Association between 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 and PCB congeners. Follow-up sampling in 1997 included examination of liver histopathology, 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 (4.6-8.6 and 4.1-7.9 mg/kg wet weight, respectively) compared to PCB concentrations in reference areas (e.g., 0.04 mg/kg in walleye fillets from Lake Winnebago). Mean total PCB concentrations were 87% higher in walleye collected from eastern Green Bay than in western Green Bay, a finding consistent with spatial patterns of PCB contamination in bay sediments and with walleye data collected by Connolly et al. (1992). We observed a significant ($p < 0.01$) elevation 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. 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. 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. Assessment area walleye exhibited a higher prevalence and intensity of gill parasites, whereas intestinal parasites and bacterial infections were similar in assessment area and reference area fish. 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.

Key words: PCBs, walleye, cancer, histopathology, biomarkers

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). PCB concentrations in salmonids from Green Bay are generally higher than in the Lake Michigan basin (Staggs, 1987). 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. (1997a) 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 substantially 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) shows that most chlorinated pesticides are present at 10-fold lower concentrations than PCBs and show limited if any elevation above reference areas. Thus the focus of this study was the association of PCBs with indices of health in assessment area walleye.

The objective of this study was to evaluate the association between PCB contamination and 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). Walleye

initially were collected between July and October, 1996 for determination of PCB residues in whole body and liver samples and for a limited assessment of liver histopathological lesions. 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. Because PCBs and histological lesions were detected in assessment area walleye in 1996, supplemental sampling was performed during August and September, 1997 to include an expanded suite of biochemical, physiological, histological, and fish health measurements. Walleye were assessed for parasites, bacterial and viral infections, liver histopathological lesions, hepatic EROD activity, plasma vitellogenin levels, and kidney and blood immunological parameters.

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. Appendix A (Table A-1) and Appendix B provide additional sample location information.

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 but have not yet been analyzed. 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 two 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. In 1996,

Figure 1
Study Area and Walleye Collection Locations

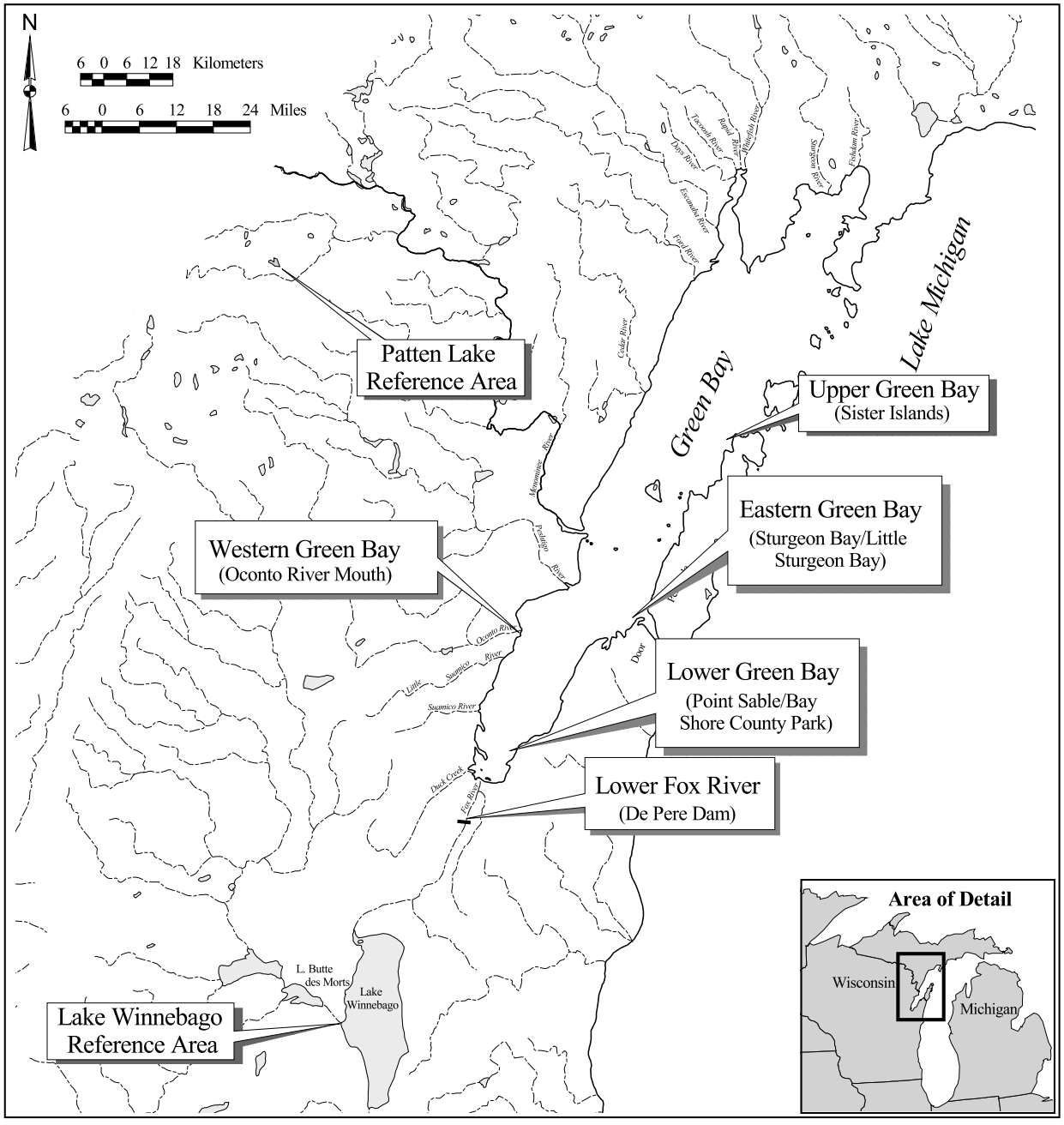


Table 1
Summary of Collection Locations, Sample Type, and Number[1]

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*	—
	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	—
	Health Exam	mult	—	12
	Histopathology	liver	4	12
	EROD	liver	—	12
Eastern Green Bay	PCBs	whole	11	—
		liver	4	—
	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	—
	Histopathology	liver	4	14
	EROD	liver	—	10

Table 1 (cont.)
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)
Upper Green Bay	PCBs	whole	3	—
		liver	4	—
	Histopathology	liver	4	—
Reference Areas				
Lake Winnebago	Health Exam	mult	—	12
	Histopathology	liver	—	21
	Immunology	mult	—	13
	EROD	liver	—	12
Patten Lake	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. Tissue samples were collected in 1997 but analytical results are not available.
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.

fish were sampled by electroshocking or gill nets. In 1997, all fish were collected by electroshocking from boats and then held in live wells. Fish were anesthetized with lethal levels of sodium bicarbonate-buffered MS-222 before tissue processing to minimize capture stress. To minimize age-related variance in measurements, we targeted adult fish 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. Appendix A provides size and age information for walleye samples.

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.

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.

Analytical Chemistry

Walleye collected in 1996 from the Lower Fox River and from lower, eastern, western, and upper Green Bay were analyzed for concentrations of total PCBs in liver samples and PCB congeners in composites of whole body samples. Full analytical methods are provided in Appendix C. 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 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. Appendix C provides documentation of the analytical chemistry methods and quality assurance and quality control procedures.

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 (number 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 incidence 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 Colme, 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 Homes (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 immune alterations using a 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 (Appendix D). 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.

Vitellogenin Assays

Walleye collected in 1997 from the Lower Fox River and eastern Green Bay were assessed for plasma levels of vitellogenin (Vtg) to screen for estrogenic effects. 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

Differences in mean PCB concentrations in walleye livers and whole body samples between collection regions were assessed by one way analysis of variance. Regional differences in histological observations that were expressed as counts of cellular features (i.e., FCA and HT lesions) and other biomarker responses (i.e., EROD activity; liver:body weight ratio; condition

factor; immumological parameters) 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 (i.e., 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 5-8 years. All other comparisons (i.e., 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 whole body PCB concentrations across sampling locations were not significantly different ($p = 0.10$). However, mean whole body total PCB concentrations were approximately 87% higher in eastern Green Bay walleye than in western Green Bay fish, a pattern that is consistent with spatial patterns of PCB sediment contamination (Manchester-Neesvig et al., 1996). Mean concentrations of PCBs (wet weight) in liver samples were similar to whole body concentrations and ranged from 4.1 mg/kg (western Green Bay) to 7.9 mg/kg (eastern Green Bay). PCB concentrations in liver samples were not significantly different across locations on a wet weight basis ($p = 0.2$) but were different on a lipid normalized basis ($p = 0.02$) due to higher concentrations in eastern Green Bay walleye. PCB concentrations measured in all samples are provided in Appendix A (Table A-2, 1996 liver data; Table A-4, 1996 whole body composite data).

Congener patterns in walleye collected from all five assessment area locations were generally similar (Figures 2a through 2e), with the exception of the Lower Fox River. 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

Table 2
Mean and Standard Deviation (SD) of Concentrations of Total PCBs and Nonortho PCB Congeners in Walleye Collected from the Assessment Area in 1996

Sample Location	Tissue	n	Total PCBs		PCB Congener[3] (µg/kg ww)			
			mg/kg ww	µg/g Lipid	77	81	126	169
Lower Fox River	whole[1]	7	6.0 (2.2)	47.5 (13.7)	8.1	0.9	0.7	0.1
	liver[2]	1	4.9	32.3	—[4]	—	—	—
Lower Green Bay	whole[1]	6	5.7 (2.9)	34.0 (15.0)	11.2	1.4	1.1	0.1
	liver	4	4.9 (2.2)	37.4 (7.1)	—	—	—	—
Eastern Green Bay	whole[1]	11	8.6 (3.6)	52.9 (21.8)	4.8	ND[4]	0.6	0.1
	liver	4	7.9 (2.5)	57.4 (17.0)	—	—	—	—
Western Green Bay	whole[1]	4	4.6 (0.6)	30.8 (6.6)	7.5	0.5	0.8	0.1
	liver	4	4.1 (2.5)	28.2 (6.8)	—	—	—	—
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)	—	—	—	—

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.

Figure 2a
PCB Congener Pattern in Walleye (Whole Body Composite) Collected from the Lower Fox River

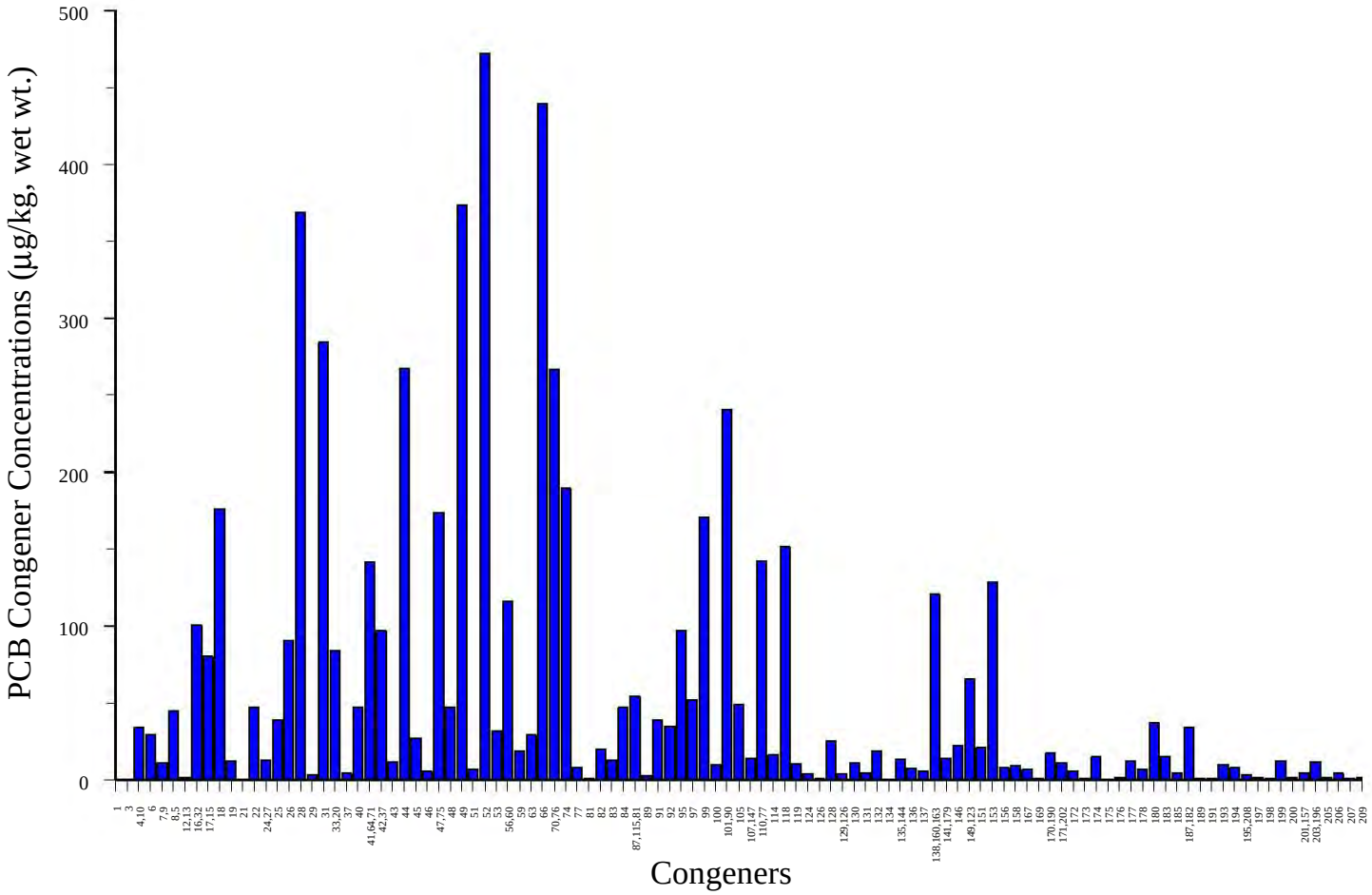


Figure 2b
PCB Congener Pattern in Walleye (Whole Body Composite) Collected from Lower Green Bay

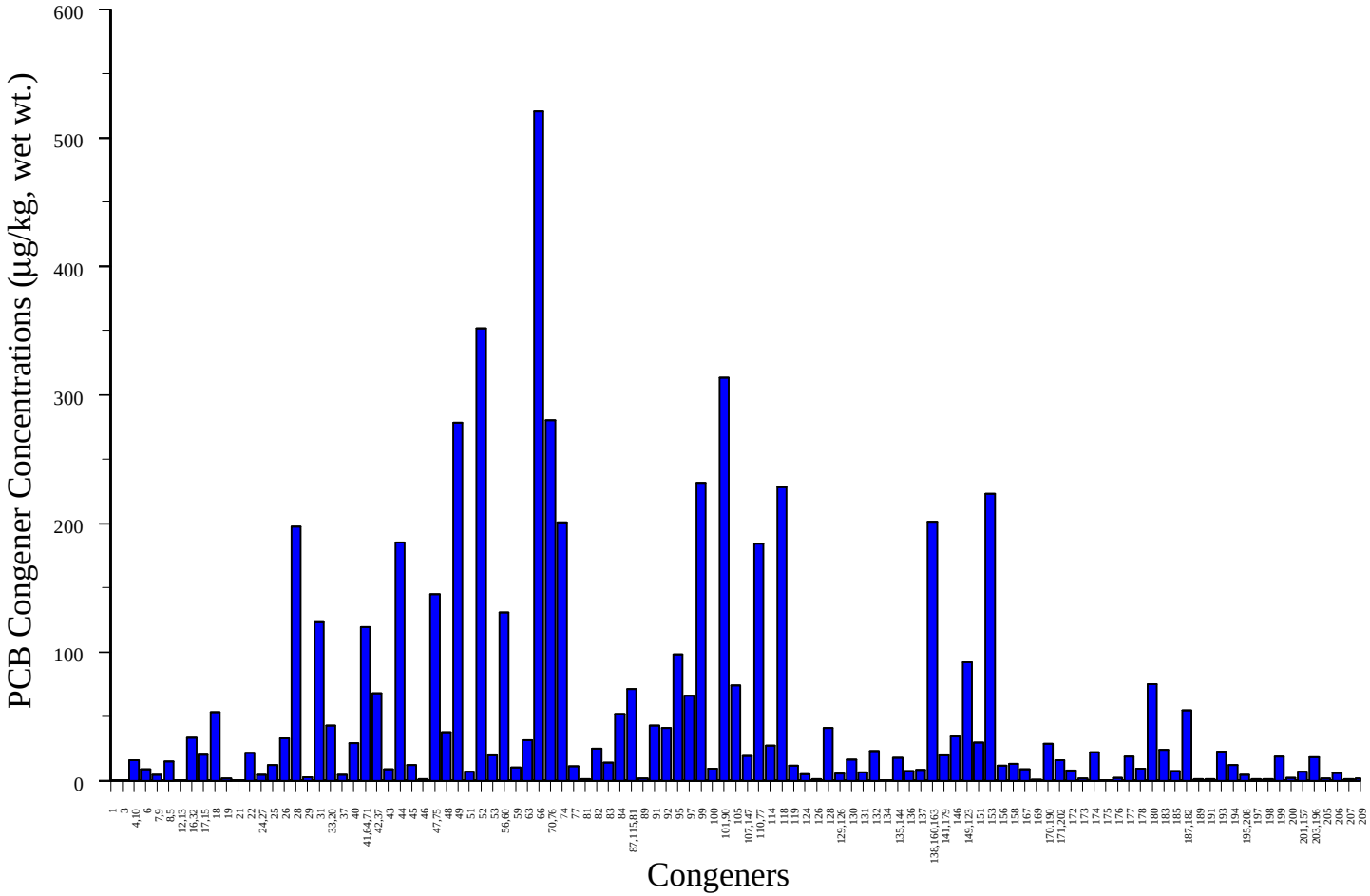


Figure 2c
PCB Congener Pattern in Walleye (Whole Body Composite) Collected from Eastern Green Bay

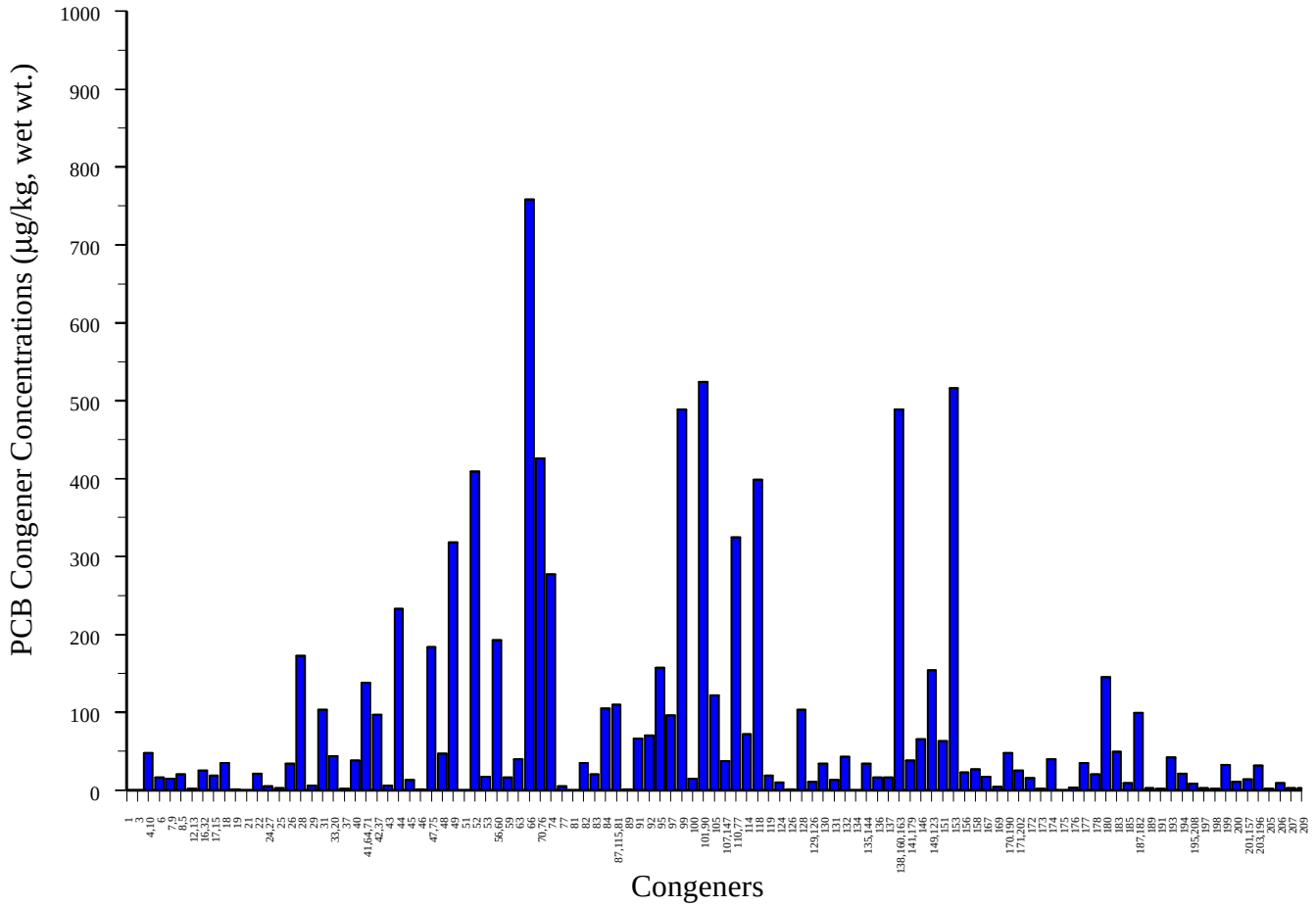


Figure 2d
PCB Congener Pattern in Walleye (Whole Body Composite) Collected from Western Green Bay

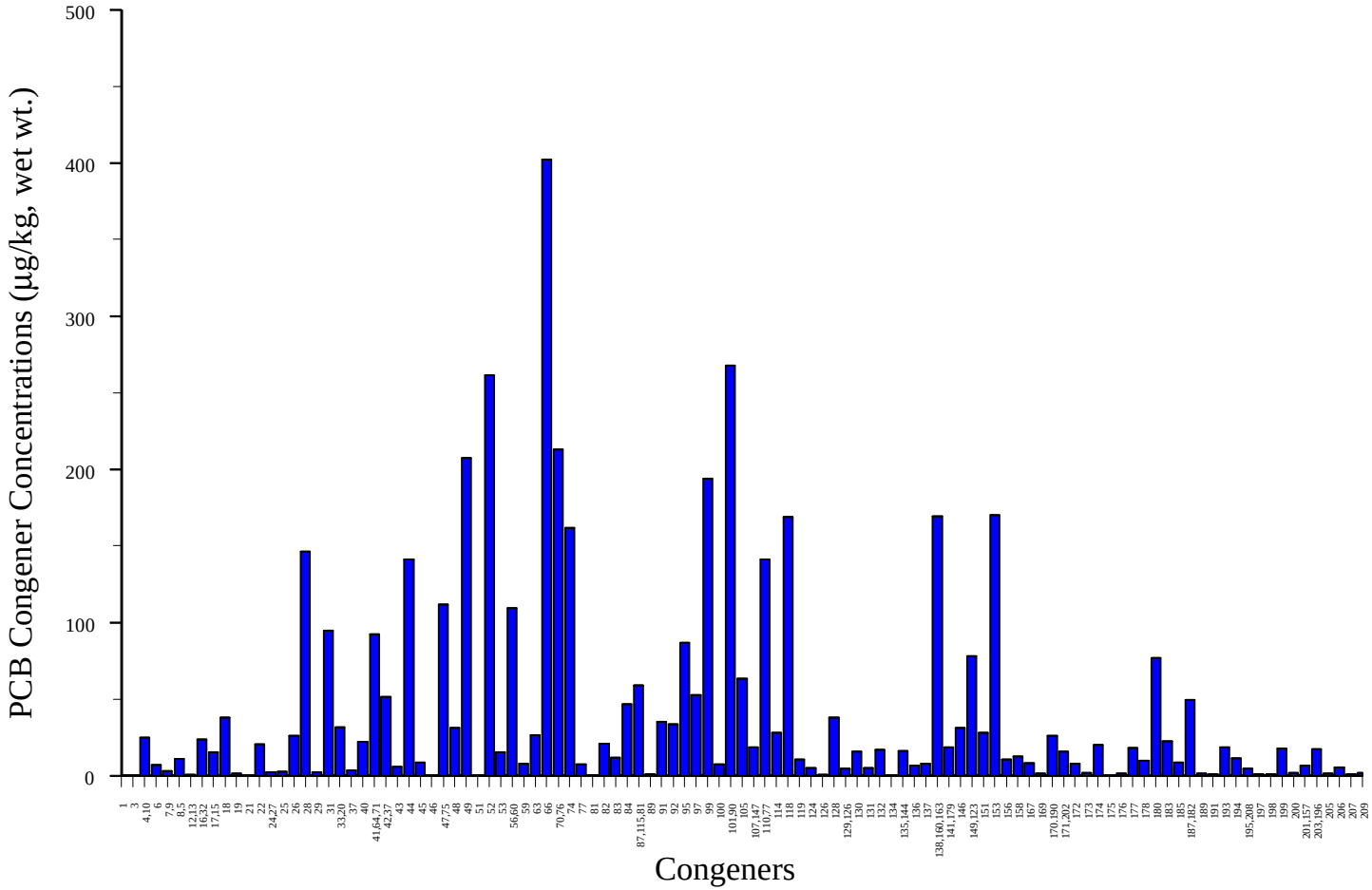
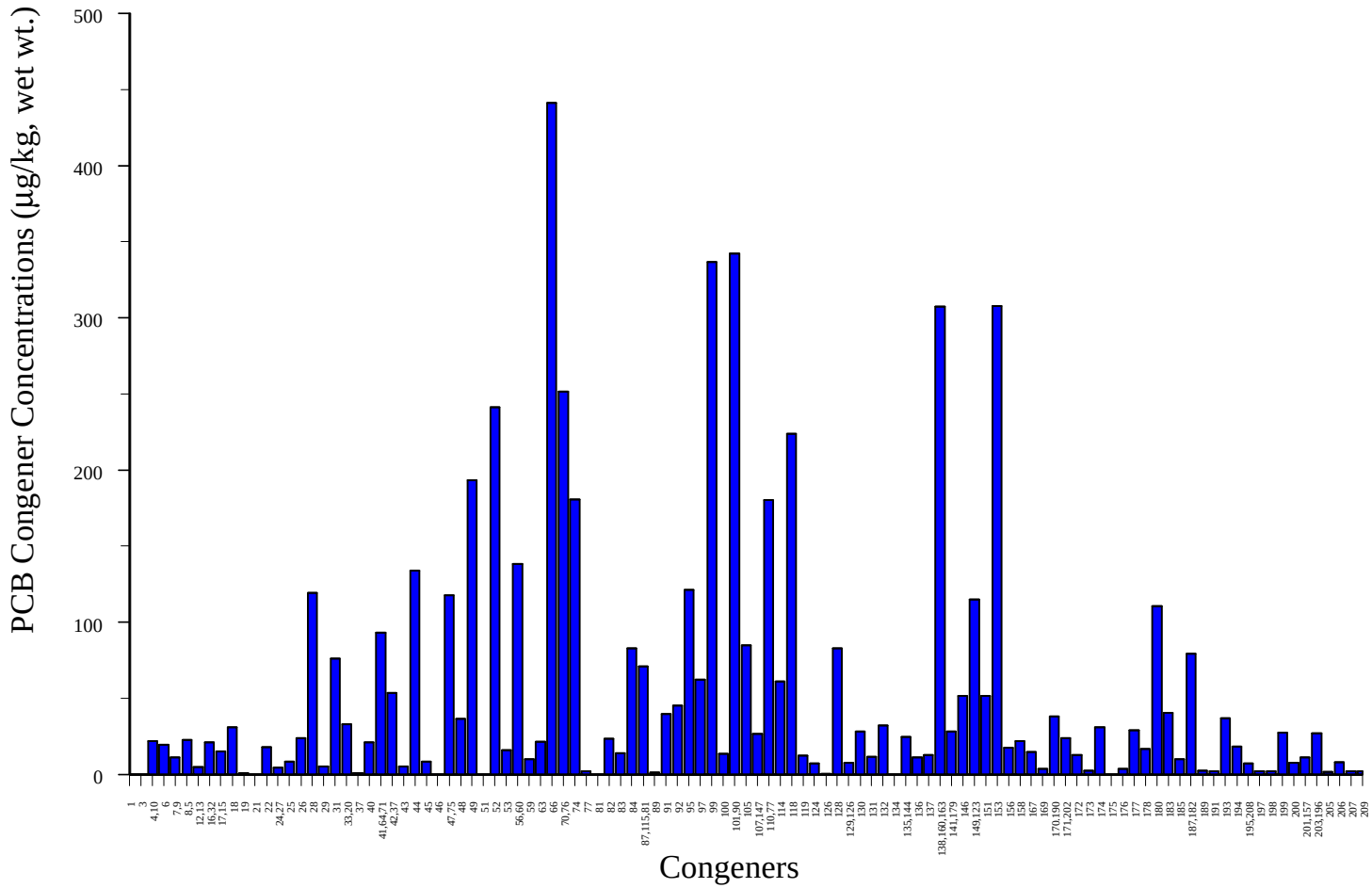


Figure 2e
PCB Congener Pattern in Walleye (Whole Body Composite) Collected from Upper Green Bay



both 1996 and 1997 (Table 3; Figure 3). Appendix A provides information on individual fish and Appendix E provides pathology reports.

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; data for individual fish are provided in Appendix A, Table A-2). 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; data for individual fish collected in 1997 are provided in Appendix A, Table A-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 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, we also compared the prevalence of FCA and hepatic tumors for each age class of walleye sampled (Figure 4) and, within common age classes, for males and females (Table 4). Figure 4 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 (i.e., 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 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

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[1,2]

	1996						1997					
		Fish	% Prevalence		Lesions per Liver[3]			Fish	% Prevalence		Lesions per Liver[3]	
Sampling Location	n	Weight (kg)	FCA	HT	FCA	HT	n	Weight (kg)	FCA	HT	FCA	HT
Lower Fox River	4	1.08 (0.35)	0	0	—[4]	—	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[4]	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. Data for 1996 fish are provided in Teh et al. (1997b); data for 1997 fish are provided in Teh et al. (1998). See Appendix E.
2. FCA: foci of cellular alteration; HT: hepatocellular tumor.
3. Average FCA or HTs per liver sample of fish containing these lesions.
4. — : Not applicable (0% prevalence); NS: not sampled.

Figure 3
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

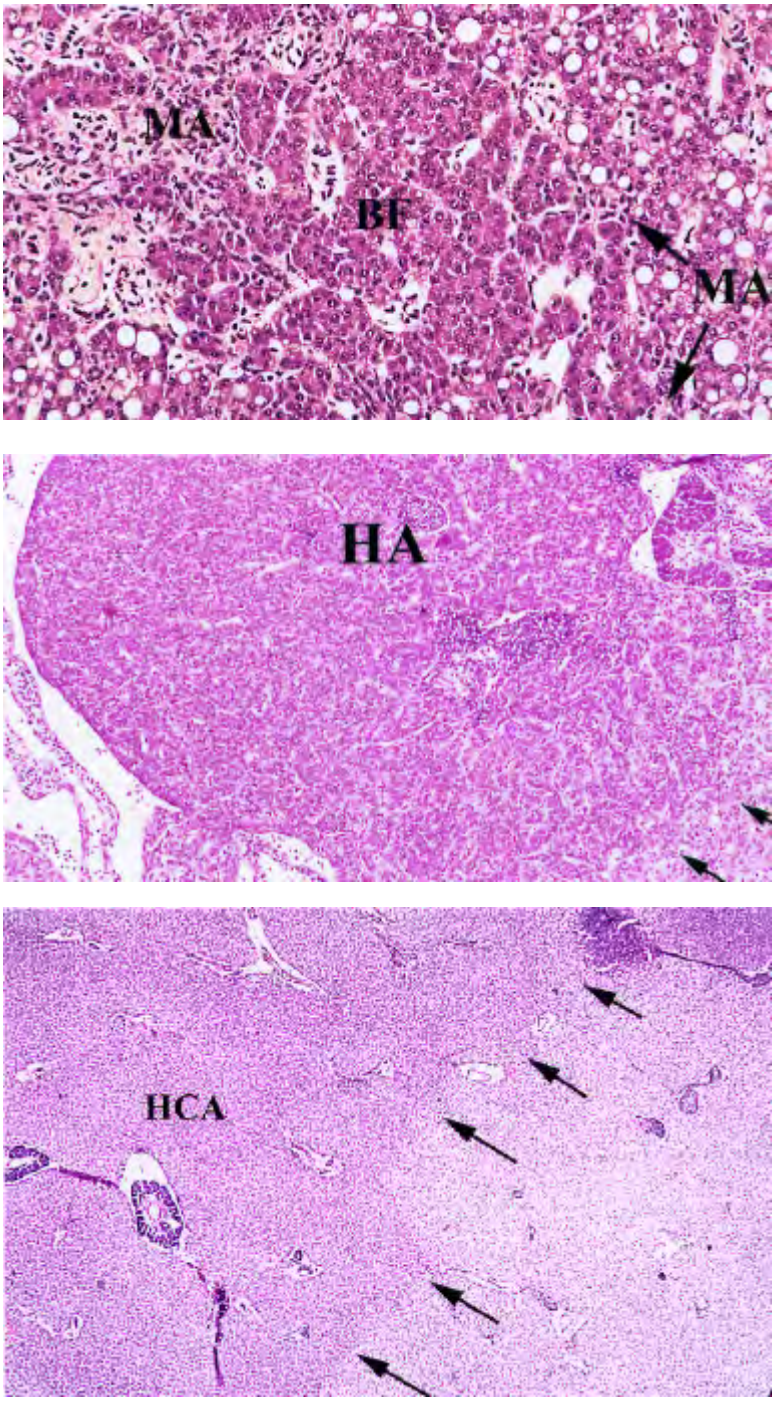
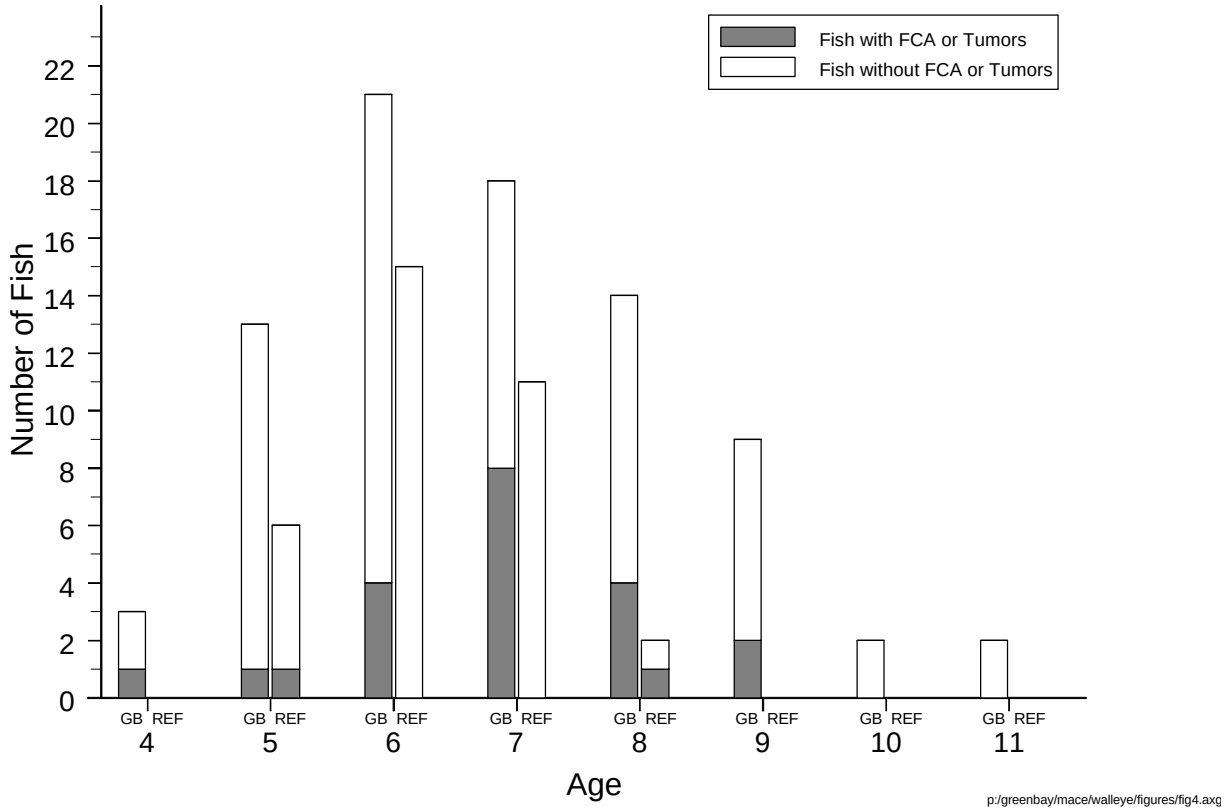


Figure 4
Prevalence of Hepatic FCA and Neoplasms in Walleye from the Assessment Area (GB: left-hand bars; data for 1996 and 1997; all locations combined) and Reference Areas (REF: right-hand bars; data for Patten Lake and Lake Winnebago combined) by Age Class



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 ($p = 0.001$).

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 (%)				Mean Lesions per Liver Sample[2]	
				FCA and/or HT	FCA	HT	No FCA and/or HT	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)

Table 5 (cont.)
Liver Histologic Scores of Walleye (5-8 years old) Collected from Green Bay and Reference Areas in 1996 and 1997

Lesion Descriptor[1]	Green Bay Number of Fish (%)	Reference Area Number of Fish (%)
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. Fisher's Exact Test.		

Other Biomarker Responses

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; Appendix D provides a full report on the immunological assessment of walleye).

Walleye (all ages and sexes combined) were subjected to virology, bacteriological (kidney), and parasitology (gills and intestinal tract) evaluations (Table 7; Appendix F provides a full report on the health assessment for walleye). 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 6
Mean Immunological Responses (SD) of Walleye from Assessment Area and Reference Areas Collected in 1997[1]

Sample Location	n[2]	HC[3] (%)	LC[4] (%)	Blood Counts (%)[5]						SOA[6]			LP[7]			Phagocytic Activity		
				M	S	L	P	B	G	U	S	S-U	U	S	S-U	I[8]	C3[9]	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)[10]	0.30 (0.13)	2.90 (2.07)[10]	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)[10]	0.41 (0.18)[10]	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. Data are provided in Zelikoff (1999). See Appendix D.
2. Number of fish sampled for immunological analyses.
3. Blood hematocrit.
4. Blood leucocrit.
5. Differential blood counts. M: monocytes; S: small lymphocytes; L: large lymphocytes; P: polymorphonuclear leukocytes; B: blast cells; G: granulocytes.
6. Intracellular superoxide anion production in head kidney (optical density units). U: unstimulated; S: stimulated with phorbol myristate acetate. Optical Density × 15.87.
7. 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.
8. I: phagocytic index [(total number of cells with particles/total number of cells counted) × 100].
9. Phagocytic capacity. C3: % phagocytically active cells containing 1 to 3 particles; C4: % cells containing ≥ 4 particles.
10. 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[1]

Sample Location	n	% with Kidney Bacterial Growth	Gill Parasites			Intestinal Tract Parasites			
			Species[2]	% Prevalence	Intensity (range)	Species[2]	% Prevalence	Intensity (range)	Viruses[3]
Lower Fox River	20	50%	E[4]	40.0%	29.8 (1-132)	B	60	9.4 (2-26)	ND
Lower Green Bay	12	100%	E[4]	58.3%	26.3 (26-37)	B	58.3	6.3 (3-13)	ND
Eastern Green Bay	17	53%	E[4]	94.1%	19.1 (2-79)	B N L	17.6 11.8 5.9	4.7 (3-10) 8.0 (2-16) 2.0 (2-2)	ND
Lake Winnebago	12	33%	M	8.3%	1.0 (1-1)	B P	58.3 8.3	9.2 (1-22) 1.0 (1-1)	ND
Patten Lake	13	100%	M	15.4%	1.0 (1-1)	B C	58.3 7.7	16.3 (1-91) 1.0 (1-1)	ND

1. Data are provided in Woolley et al. (1998). See Appendix F.
2. E: *Ergasilus luciopercarum;* M: *Monogenea sp*; B: *Bothriocephalus cuspidatus*; N: *Neoechinorhynchus cylindratum*; L: *Leptorhynchoides thecatum*; P: *Proteocephalus sp.*; C: *Crepidostomum sp.*
3. ND: not detected.
4. *Argulus sp.* detected in gills, but not reported (primarily occurs on skin and fins not assessed in this study).

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. Appendix G provides full reports on measurements of EROD activity.

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. Appendix H summarizes the results of the Vtg analysis.

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).

Table 8
Mean and Standard Deviation (SD) of Hepatic EROD Activity in Walleye from Assessment and Reference Areas Collected in 1997[1]

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	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	25	29.0	11.3	12-56

1. Data for all samples are provided Alt and Tillitt (1998a, b, c). See Appendix G.

Table 9
Mean and Standard Deviation (SD) of Plasma Vitellogenin in Walleye

Sample Location	Sex	n	Vtg (mg/mL)[1]		
			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

1. Data are provided in Denslow (1998). See Appendix H.

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 $(cm)^3$] × 1000.
2. Significantly different from reference area average ($p < 0.001$; Mann Whitney test).

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 5 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 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).

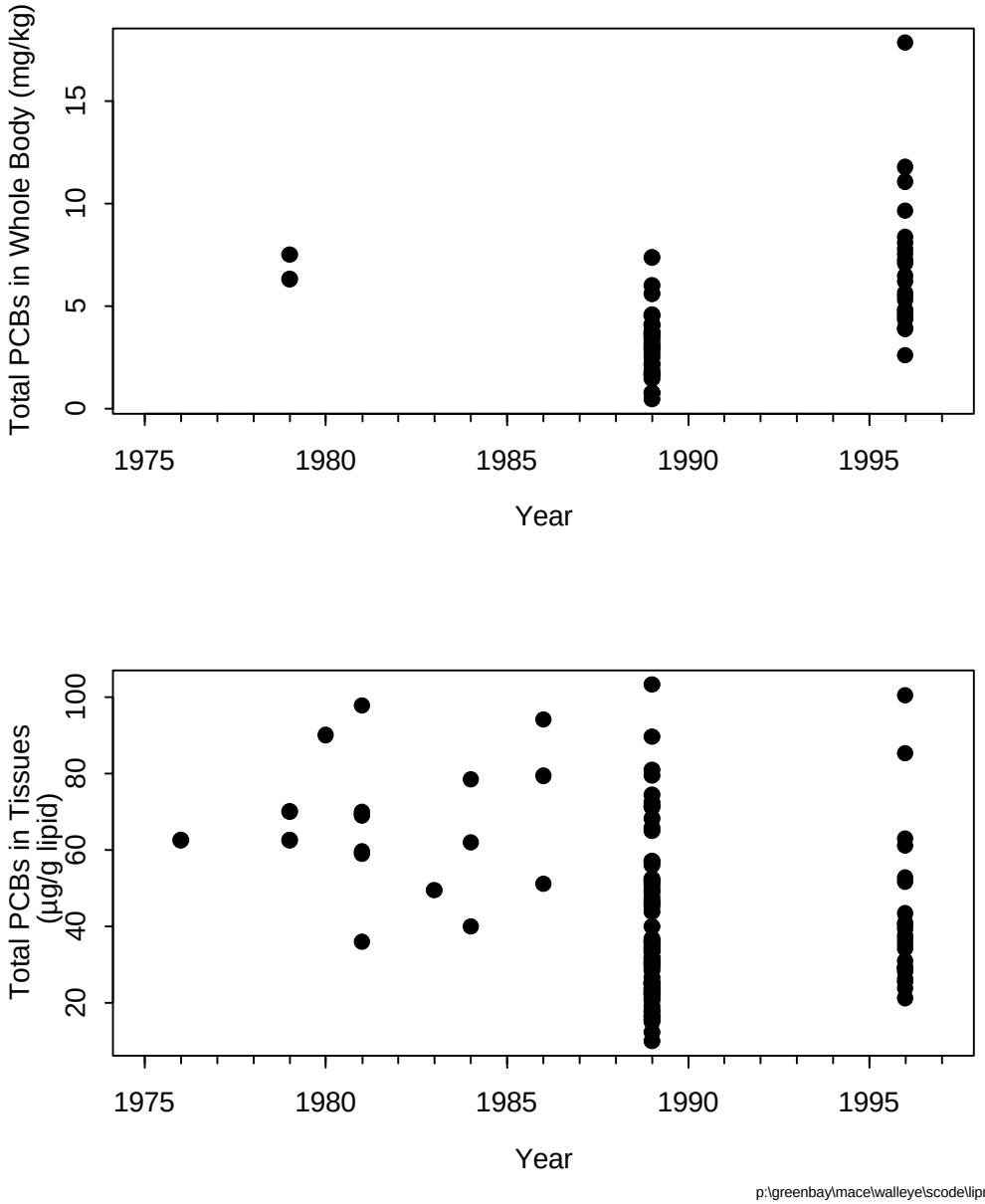
PCB concentrations in walleye whole body and liver samples we collected in 1996 were within tenfold of each other at all assessment area collection locations. ANOVA of lipid normalized liver concentrations indicated a significant ($p = 0.02$) influence of collection location on PCB concentrations, whereas wet weight PCB concentrations did not ($p > 0.05$). Although walleye are mobile, walleye from eastern Green Bay had the greatest PCB concentrations in both whole body and liver samples compared to other areas of Green Bay. For example, mean total PCB concentrations in walleye whole body samples from eastern Green Bay were 87% higher than in western Green Bay, a finding consistent with spatial patterns of PCB contamination in bay sediments and with assessment area walleye data collected by Connolly et al. (1992).

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). The dioxin-like coplanar PCBs 77 and 126 were observed in walleye collected from all assessment area locations.

Preneoplastic and Neoplastic Lesions in Walleye Livers

The most remarkable liver lesions observed in walleye from the assessment area were FCA, hepatocellular adenomas, and hepatocellular carcinomas. FCA are preneoplastic lesions that can develop into tumors. 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 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

Figure 5
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 (µg/g lipid). Data sources: Wisconsin DNR (1996); Connolly et al. (1992); this study.



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. In general, lower chlorinated congeners dominate in the Lower Fox River whereas higher chlorinated congeners are predominant in upper areas of Green Bay (Connolly et al, 1992). Higher chlorinated PCB congeners are associated with increased liver tumors in rodents (IARC, 1999). Therefore, this compositional difference may partially explain the observed general trend of increased FCA and tumors from the lower to upper bay for walleye of similar age.

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. (1997a) 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. (1997a) 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 chlorinated pesticides and mercury (U.S. EPA, 1992; 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. Therefore, the data indicate that the increased

incidence of preneoplastic and neoplastic lesions in assessment area walleye is associated with elevated exposure to and accumulation of PCBs.

Other Biomarker Responses

Our data did not show any clear distinctions in the incidence of disease or immunological responses between reference and assessment area walleye. 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).

The significant ($p = 0.002$) increase in hematocrit values observed in assessment area fish could be due to a number of factors, including premature release into the circulation of immature red blood cells from the primary organ of hematopoiesis (i.e., kidney). Reduced leukocrit values, due possibly to 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. Along these lines, walleye from the assessment area exhibited a greater incidence of gill parasite infection compared to fish from the Lake Winnebago reference area. 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). We did not observe any significant ($p > 0.05$) differences 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 10-100 pmol/min/mg in males and 15-40 pmol/min/mg in females.

Potential explanations for these results include inhibition of EROD activity by PCBs (e.g., Besselink et al., 1998), 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 of , based on a lack of response toward induction observed in offspring of the New Bedford Harbor fish that were exposed to AhR agonists (Bellos et al., 1998). 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.

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. We observed no detectable Vtg in male fish from the Lower Fox River and eastern Green Bay at a detection level of 0.001 mg/mL. We did measure elevated levels of Vtg (0.25 to 8.4 mg/mL) 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. 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 (e.g., 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). The role of PCBs in controlling Vtg in male or female walleye has not yet been elucidated.

CONCLUSIONS

The results of this investigation demonstrate that PCBs remain elevated in assessment area walleye. Further, we observed a substantial elevation of preneoplastic lesions and hepatic tumors in walleye from the assessment area relative to walleye from reference areas. These lesions are consistent with long-term exposure to tumor promoters such as PCBs. The high levels of PCBs measured in walleye tissues and the known carcinogenic effects of PCBs suggest that the elevation of hepatic FCA and tumors are strongly associated with PCB exposure.

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APPENDICES
APPENDIX A: DATA TABLES

Table A-1
Sample Locations Information for Walleye Collected in 1996 and 1997

Year	Sample Location	Site Description	Latitudinal Range (decimal degrees)	Longitudinal Range (decimal degrees)	Area	Approx. Depth
1996	Lower Fox River	Lower Fox River below DePere Dam	4432.130-4431.505	-8800.381--8800.592	From Coast Guard launch area to Walnut Bridge	6-16 ft.
1996	Lower Green Bay	Bay Shore County Park	NR[1]	NR	From Pt. Sable north to Bay Shore County Park	3-10 ft.
1996	Eastern Green Bay	Sturgeon Bay	4453.09-4454.01	-8724.48--8724.91	Offshore of Quarry Point and Sawyer Harbor	3-35 ft.
1996	Western Green Bay	Mouth of Oconto River	NR	NR	Breakwater north of mouth of Oconto River	10 ft.
1996	Upper Green Bay	Sister Islands	~4517.67-4517.70	-8701.66--8701.94	Rocky shoals and bottom and Sister Islands	2-3 ft.
1997	Lake Winnebago Reference Area	South Asylum Bay	4403.517-4403.880	-8831.445--8830.983	Asylum Point to Libby Point, emergent and submergent aquatic vegetation habitat	1.5-4 ft.
1997	Patten Lake Reference Area	Patten Lake	4550.900-4551.972	-8824.817--8825.701	West end of Patten Lake	2-8 ft.
1997	Lower Fox River	Lower Fox River below DePere Dam	44269.96-44270.23	-8803.794--8804.020	Directly below De Pere Dam and spillway	2-6 ft.
1997	Lower Green Bay	Bay Shore County Park	4438.203-4438.299	-8748.278--8748.828	Shoreline adjacent to ramp; 1/2 mi. to north- 3/4 mi. to south	1-4 ft.
1997	Eastern Green Bay	Sturgeon Bay/ Little Sturgeon Bay	4452.289-4453.061	-8724.997--8726.042	Submerged weeds inside of Sawyer Harbor and SW shoreline of the shipping canal on both sides of Sawyer Harbor	2-10 ft.
1997	Western Green Bay	Oconto River Estuary	4453.801-4453.913	-8749.346--8749.606	Rip-rapped shorelines along interior of pier heads from boat launch to mouth of Oconto River	3-13 ft.

1. NR: not recorded. Locations specified in Figure 1 and Appendix B.

Table A-2
Fish Identification and Information for Walleye Collected during 1996

Sample Location	Fish Identification[1]	Sex[2]	Age (years)	Weight (kg)	Length (cm)	FCA[4]	HT[5]	Total PCBs in Liver	
								mg/kg[6]	µg/g lipid
Lower Fox River	WEFR01	F	5	1.35	51.1	0	0	4.9[7]	32.3
	WEFR02	F	6	1.4	51.2	0	0	—	—
	WEFR03	—[3]	5	0.7	43.5	0	0	—	—
	WEFR04	—	5	0.85	45.6	0	0	—	—
Lower Green Bay	WELG01	F	6	1.56	54.5	0	0	4.1	33.8
	WELG02	F	6	2.12	58.3	1	0	2.5	31.0
	WELG03	F	7	1.94	56.9	1	3	5.5	37.4
	WELG04	M	5	1.18	48.5	0	0	7.7	47.3
Eastern Green Bay	WEEG01	M	9	2.62	59.5	0	0	5.5	60.0
	WEEG02	M	9	2.5	60.1	0	0	9.5	36.3
	WEEG03	M	8	2	57.5	0	0	6.1	80.3
	WEEG04	M	7	1.5	51.4	1	0	10.6	42.9
Western Green Bay	WEWG01	F	8	1.74	57.5	0	0	2.6	22.6
	WEWG02	M	8	1.52	51.7	0	0	7.9	37.6
	WEWG03	F	9	2.14	59.5	0	0	3.4	23.6
	WEWG04	F	8	1.78	56.6	0	4	2.6	28.9
Upper Green Bay	WEUG01	F	7	2.3	59.1	0	1	5.2	28.5
	WEUG02	F	8	2.38	60.6	0	1	2.7	36.8
	WEUG03	M	10	3.44	65.7	0	0	5.8	43.0
	WEUG04	M	9	2.88	59.6	0	0	3.9	21.4

1. Identification code used in field collections.
2. F: female; M: male.
3. — = not determined.
4. Foci of cellular alteration in liver.
5. Hepatic tumors.
6. Wet weight basis.
7. Composite of four livers from fish WEFR13, WEFR14, WEFR16, and WEFR26.

Table A-3
Fish Identification and Information for Walleye Collected during 1997

Sample Location	Fish Identification[1]	Sex[2]	Age (years)	Body Weight (kg)	Liver Weight (g)	Total Length (cm)	Assays Conducted					
							Immune[4]	F[5]	T[6]	E[7]	H[8]	V[9]
Lake Winnebago	WELW08/0601	F	7	—[3]	—	49	—	0	0	—	—	—
	WELW08/0602	F	7	—	—	50	(LW-02)	0	0	—	—	—
	WELW08/0603	F	7	—	—	50.5	(LW-03)	0	0	—	—	—
	WELW08/0604	F	6	—	—	47	—	0	0	—	—	—
	WELW08/0605	F	6	—	—	43.5	(LW-05)	0	0	—	—	—
	WELW08/0606	F	6	—	—	44	—	0	0	—	—	—
	WELW08/0607	F	5	—	—	47	—	0	0	—	—	—
	WELW08/0608	F	7	—	—	44.5	—	0	0	—	—	—
	WELW08/0609	M	7	—	—	49	—	0	0	—	—	—
	WELW08/1101	F	6	0.86	6.59	46.5	1 (LW-01)	0	0	34	Y	—
	WELW08/1102	F	5	0.7	4.15	44	1 (LW-02)	1	0	13	Y	—
	WELW08/1103	F	6	0.8	4.55	46	1 (LW-03)	0	0	27	Y	—
	WELW08/1104	F	7	1.08	7.88	51	1 (LW-04)	0	0	12	Y	—
	WELW08/1105	F	6	0.72	3.68	44	1 (LW-05)	0	0	31	Y	—
	WELW08/1106	F	6	0.98	7.28	48.5	1 (LW-06)	0	0	30	Y	—
	WELW08/1107	F	7	0.94	7.09	49	1 (LW-07)	0	0	21	Y	—
	WELW08/1108	F	7	0.74	3.91	47	1 (LW-08)	0	0	16	Y	—
	WELW08/1109	F	7	0.9	5.75	48.5	1 (LW-09)	0	0	29	Y	—
	WELW08/1110	M	8	1.02	6.17	52.1	—	0	0	19	Y	—
	WELW08/1111	F	7	0.92	5.86	49	1 (LW-11)	0	0	21	Y	—
	WELW08/1112	F	8	1.12	5.96	51.8	—	1	0	26	Y	—
Lower Fox River	WEFR08/1201	F	8	1.3	11.27	50	2 (FR-01)	0	0	16	Y	0
	WEFR08/1202	F	—	1.2	15.00	48	2 (FR-02)	0	0	14	Y	0
	WEFR08/1203	F	6	1.66	13.45	55.1	2 (FR-03)	4	2	39	Y	0
	WEFR08/1204	F	6	1.06	9.96	48.5	2 (FR-04)	0	0	19	Y	0
	WEFR08/1205	F	5	1.06	11.50	37.5	2 (FR-05)	0	0	51	Y	0
	WEFR08/1206	F	6	1.36	10.08	52.5	2 (FR-06)	0	0	25	Y	0
	WEFR08/1207	F	6	1.44	10.53	51	2 (FR-07)	0	0	53	Y	0
	WEFR08/1208	M	6	1.24	9.03	51.4	—	0	0	25	Y	0
	WEFR08/1209	F	5	1.28	9.37	50.5	2 (FR-09)	0	0	44	Y	0
	WEFR08/1210	F	5	1.16	12.64	50	2 (FR-10)	0	0	15	Y	0
	WEFR08/1211	F	7	1.44	9.49	54.2	2 (FR-11)	0	0	38	Y	0
	WEFR08/1212	M	6	0.96	11.31	47	—	0	0	46	Y	0

Table A-3 (cont.)
Fish Identification and Information for Walleye Collected during 1997

Sample Location	Fish Identification[1]	Sex[2]	Age (years)	Body Weight (kg)	Liver Weight (g)	Total Length (cm)	Assays Conducted					
							Immune[4]	F[5]	T[6]	E[7]	H[8]	V[9]
Lower Fox River (cont.)	WEFR08/1213	F	6	0.94	5.36	46	—	0	0	41	Y	0
	WEFR08/1214	M	6	0.92	12.96	46	—	0	0	30	Y	0
	WEFR08/1215	M	4	0.82	4.49	44	—	0	0	54	Y	0
	WEFR08/1216	M	5	0.7	8.01	43	—	0	0	49	Y	0
	WEFR08/1217	F	5	0.74	4.69	44	—	1	0	37	Y	0
	WEFR08/1218	M	5	0.76	10.91	43.5	—	0	0	73	Y	0
	WEFR08/1219	M	6	0.94	6.55	47.1	—	0	0	19	Y	0
	WEFR08/1220	F	5	0.82	6.07	45.7	—	0	0	15	Y	0
Eastern Green Bay	WEEG08/2701	M	6	0.92	10.26	46.0	—	2	0	32	Y	0
	WEEG08/2702	M	9	2.20	16.45	57.5	—	0	0	18	Y	0
	WEEG08/2703	M	11	2.12	25.93	56.8	—	0	0	7	Y	0
	WEEG08/2704	M	6	0.82	5.14	41.8	—	0	0	7	Y	0
	WEEG08/2805	F	11	3.72	24.03	70.9	3 (FR-01a)	0	0	5	Y	1.4
	WEEG08/2806	F	10	4.74	47.22	74.4	3 (FR-02a)	0	0	2	Y	0.25
	WEEG08/2907	F	7	2.22	13.34	61.6	4 (FR-07)	1	1	9	Y	0.46
	WEEG08/2908	M	6	1.32	14.92	50.7	4 (FR-08)	0	0	33	Y	0
	WEEG08/2909	F	7	1.84	31.12	55.2	4 (FR-09)	0	0	28	Y	0.38
	WEEG08/2910	M	6	1.34	11.40	52.7	—	0	0	39	Y	0
	WEEG08/2911	F	9	3.62	23.26	66.7	4 (FR-11)	0	0	22	Y	2.5
	WEEG08/2912	M	5	1.1	11.30	46.9	4 (FR-12)	0	0	40	Y	0
	WEEG08/2913	F	9	2.36	33.50	61.1	4 (FR-13)	0	0	9	Y	8.5
	WEEG09/1514	M	6	0.68	4.96	42.0	5 (FR-01)	3	0	30	Y	—
	WEEG09/1515	F	4	0.82	6.70	45.0	5 (FR-02)	0	0	60	Y	—
	WEEG09/1516	M	8	1.74	21.41	53.0	5 (FR-03)	0	0	15	Y	—
	WEEG09/1517	M	4	0.82	6.68	43.0	5 (FR-04)	3	0	34	Y	—

Table A-3 (cont.)
Fish Identification and Information for Walleye Collected during 1997

Sample Location	Fish Identification[1]	Sex[2]	Age (years)	Body Weight (kg)	Liver Weight (g)	Total Length (cm)	Assays Conducted					
							Immune[4]	F[5]	T[6]	E[7]	H[8]	V[9]
Lower Green Bay	WELG08/2601	F	7	1.34	16.46	52.3	—	0	0	21	Y	—
	WELG08/2602	F	6	1.56	7.63	54.9	—	0	0	31	Y	—
	WELG08/2603	F	6	1.3	16.77	49.5	—	0	0	45	Y	—
	WELG08/2604	M	8	1.46	11.06	52.7	—	0	0	7	Y	—
	WELG08/2605	F	8	1.9	21.60	58.7	—	0	0	14	Y	—
	WELG08/2606	F	6	1.4	18.24	53	—	0	0	24	Y	—
	WELG08/2607	M	6	1.32	10.28	50.7	—	0	0	22	Y	—
	WELG08/2608	F	7	1.38	15.19	52.4	—	0	0	24	Y	—
	WELG08/2609	F	5	1.04	10.67	49.7	—	0	0	21	Y	—
	WELG08/2610	F	7	1.69	10.65	56.2	—	3	0	13	Y	—
	WELG08/2611	F	7	1.7	15.57	57.5	—	0	0	18	Y	—
	WELG08/2612	F	9	2.64	18.29	64	—	3	0	18	Y	—
Western Green Bay	WEWG08/1001	M	7	1.64	17.07	51	—	0	0	18	—	—
	WEWG08/1002	F	8	2.06	26.19	57.2	—	4	0	25	—	—
	WEWG08/1003	M	8	1.9	16.57	57	—	0	0	25	—	—
	WEWG08/1004	F	7	1.74	16.58	57	—	0	0	7	—	—
	WEWG08/1005	F	8	1.98	26.48	57	—	0	0	25	—	—
	WEWG08/1006	F	7	2	22.17	57.8	—	0	0	33	—	—
	WEWG08/1007	M	7	1.7	15.42	54	—	0	0	23	—	—
	WEWG08/1008	F	7	1.78	17.37	55.5	—	>10	2	30	—	—
	WEWG08/1009	F	7	1.92	15.04	58	—	5	0	30	—	—
	WEWG08/1010	F	9	2.38	24.34	61.5	—	1	0	6	—	—
	WEWG08/1311	M	8	1.94	16.62	56.4	—	0	0	—	—	—
	WEWG08/1312	F	8	2.74	30.89	64.5	—	11	0	—	—	—
	WEWG08/1313	F	7	2.08	17.21	58.1	—	>10	0	—	—	—
	WEWG08/1314	F	7	2.08	17.94	60.7	—	0	0	—	—	—

Table A-3 (cont.)
Fish Identification and Information for Walleye Collected during 1997

Sample Location	Fish Identification[1]	Sex[2]	Age (years)	Body Weight (kg)	Liver Weight (g)	Total Length (cm)	Assays Conducted					
							Immune[4]	F[5]	T[6]	E[7]	H[8]	V[9]
Patten Lake	WELP09/1601	F	6	1.04	8.75	50.5	—	0	0	31	Y	—
	WELP09/1602	M	6	0.80	4.95	44.8	—	0	0	24	Y	—
	WELP09/1603	M	5	0.72	4.75	43.1	—	0	0	17	Y	—
	WELP09/1604	M	5	0.68	4.00	42.7	—	0	0	49	Y	—
	WELP09/1605	F	7	1.02	10.14	49.7	—	0	0	24	Y	—
	WELP09/1606	F	6	0.80	7.88	46.2	—	0	0	32	Y	—
	WELP09/1607	F	5	0.82	8.09	45.3	—	0	0	32	Y	—
	WELP09/1608	F	6	0.80	8.16	45.0	—	0	0	33	Y	—
	WELP09/1609	F	6	0.78	5.54	45.3	—	0	0	30	Y	—
	WELP09/1610	F	6	0.76	6.50	43.5	—	0	0	56	Y	—
	WELP09/1611	F	5	0.66	6.77	44.5	—	0	0	34	Y	—
	WELP09/1612	F	6	0.8	10.14	43.5	—	0	0	55	Y	—
	WELP09/1613	F	6	0.72	7.45	43.1	—	0	0	29	Y	—

1. Identification code used in field collections.
2. F: female; M: male.
3. — : not determined.
4. Blood and kidney immunoassays. Identification code shown [Group number (fish code)] as used in Zelikoff (1999; Appendix D).
5. Foci of cellular alteration in liver.
6. Hepatic tumors.
7. Liver EROD activity (pmol/min/mg microsomal protein).
8. Y: fish screened for spleen and kidney viruses, kidney bacteria, and gill/intestinal parasites.
9. Plasma vitelogenin level (mg/mL).

Table A-4
1996 Whole Body Composites Analyzed for PCBs

Sample I.D.	Sampling Area and Section[1]	Sex[2]	Body Weight (kg)	Total Length (cm)	Reporting I.D.	Analysis Requested		Total PCB[3]	
						Base 106 Congeners	Coplanar Congeners	mg/kg ww	µg/g lipid
wefr23wf	Area I, Section 1	U	0.4	38	wefr01cp	✓		5.40	59.71
wefr25wf	Area I, Section 1	U	0.5	38					
wefr19wf	Area I, Section 1	U	0.55	40					
wefr15wf	Area I, Section 1	U	0.7	45					
wefr20wf	Area I, Section 1	U	0.80	45					
wefr18wf	Area I, Section 1	U	0.85	44	wefr02cp	✓		5.30	58.48
wefr24wf	Area I, Section 1	U	1.00	46					
wefr22wf	Area I, Section 1	U	1.15	49					
wefr21wf	Area I, Section 2	U	0.55	39	wefr03cp	✓		6.45	53.36
wefr17wf	Area I, Section 2	U	0.70	42					
wefr12wf	Area I, Section 2	U	0.75	43					
wefr14wf	Area I, Section 2	U	0.75	42	wefr04cp	✓		3.36	19.07
wefr13wf	Area I, Section 2	U	0.80	44					
wefr26wf	Area I, Section 2	U	0.85	46					
wefr16wf	Area I, Section 2	U	0.85	46					
wefr44wf	Area I, Section 3	U	0.48	37	wefr05cp	✓		5.45	44.61
wefr36wf	Area I, Section 3	U	0.56	41					
wefr35wf	Area I, Section 3	U	0.56	41					
wefr37wf	Area I, Section 3	U	0.82	45					
wefr43wf	Area I, Section 3	U	0.94	45					
wefr38wf	Area I, Section 4	U	1.24	50	wefr06cp	✓		5.83	48.44
wefr41wf	Area I, Section 4	U	1.30	50					
wefr42wf	Area I, Section 4	U	1.76	55					
wefr34wf	Area I, Section 4	U	1.82	53	wefr07cp	✓	✓	10.47	48.91
wefr39wf	Area I, Section 4	U	2.24	60					
wefr40wf	Area I, Section 4	U	2.88	67					
welg04wf	Area IIB, Section 3	M	1.18	49	welg01cp	✓		6.21	23.87
welg01wf	Area IIB, Section 3	F	1.56	55					
welg03wf	Area IIB, Section 3	F	1.94	57					
welg02wf	Area IIB, Section 3	F	2.12	58					

Table A-4 (cont.)
1996 Whole Body Composites Analyzed for PCBs

Sample I.D.	Sampling Area and Section[1]	Sex[2]	Body Weight (kg)	Total Length (cm)	Reporting I.D.	Analysis Requested		Total PCB[3]	
						Base 106 Congeners	Coplanar Congeners	mg/kg ww	µg/g lipid
welg07wf	Area IIB, Section 3	U	0.48	40	welg02cp	✓		2.60	21.27
welg15wf	Area IIB, Section 3	U	0.68	41					
welg23wf	Area IIB, Section 3	U	0.72	42					
welg08wf	Area IIB, Section 3	U	0.78	45					
welg18wf	Area IIB, Section 3	U	0.82	44					
welg11wf	Area IIB, Section 3	U	0.82	43	welg03cp	✓		3.93	29.48
welg06wf	Area IIB, Section 3	U	0.84	43					
welg27wf	Area IIB, Section 3	U	0.96	45					
welg21wf	Area IIB, Section 3	U	1.06	46					
welg12wf	Area IIB, Section 3	U	1.10	47					
welg16wf	Area IIB, Section 3	U	1.30	48	welg04cp	✓		4.82	30.96
welg24wf	Area IIB, Section 3	U	1.48	51					
welg25wf	Area IIB, Section 3	U	1.50	53					
welg28wf	Area IIB, Section 3	U	1.66	53					
welg29wf	Area IIB, Section 3	U	1.74	54					
welg19wf	Area IIB, Section 3	U	1.74	54	welg05cp	✓		5.48	35.40
welg05wf	Area IIB, Section 3	U	1.82	56					
welg13wf	Area IIB, Section 3	U	1.84	54					
welg09wf	Area IIB, Section 3	U	2.02	56					
welg10wf	Area IIB, Section 3	U	2.12	58					
welg20wf	Area IIB, Section 3	U	2.16	57	welg06cp	✓	✓	11.06	62.90
welg26wf	Area IIB, Section 3	U	2.44	61					
welg14wf	Area IIB, Section 3	U	2.80	65					
welg17wf	Area IIB, Section 3	U	4.30	70					
wewg02wf	Area IIIA, Section 3	M	1.52	52	wewg01cp	✓		4.34	28.16
wewg01wf	Area IIIA, Section 3	F	1.74	58					
wewg04wf	Area IIIA, Section 3	F	1.78	57					
wewg03wf	Area IIIA, Section 3	F	2.14	60					
wewg05wf	Area IIIA, Section 3	U	0.84	45	wewg02cp	✓		5.31	40.51
wewg08wf	Area IIIA, Section 3	U	0.96	45					
wewg07wf	Area IIIA, Section 3	U	1.00	44					
wewg11wf	Area IIIA, Section 3	U	1.30	51					
wewg14wf	Area IIIA, Section 3	U	1.46	52					

Table A-4 (cont.)
1996 Whole Body Composites Analyzed for PCBs

Sample I.D.	Sampling Area and Section[1]	Sex[2]	Body Weight (kg)	Total Length (cm)	Reporting I.D.	Analysis Requested		Total PCB[3]	
						Base 106 Congeners	Coplanar Congeners	mg/kg ww	µg/g lipid
wewg20wf	Area IIIA, Section 3	U	1.58	55	wewg03cp	✓		4.78	29.09
wewg16wf	Area IIIA, Section 3	U	1.64	53					
wewg15wf	Area IIIA, Section 3	U	1.74	59					
wewg13wf	Area IIIA, Section 3	U	1.88	57					
wewg19wf	Area IIIA, Section 3	U	1.96	56					
wewg12wf	Area IIIA, Section 3	U	1.98	57	wewg04cp	✓	✓	3.88	25.53
wewg18wf	Area IIIA, Section 3	U	2.06	57					
wewg06wf	Area IIIA, Section 3	U	2.23	58					
wewg17wf	Area IIIA, Section 3	U	2.44	60					
wewg10wf	Area IIIA, Section 3	U	2.48	63					
wewg09wf	Area IIIA, Section 3	U	2.92	63					
weeg04wf	Area IIIB, Section 2	U	1.50	51	weeg01cp	✓		7.78	61.09
weeg03wf	Area IIIB, Section 2	U	2.00	58					
weeg02wf	Area IIIB, Section 2	U	2.50	60					
weeg01wf	Area IIIB, Section 2	U	2.62	60					
weeg30wf	Area IIIB, Section 2	U	0.54	39	weeg02cp	✓		4.46	29.17
weeg29wf	Area IIIB, Section 2	U	0.58	37					
weeg39wf	Area IIIB, Section 2	U	1.18	47					
weeg35wf	Area IIIB, Section 2	U	1.18	47					
weeg37wf	Area IIIB, Section 2	U	1.34	50					
weeg24wf	Area IIIB, Section 2	U	1.36	50	weeg03cp	✓		8.36	51.7
weeg28wf	Area IIIB, Section 2	U	1.42	52					
weeg17wf	Area IIIB, Section 2	U	1.44	51					
weeg33wf	Area IIIB, Section 2	U	1.48	50					
weeg08wf	Area IIIB, Section 2	U	1.60	52					
weeg42wf	Area IIIB, Section 2	U	1.62	52	weeg04cp	✓		6.46	36.54
weeg27wf	Area IIIB, Section 2	U	1.68	52					
weeg41wf	Area IIIB, Section 2	U	1.68	52					
weeg13wf	Area IIIB, Section 2	U	1.76	52					
weeg49wf	Area IIIB, Section 2	U	1.78	56					

Table A-4 (cont.)
1996 Whole Body Composites Analyzed for PCBs

Sample I.D.	Sampling Area and Section[1]	Sex[2]	Body Weight (kg)	Total Length (cm)	Reporting I.D.	Analysis Requested		Total PCB[3]	
						Base 106 Congeners	Coplanar Congeners	mg/kg ww	µg/g lipid
weeg05wf	Area IIIB, Section 2	U	1.84	53	weeg05cp	✓		8.08	43.34
weeg22wf	Area IIIB, Section 2	U	1.86	53					
weeg10wf	Area IIIB, Section 2	U	1.86	44					
weeg15wf	Area IIIB, Section 2	U	1.92	54					
weeg16wf	Area IIIB, Section 2	U	1.92	57					
weeg48wf	Area IIIB, Section 2	U	1.94	53	weeg06cp	✓		7.52	40.91
weeg44wf	Area IIIB, Section 2	U	1.96	57					
weeg45wf	Area IIIB, Section 2	U	2.00	54					
weeg11wf	Area IIIB, Section 2	U	2.04	58					
weeg34wf	Area IIIB, Section 2	U	2.14	56					
weeg46wf	Area IIIB, Section 2	U	0.00	55	weeg07cp	✓		11.77	85.18
weeg14wf	Area IIIB, Section 2	U	2.14	57					
weeg52wf	Area IIIB, Section 2	U	2.20	58					
weeg38wf	Area IIIB, Section 2	U	2.20	60					
weeg12wf	Area IIIB, Section 2	U	2.24	58					
weeg09wf	Area IIIB, Section 2	U	2.34	57					
weeg21wf	Area IIIB, Section 2	U	2.34	57	weeg08cp	✓		9.64	52.72
weeg53wf	Area IIIB, Section 2	U	2.35	57					
weeg23wf	Area IIIB, Section 2	U	2.40	58					
weeg43wf	Area IIIB, Section 2	U	2.52	58					
weeg54wf	Area IIIB, Section 2	U	2.54	59					
weeg20wf	Area IIIB, Section 2	U	2.54	62	weeg09cp	✓	✓	7.08	37.64
weeg06wf	Area IIIB, Section 2	U	2.62	59					
weeg18wf	Area IIIB, Section 2	U	2.68	64					
weeg51wf	Area IIIB, Section 2	U	2.88	60					
weeg40wf	Area IIIB, Section 2	U	2.96	64					
weeg25wf	Area IIIB, Section 2	U	3.02	63	weeg10cp	✓		17.83	100.3
weeg31wf	Area IIIB, Section 2	U	3.14	61					
weeg07wf	Area IIIB, Section 2	U	3.36	66					
weeg26wf	Area IIIB, Section 2	U	3.40	67					
weeg50wf	Area IIIB, Section 2	U	3.40	68					

Table A-4 (cont.)
1996 Whole Body Composites Analyzed for PCBs

Sample I.D.	Sampling Area and Section[1]	Sex[2]	Body Weight (kg)	Total Length (cm)	Reporting I.D.	Analysis Requested		Total PCB[3]	
						Base 106 Congeners	Coplanar Congeners	mg/kg ww	µg/g lipid
weeg32wf	Area IIIB, Section 2	U	3.40	66	weeg11cp	✓		5.48	43.41
weeg47wf	Area IIIB, Section 2	U	3.54	68					
weeg36wf	Area IIIB, Section 2	U	3.68	66					
weeg19wf	Area IIIB, Section 2	U	3.88	68					
weug01wf	Area IV, Section 1	F	2.30	59	weug01cp	✓		5.65	39.37
weug02wf	Area IV, Section 1	F	2.38	61					
weug04wf	Area IV, Section 1	M	2.88	60					
weug03wf	Area IV, Section 1	M	3.44	66					
weug10wf	Area IV, Section 1	U	1.92	57	weug02cp	✓	✓	4.61	26.28
weug05wf	Area IV, Section 1	U	2.06	58					
weug08wf	Area IV, Section 1	U	2.34	62					
weug09wf	Area IV, Section 1	U	2.52	54	weug03cp	✓		7.26	34.26
weug07wf	Area IV, Section 1	U	4.54	72					
weug06wf	Area IV, Section 1	U	5.22	72					

1. Sampling areas corresponding to fish collection locations used in the Green Bay Mass Balance Study (Connolly et al., 1992).
2. F: female; M: male; U: undetermined.
3. Sum of congeners, excluding the reported value for PCB 85.