptorhynchoides thecatum*; P: *Proteocephalus* sp.; C: *Crepidostomum* sp.
[2]ND: not detected.
[3]*Argulus sp.* detected in gills, but not reported (primarily occurs on skin and fins not assessed in this study).

and HT was higher in female walleye than in male fish. However, the proportion of females was higher in the sample from the reference area than that from the assessment area (Table 4), indicating that the observed difference in FCA and HT prevalence between the two areas is not due to sex-skewed samples. Among females alone, FCA and HT prevalence was also significantly ($p = 0.003$) higher in fish from the assessment area compared to fish from the reference areas.

Although sex specific tumor incidences have been previously reported in other species, information in fish is limited (U.S. EPA 1998). The lesions observed in walleye are consistent with exposure to xenobiotic carcinogens or tumor promoters. Additionally, liver weights relative to body weights were significantly ($p = 0.003$; combined sex and ages) elevated in assessment area fish, consistent with the reported effects of PCBs in mammals and fish (Safe 1994, Niimi 1996). PCBs are known to cause cellular changes at low mg/kg concentrations (Niimi 1996). Dietary exposure of PCBs has been demonstrated to increase hepatic tumors in several species (IARC 1999). For example, dietary administration of Aroclor 1254 significantly increased the incidence of hepatocellular adenomas and carcinomas in mammals (IARC 1999), the same hepatic neoplasms observed in assessment area walleye.

Environmental PCB contamination has been associated by other authors with increased tumor frequencies and other histological lesions in feral fish, including ovarian atresia and hepatocellular lesions (Niimi 1996). For example, Teh *et al.* (1997) reported severe lipidosis and vacuolated and basophilic FCA in largemouth bass collected from a PCB contaminated reservoir, whereas reference area fish did not contain these lesions. Baumann *et al.* (1991) reported neoplasms in walleye from the Lower Fox River, as well as other Great Lakes locations. Laboratory studies have generally shown that PCB exposures of one year or less do not result in an increase in liver lesions. The results of carcinogenic studies on rainbow trout are consistent with those of studies on mammals that show PCBs have poor tumor initiation properties, but are tumor promoters (Niimi 1996). Teh *et al.* (1997) concluded that the finding of specific lesions only in fish from contaminated sites suggests a contaminant etiology.

Other potentially carcinogenic contaminants are present in the assessment area, including TCDD, chlorinated pesticides and mercury (U.S. EPA 1992,

TABLE 8. Mean and standard deviation (SD) of hepatic EROD activity in walleye from assessment and reference areas collected in 1997.

Sample Location	n	EROD (pmol/min/mg microsomal protein) Mean	SD	Range
Lower Fox River	20	35.2	16.5	14–73
Lower Green Bay	12	21.5	9.6	7–45
Eastern Green Bay	17	22.9	15.9	2–60
Western Green Bay	10	22.2	9.3	6–33
Assessment Area Average	59	26.7	15.1	2–73
Lake Winnebago	12	23.3	7.3	12–34
Patten Lake	13	34.3	11.9	17–56
Reference Area Average	25	29.0	11.3	12–56

TABLE 9. Mean and standard deviation (SD) of plasma vitellogenin (Vtg) in walleye.

Sample Location	Sex	n	Vtg (mg/mL) Mean	SD	Range
Lower Fox River	M	7	< 0.001	0	—
	F	13	< 0.001	0	—
Eastern Green Bay	M	7	< 0.001	0	—
	F	6	2.24	3.16	0.25–8.4

TABLE 10. Mean and standard deviation (SD) liver to body weight ratios in assessment area and reference area walleye collected in 1997.

Sample Location	Sample Size	% Liver: Body Weight[1]
Lower Fox River	20	0.90 (0.27)
Lower Green Bay	12	0.95 (0.27)
Eastern Green Bay	17	0.96 (0.31)
Western Green Bay	14	1.00 (0.17)
Assessment Area Average	63	0.95 (0.26)[2]
Lake Winnebago	12	0.63 (0.09)
Patten Lake	13	0.89 (0.20)
Reference Area Average	25	0.83 (0.23)

[1]Calculated as: [liver weight (kg)/body weight (kg)] × 100.
[2]Significantly different from reference area average ($p = 0.003$; Mann Whitney test).

TABLE 11. Mean and standard deviation (SD) condition factor in assessment area and reference area walleye collected in 1997.

Sample Location	Sample Size	Condition Factor[1]
Lower Fox River	20	10.0 (2.5)
Lower Green Bay	12	9.6 (0.6)
Eastern Green Bay	17	10.5 (1.0)
Western Green Bay	14	10.5 (0.8)
Assessment Area Average	63	10.2 (1.6)[2]
Lake Winnebago	12	8.0 (0.5)
Patten Lake	13	8.7 (0.6)
Reference Area Average	25	8.4 (0.6)

[1]Calculated as: [body weight (g)/length (cm)^3] × 1,000.
[2]Significantly different from reference area average ($p < 0.001$; Mann Whitney test).

Wisconsin DNR 1996). However, relative to PCB levels, concentrations of other contaminants are substantially less elevated above those of reference areas. For example, inspection of the Wisconsin Department of Natural Resources fish contaminant database (Wisconsin DNR 1996) shows that most chlorinated pesticides are present at 10- to 100-fold lower concentrations than PCBs and show limited if any elevation above that of reference areas. Additionally, pesticides such as dieldrin and DDE have the same order-of-magnitude carcinogenic potency as PCBs (U.S. EPA 1998, IARC 1999), but are present at substantially lower concentrations in walleye. Polycyclic aromatic hydrocarbons (PAHs) can

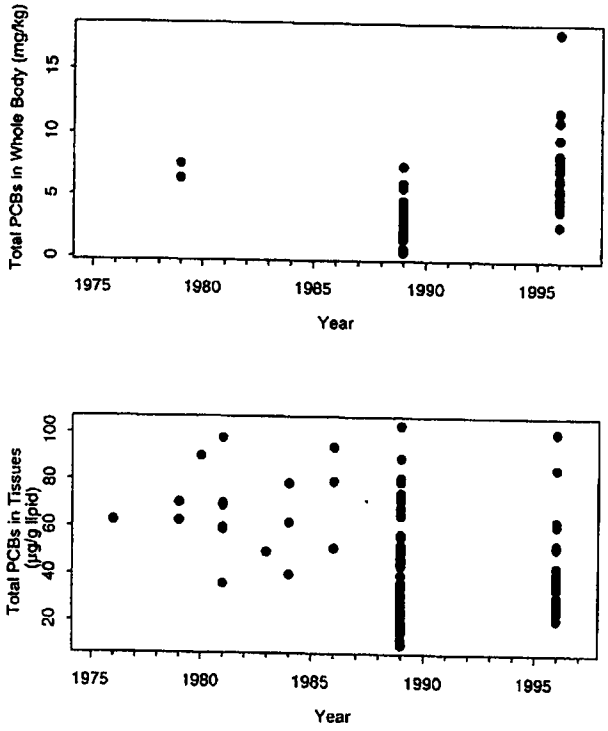

FIG. 6. Total PCB concentrations in walleye collected from the assessment area (all collection locations combined). Top panel: PCBs in whole body (mg/kg wet weight). Bottom panel: PCBs in whole body and fillets (g/g lipid). Data sources: Wisconsin DNR (1996), Connolly* et al. *(1992), this study.

be elevated in sediments in proximity to industrial areas, but bioaccumulation of the larger carcinogenic PAHs is generally limited in fish (Varanasi and Stein 1991). An association was observed between PCBs and elevated tumor prevalence in Green Bay compared to reference areas, but there was no association between PCB concentrations and presence/absense of tumors at the level of individual fish.

Other Biomarker Responses

The biomarker indices measured in this study are sensitive to fish handling and sample holding times. For example, sampling stress may alter immune parameters such as blood cell counts, macrophage function, or lymphocyte function (Anderson *et al.* 1997). Therefore, fish collected for biomarker analyses were subject to standardized fish handling procedures to minimize stress and insure that all fish (reference and assessment sites) were collected in a consistent manner. Immune biomarker validation studies were conducted in the laboratory to show that sample holding times before analysis (12 to 16 hours for kidney cell suspensions or blood samples on wet ice) would not significantly alter cell viability or blood cell measurements.

The data did not show any clear distinctions in the prevalence of disease or immunological responses between reference and assessment area walleye. However, not all aspects of disease or immune function were evaluated. Viruses were not detected in any walleye samples. A large number of isolates of an unidentified yeast and one identified mold were recovered from walleye from the Lower Fox River, lower Green Bay, and eastern Green Bay. There is some evidence that fungal infections in fish are associated with immunosuppression (Roberts 1989, Noga 1996). Elevated prevalence of parasitic copepods in Green Bay walleye could be simply from ecological differences in the smaller reference lakes.

The significant ($p = 0.002$) increase in hematocrit values observed in assessment area fish could be because of a number of factors, including premature release into the circulation of immature red blood cells from the primary organ of hematopoiesis (kidney). Hematocrit values in assessment area fish were consistent with values reported for this species (Wededmeyer and Yasutake 1977). Reduced leukocrit values, possibly because of the significant ($p = 0.002$) reduction in the relative percentages of circulating monocytes, indicate a reduction in those cells responsible for host defense against infectious agents. Under many conditions, this decrease in circulating white blood cells could lead to an increased risk of infectious disease in feral populations. Conversely, the significant ($p = 0.02$) increase in lymphoproliferative capacity of kidney T-cells in assessment area fish may suggest an enhancement in cells responsible for host defense (Snarski 1982, Rice and Schlenk 1995). Walleye from the assessment area exhibited a greater incidence of gill parasite infection compared to fish from the Lake Winnebago reference area, which may be related to habitat differences. While high tissue levels of PCBs were measured in exposed walleye, effects on immune responses were generally marginal. However, given that sensitivity to many contaminants appears to be species/strain-dependent, it is possible that walleye are relatively insensitive to the immunomodulating effects of

PCBs, at least under the conditions to which the fish were exposed in this study.

Fish exposed to PCBs generally exhibit EROD induction, and may exhibit modulation of other enzymes involved in steroid metabolism (Niimi 1996). Hepatic EROD induction has been associated with greater DNA adduct formation in fish exposed to carcinogens (Watson and Di Giulio 1997). No significant ($p > 0.05$) differences were observed in hepatic EROD activity between assessment area and reference area walleye, despite substantial differences in PCB exposure. However, laboratory studies to understand the susceptibility of walleye toward induction of EROD have not been conducted. Only one reference to the measurement of EROD in walleye is currently found in the literature. Williams *et al.* (1997) measured EROD activity in walleye collected from Leland Lake, Northwest Territory, Canada, and found a range of 10 to 100 pmol/min/mg in males and 15 to 40 pmol/min/mg in females.

Potential explanations for these results include inhibition of EROD activity by PCBs or other contaminants (Besselink *et al.* 1998), natural factors (sex or age effects, environmental conditions), or alteration of EROD activity during fish capture procedures (Machala *et al.* 1997). For example, PCBs may impair the ability of fish to elicit a physiological response normally associated with cortisol stimulation (Vijayan *et al.* 1997). Alternatively, fish chronically exposed to elevated amounts of contaminants, including PCBs and dioxins, have shown a reduced responsiveness toward EROD induction (Prince and Cooper 1995, Förlin and Celander 1995). Bellos *et al.* (1998) proposed a genetic basis for the resistance toward EROD induction in a chronically exposed population, based on a lack of response toward induction observed in offspring of the New Bedford Harbor fish that were exposed to AhR agonists. There have been other studies in which EROD activity in fish populations with substantially different exposures to AhR agonists were not different. The activity of mixed function oxidases in lake trout taken from Lake Ontario were observed to be similar to EROD activity in lake trout collected from Lake Superior (Palace *et al.* 1998). Thus, within the Great Lakes there have been examples of an apparent lack of differential EROD responses to known differences in PCB exposure. Future studies should consider the use of P450 content in addition to measuring P450 activity such as EROD responses (Anderson *et al.* 1997).

Vtg was determined in a limited number of male and female fish at only two locations (Fox River and eastern Green Bay) as an initial screening evaluation. Vtg has previously been used as a biomarker of estrogenic effects in fish (Goodbred *et al.* 1997). For example, Folmar *et al.* (1996) measured elevated Vtg in the serum of male carp below a sewage treatment plant relative to male carp from a reference area. No detectable Vtg was observed in male fish from the Lower Fox River and eastern Green Bay at a detection level of 0.001 mg/mL. Elevated levels of Vtg (0.25 to 8.4 mg/mL) were measured in all female fish collected from eastern Green Bay during late August 1997. This period is normally associated with low or nondetectable Vtg in early recrudescent fish (van Bohemen and Lambert 1981). Walleye are spring spawners that do not begin gonad maturation until after the winter recrudescent period (typically in March and April in the Great Lakes). A weak estrogenic response to contaminants in fish may include an elevation of Vtg in female fish, but an undetectable response in male fish. Additionally, prior exposure to endogenous estrogens in fish (prior spawning cycles) may increase their responsiveness to secondary stimulation (Pakdel *et al.* 1991). PCBs are potential endocrine disrupting chemicals with both estrogenic and antiestrogenic effects (Safe 1994, Niimi 1996, Goodbred *et al.* 1997), however, the role of PCBs in controlling Vtg in male or female walleye has not yet been elucidated.

CONCLUSIONS

The intent of this study was to broadly evaluate the responses of a suite of biomarkers that were known to be responsive to contaminant exposure. The work focused on PCBs because they are substantially elevated in the sediment and food web of Green Bay relative to most other contaminants. The results of this investigation demonstrate that PCBs remain elevated in walleye from Green Bay. Further, a substantial elevation of preneoplastic lesions and hepatic tumors in walleye from Green Bay areas was observed relative to walleye from reference areas. These lesions are consistent with long-term exposure to tumor promoters such as PCBs. However, this field study did not establish a causal link between tumors and PCBs in individual fish from Green Bay. Other contaminants elevated in Green Bay fish relative to reference areas included 2,3,7,8-TCDD, chlorinated specific chlorinated pesticides (DDT and metabolites), and mercury. Despite previous associations between PAHs and

tumors in fish, PAHs are not considered to be likely causative agents of tumors in Green Bay walleye. PAHs are typically associated with tumors in benthic fish, rather than pelagic fish, and carcinogenic PAHs are less likely to be trophically transferred to pelagic piscivorous fish (Varanasi and Stein 1991) such as walleye. Ecological factors (viruses) may play a role in the elevated tumor incidence in Green Bay; however prevalence of virus was similar to reference areas. Additional sampling and analyses would be needed to statistically evaluate the association between PCBs and elevated tumors in Green Bay fish, and to elucidate the causal mechanisms underlying the neoplastic and preneoplastic lesions observed in Green Bay walleye.

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