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Biochemical and toxicopathic biomarkers assessed in smallmouth bass recovered from a polychlorinated biphenyl-contaminated river

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Smallmouth bass (*Micropterus dolomieu*) were collected to quantify the nature and prevalence of biomarker responses, including biochemical indices, toxicopathic lesions and general health indices, among fish collected from polychlorinated biphenyl (PCB)-contaminated and nearby uncontaminated reaches of the Kalamazoo River, Michigan, USA. Blood and tissue samples (gill, liver, spleen, head kidney, trunk kidney, thyroid and gonads) were collected and preserved at necropsy for biochemical and histological analyses. The body condition factor and liver somatic index were significantly lower in fish collected from the downstream, contaminated site. Plasma vitellogenin was not detected in male fish collected from either site. Liver ethoxyresorufin-*O*-deethylase activity and liver and spleen superoxide dismutase activity were significantly depressed in fish collected from the downstream site. Significant toxicopathic lesions such as glycogen depletion, enhanced macrophage aggregates, hepatic foci of cellular alteration (i.e. preneoplastic lesions) and neoplasia were also detected in the liver of fish collected from the downstream site. This study indicates that many of the biochemical and histopathological biomarker responses were associated with liver and body tissue PCB concentrations. Taken together, the biomarkers of exposure and effect strongly suggest that fish within the downstream site are adversely affected by PCBs and other chemical stressors.

Keywords: ethoxyresorufin-O-deethylase, histopathology, superoxide dismutase, vitellogenin, smallmouth bass.

Introduction

Fisheries-related contaminant monitoring programmes commonly include only an assessment of chemical residues in fish tissues. Although tissue residues provide important information for resource managers concerning the protection of human health from consumption of potentially harmful xenobiotics, they do not necessarily provide a clear understanding of the impacts of chemical exposure on fish health. Integrated biomarker studies have shown promise as an assessment tool

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for evaluating the impact of contaminants on fisheries resources (Adams 1990, Anderson *et al*. 1997, Teh *et al*. 1997). We investigated the health of smallmouth bass (*Micropterus dolomieu*), an important predatory and sport fishery species, collected from the Kalamazoo River, Michigan, USA, using a suite of biomarkers of exposure and effect that evaluate organochlorine contaminant impacts on fish (Anderson *et al*. 1997).

The Kalamazoo River drainage basin encompasses approximately 5180 km^2 (2000 square miles) (Figure 1). The main stem of the river is approximately 195 km^2 (120 miles) long and flows from the town of Albion, Michigan, to Lake Michigan near the city of Saugatuck. Within and near the city of Kalamazoo, several pulp and paper mills release polychlorinated biphenyls (PCBs) into the Kalamazoo River through the discharge of wastes produced during the de-inking and/or repulping of recycled carbonless copy paper. Since the early 1900s these mills have been used primarily for pulp recycling (Blasland & Bouck Engineers., 1992), so other chlorinated compounds such as dioxins and furans are not an environmental issue here as they are at other pulp and paper mill sites where the bleached kraft process is used on virgin pulp.

PCBs were the contaminant of primary interest in this study because of their distribution and high concentration within the sediments of the study area (Blasland, Bouck & Lee 2000). PCBs are biomagnified within the aquatic food chain because of their lipophilic (log octanol–water partitioning coefficient [log K_{ow}] > 4) and chemically persistent nature (Barron 1990, Rasmussen *et al*. 1990). PCB exposure in fish has been associated with immune system (Zelikoff 1994, Rice and Schlenk 1995, Duffy *et al*. 2002, 2003), antioxidant (Otto *et al*. 1996) and hormone modulation (Anderson *et al*. 1996, Niimi 1996), histopathological lesions

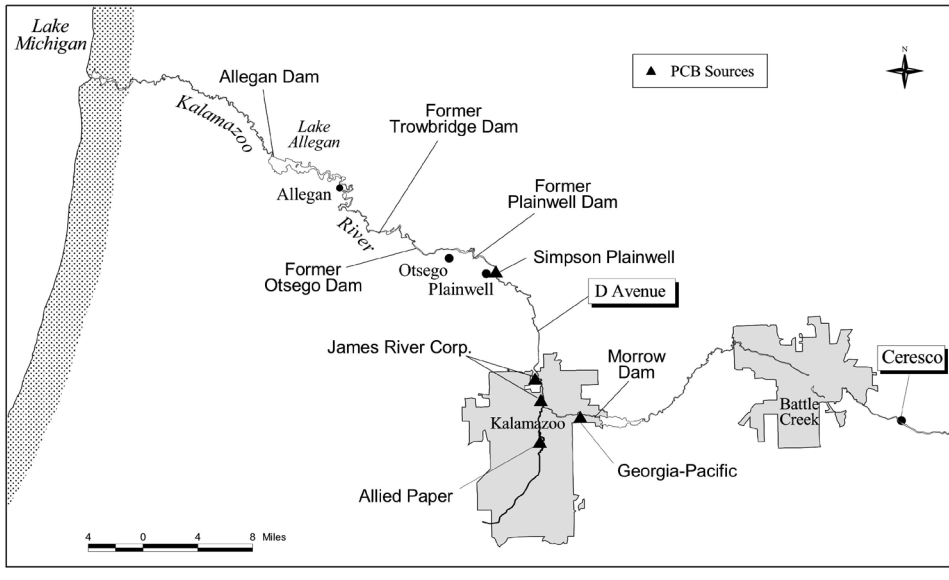

Figure 1. The two sampling areas for smallmouth bass on the Kalamazoo River, located at Ceresco and D Avenue, and associated sources of PCB contamination.

(Niimi 1996, Teh *et al*. 1997, Barron *et al*. 2000), and reproductive and developmental impairments (Niimi 1996, Walker *et al*. 1996).

In this field study we evaluated the health of fish in a PCB-contaminated river using a suite of biomarkers of exposure and effect. We described and evaluated multiple biomarker responses in smallmouth bass and their association with PCB residues in tissue.

Materials and methods

Sampling locations and fish collection

Smallmouth bass were collected over a 4-day period from two sites on the Kalamazoo River in September 1995. The upstream site was located near the city of Ceresco and the downstream site was a reach of the river downstream of the city of Kalamazoo near D Avenue (Figure 1). The upstream site served as a reference area for the assessment of biomarker status because sediments of the downstream site were known to be contaminated with PCBs, while sediments of the upstream site were known to be relatively uncontaminated by PCBs (Blasland, Bouck & Lee 2000). The presence of a dam at Morrow Lake (Figure 1) prevents fish from migrating from the more contaminated site to the upstream site, but downstream migration over the dam is not precluded.

Fish were collected from a boat equipped with electroshocking apparatus using pulsed direct current (4A, 30V, 120pulses s^{-1}) to temporarily immobilize the fish. The fish were then dip-netted and placed in a live well at a density of one fish per 8l of water; the water was renewed every 15–30min. Fifteen fish were captured over 90min intervals at each sampling location and transferred to an in-river hoop net and held for approximately 14–20h before blood collection and necropsy. The water temperature at each sampling location was approximately 20°C.

To minimize age-related variance in measurements, adult fish were targeted for capture (>240mm in total length; Scott and Crossman 1973). A total of 30 fish were retained and examined in the biomarker analyses, all of which were aged 3–5 years.

Weight, length and age determination, and sampling procedures

Fish were euthanized with 200mg l^{-1} phosphate buffered tricaine methane sulphonate (MS222), measured for total length (to the nearest 1.0mm) and weighed (to the nearest 0.1g), and 10–15 scales beneath the dorsal fins and below the lateral line were removed for age determination. External observations, including body shape and appearance, gill structure and colour, presence of external parasites (located on the gill or outer body), and gross morphological superficial alterations, were then recorded. After this examination, tissue samples were collected as described below. To prevent cross-contamination between fish, any tools used on more than one fish were cleaned between uses by sequential rinses with acetone, hexane and deionized water.

Blood sampling

Blood was obtained by caudal venipuncture using a 10cc tuberculin syringe equipped with a 22 gauge needle, and was stored on ice at 4°C in heparinized Vacutainer™ tubes before centrifugation. To prevent protein degradation, an aprotinin solution (Sigma Chemical Company, St Louis, Missouri, USA) containing 5–10 trypsin inhibitor units was added to each 2ml of blood. Plasma was prepared in the field 6–8h following blood withdrawal by centrifuging whole blood for 10min at $\sim 1000g$. Aliquots of the resultant plasma were immediately frozen on dry ice.

Tissue sampling

Tissues were removed from each fish in the following order: gill, gonad, spleen, liver, gastrointestinal tract, kidney, thyroid and skeletal muscle. All tissues for histopathology were fixed in 10 volumes of 10% buffered formalin. After removal, the gonads, spleen and liver were blotted dry with Kimwipes™ and weighed (to the nearest 0.1g). Care was taken to collect tissue samples from similar locations within each organ. For example, two sections of gill (second and third right-side gill arches) and three transverse slices of gonads representing the cranial, middle and caudal thirds were collected; spleens were divided in half for morphological analysis and biochemical evaluation. Livers were first trimmed of attached mesentery and gall bladder, and then apportioned for biochemical, histopathological or PCB congener analysis. The subsections of liver for PCB analysis were place in precleaned, certified glass jars. The thyroid gland was removed with the attached gill arch and ventral aorta. Transverse slices were taken from the midpoint of the stomach, head kidney and trunk kidney, and fixed. Lateral skeletal musculature

with dermis and epidermis attached (i.e. skin-on fillet) was removed, wrapped in aluminium foil, placed in a coded plastic bag, and frozen for total PCB analysis. Tissues collected for biochemical and contaminant analysis were immediately placed on dry ice and frozen. All samples were shipped by overnight courier under chain-of-custody to the appropriate laboratory. On arrival, samples were preserved at -80°C until analysis. Histopathology samples were maintained at room temperature in fixative until embedding with paraffin and subsequent processing took place. Contaminant analysis samples were stored at -20°C until analysis.

Liver and fillet PCB congener and total PCB analysis

Livers from each fish were analysed for 18 ortho-substituted congeners (PCB 8, 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 170, 180, 187, 195, 206 and 209) using gas chromatography and electron capture detection. Samples were quantified using a DB-5 capillary column; data were acquired simultaneously from a second, DB-17 column but not used for quantification. Calibration solutions containing the 18 congeners and internal standards were used. Analytical detection limits ranged from 0.016–0.96ng g^{-1} dry weight (congeners 180 and 170, respectively). Livers were also analysed for five non-ortho-substituted PCB congeners (PCB 37, 77, 81, 126 and 169) using the same analytical methods on tissue extracts that had been subjected to a carbon column clean-up step. Skin-on fillet samples were analysed for PCB Aroclors using the methods of Webb and McCall (1973) for Aroclor quantification and activated silica gel for clean-up.

Percentage moisture and lipid content were determined in all samples. PCB concentration results were expressed on both a wet weight and a lipid-normalized basis. Total PCBs in liver samples were expressed as the sum concentration of the measured congeners (sum PCB congeners). Quality assurance and quality control procedures included the use of procedural blanks, blank spikes, instrument blanks, certified reference material and duplicate samples.

Biochemical analyses

Plasma vitellogenin. Plasma was assessed for levels of vitellogenin, a marker of oestrogenic effects in male and female fish. Vitellogenin was quantified using a monoclonal fish antisera (monoclonal antibody 3G2; HL 1393) and assayed using a capture enzyme-linked immunosorbent assay (ELISA). The vitellogenin assay procedure is described in detail by Folmar *et al*. (1996).

*Hepatic ethoxyresorufin-*O*-deethylase activity and semiquantitative analysis of CYP1A protein.* Ethoxyresorufin-*O*-deethylase (EROD) activity was determined fluorimetrically using methods described by Burke *et al*. (1985) and adapted for a microplate format (Anderson *et al*. 1996). Microsomes were prepared from frozen liver samples. A seven-point linear standard concentration curve was constructed (three replicates per concentration), and relative intensity units were converted to picomoles of resorufin. The concentration of resorufin in each well was plotted against time to observe any deviation from linearity of the reaction. A linear regression was then performed on the data from each well (three replicates per sample) to determine EROD activity (pmol min^{-1}). The amount of microsomal protein in each sample, determined using the BioRad DC Protein Assay kit (Richmond, California, USA), was used to normalize the EROD activity for that sample.

Semiquantitative analysis of liver CYP1A protein content was conducted by ELISA as described by Goksøyr (1991) and modified by Anderson *et al*. (1996). Microsomal protein from each sample was diluted in 50mM Na HCO_3 buffer, pH9.8, to coat each ELISA microtitre plate well (three replicates per sample) with 0.5μg protein. Non-specific antibody binding was then blocked by coating wells with 0.5% non-fat dry milk in phosphate buffered saline. Primary monoclonal anti-scup CYP1A1 (monoclonal antibody 1-12-3), diluted to a concentration of 10μg ml^{-1}, was applied to each well. Primary antibody binding was assessed by secondary horseradish peroxidase conjugated anti-mouse antibody (Anderson *et al*. 1996). Peroxidase substrate (3,3′,5,5′-tetramethylbenzidine) was added to each well, and after 5–10min the reaction was quenched with H_2SO_4 and the absorbance read at 450nm using a microtitre plate reader.

Hepatic and spleen superoxide dismutase activity. Subsections of liver and spleen were shipped on dry ice to the Nelson Institute of Environmental Medicine, New York University School of Medicine, Tuxedo, New York, USA, in a buffer specially prepared to minimize degradation of enzyme activity containing 50mM Tris and 0.001M ethylene diamine tetra-acetic acid (EDTA). The pH of the buffer was adjusted to 7.6 with either NaOH or HCl and, following filtration through a 0.2μm disposable filter, 10% glycerol was added. Just prior to the addition of the intact organ, 0.5ml dithiothreitol was added to the buffer.

Superoxide dismutase (SOD) activity was measured using the method described by Zelikoff *et al*. (1996). Cells (1×10^6), recovered by passing subsections of each organ through a wire mesh, were suspended in 0.1ml lysing solution (0.08% digitonin combined with 2mM EDTA, pH7.8) and incubated for 30min at $25 \pm 2^{\circ}$C. Lysis was confirmed microscopically. The cell lysate was then centrifuged at 250*g* for 10min at 4°C and the supernatant collected and diluted 1:5 with lysing solution. Enzymatic activity in the supernatant was measured by adding to solution A (9.8ml) and to 0.1ml of solution B (i) 0.1ml of double-distilled water to determine the maximal rate of autoxidation of pyrogallol;

(ii)0.1ml of a known SOD concentration to generate the standard curve; or (iii) 0.1ml of an unknown sample. Both solutions A and B were used within 30min of enrichment by or depletion of air, respectively. Aliquots (100μl) were then transferred to a cuvette and their absorbency measured for 6min at 420nm in a DU 650 spectrophotometer with kinetic capabilities. A standard curve, prepared each day for a given batch of tissue samples, was generated from a set of known bovine SOD (Sigma) concentrations and was used to derive the final SOD concentrations in each test sample. Five calibration points were used to calculate the standard curve, ranging between 0 and 15μg ml^{-1}; the correlation coefficient (r^2) was generally 0.98. Bovine SOD used for generating the standard curve was prepared by diluting a 1mg ml^{-1} stock solution in sterile, double-distilled water to yield a final working concentration between 0.5 and 15μg ml^{-1}. A typical slope of 0.02 optical density units min^{-1} was observed when no SOD was added. All of the generated values fell within the standard curve and thus extrapolation was not necessary.

Histopathology

Tissue sections were processed as previously described by Hinton and Lauren (1990). Paraffin blocks were sectioned at 5–7μm, mounted on glass slides, and then stained with haematoxylin and eosin. Fish were sexed based on gonadal structure. All tissue sections were screened and subjected to detailed, semiquantitative histopathological analyses. Data were scored from 0 to 3 depending on lesion severity. Because of the importance of numbers of foci of cellular alteration (FCAs) and hepatic tumours (HTs) in the progression of fish hepatocarcinogenesis, these lesions were counted rather than scored for severity. FCA and HT data were reported both as prevalence (the number of fish with a particular lesion) and as the number of FCAs or HTs per liver section.

Statistical analysis

Site comparisons for all endpoints that were continuous variables, including total and individual liver PCB congener concentrations, EROD activity, CYP1A protein, SOD activity, organosomatic indices and condition factor, were assessed using the Mann–Whitney test, and correlations among continuous variables were assessed using Spearman's rho (Conover 1980). PCB concentrations that were reported as below detection limits were transformed to one-half the detection limit prior to statistical analysis. Site comparisons of histological features that were expressed as categorical or semi-quantitative categories (i.e. lesion scores) were assessed as contingency tables using Fisher's exact test (Conover 1980). Statistical calculations were performed using S-PLUS (Insightful, Inc., Seattle, Washington, USA).

Results

Fillet and liver PCBs

PCBs were present in both upstream (non-contaminated) and downstream (contaminated) fish. However, mean concentrations of liver and fillet total PCBs in downstream fish were significantly ($p < 0.001$) elevated compared with the upstream fish (Table 1), as were individual PCB congener concentrations in liver (Table 2). Coplanar PCBs, including PCBs 77, 81, 126 and 169, were not detected in livers from either location (Table 2). Non-coplanar PCBs, including PCBs 18 and 28, were not detected in upstream fish livers, and PCBs 8, 195 and 209 were

Table 1. Total PCB concentrations in liver and fillet of upstream and downstream smallmouth bass.

	Upstream	Downstream
Liver concentration[a] (mg kg^{-1} wet weight)	0.26 ± 0.10 ($n = 10$)	2.19 ± 1.65** ($n = 9$)
Fillet concentration[b] (mg kg^{-1} wet weight)	0.09 ± 0.04 ($n = 15$)	1.01 ± 0.43** ($n = 15$)

Values represent mean ± SD. Fewer numbers of liver samples were obtained for PCB analysis than for fillet because the liver mass was sometimes insufficient for both biomarker and PCB analyses.

[a] Liver total PCBs estimated using the sum of congeners method (see Materials and methods).

[b] Skin-on fillet total PCBs estimated using the Aroclor method (see Materials and methods).

** Downstream liver and fillet total PCB concentrations significantly ($p < 0.001$) greater than upstream concentrations.

Table 2. Individual PCB congener concentrations in liver of upstream and downstream smallmouth bass.

PCB congener	Upstream ($n = 10$) (μg kg^{-1} wet weight)	Downstream ($n = 9$) (μg kg^{-1} wet weight)
8	39 ± 19.9	13.6 ± 3.2
18	< 58.8 ± 23	41.3 ± 12.2
28	< 39.8 ± 15.6	101.4 ± 39.4**
44	8.6 ± 8.6	83.9 ± 43.2**
52	8.6 ± 9.4	197.7 ± 126.5**
66	7.36 ± 8.2	137.4 ± 103**
77	< 2.1 ± 0.8	< 3.4 ± 1.6
81	< 2.1 ± 0.8	< 3.4 ± 1.6
101	19.4 ± 8.7	314.4 ± 247.1**
105	7.8 ± 5.5	60.3 ± 50.2**
118	15.3 ± 7.7	248 ± 205**
126	< 2.1 ± 0.8	< 3.4 ± 1.6
128	9.2 ± 11.5	59 ± 55.6**
138	32.4 ± 22.5	334.4 ± 284**
153	25.9 ± 15	338.9 ± 289.4**
169	< 0.8 ± 0.3	< 1.3 ± 0.6
170	4.1 ± 2.5	44 ± 41.0**
180	16.3 ± 8.3	103 ± 99.5**
187	12.4 ± 12.37	64.1 ± 61.1**
195	17.3 ± 12.4	6.2 ± 5.7
206	7.2 ± 8.3	8.3 ± 9.2
209	37.7 ± 19.4	56.5 ± 35.2

Values represent mean ± SD. Values qualified by < indicate that the congener was not detected (i.e. its concentration was below the detection limit).

** Congener concentration significantly greater in downstream fish livers ($p < 0.001$).

detected infrequently (< 30%) in upstream fish livers. PCB congener 209 was also detected infrequently in downstream fish livers. When pooled downstream and upstream fish PCB data were lipid-normalized, liver PCB sum congener concentrations were positively correlated with fillet total PCB concentrations ($p < 0.001$, rho = 0.85).

General indices

Table 3 shows the length, weight and age of fish collected from the upstream reference and downstream assessment sites. No overtly visible abnormalities (i.e. of the fins, body or eyes) or lesions were observed in fish from either site. Mean spleen and gonad weights given as a percentage of body weight (organosomatic indices) were not significantly different between the downstream and the upstream fish (Table 3). The mean liver organosomatic index was significantly lower ($p < 0.05$) in downstream than in upstream fish. This finding was associated with a trend in lower liver weights in downstream fish, but the difference in weight was not statistically significant (mean ± SD liver weight in downstream fish was 3.98 ± 2.83g versus 4.95 ± 3.07g in upstream fish, $p = 0.49$). Liver organosomatic indices in fish combined from both sampling locations were negatively correlated with wet weight liver sum congener concentrations ($p = 0.05$, rho = −0.45) and lipid-normalized fillet total PCB concentrations ($p < 0.01$, rho = −0.50). The condition factor, that is body weight/length^3, also was significantly lower ($p < 0.001$) in fish collected from the downstream site (Table 3). The condition factor in fish combined from

Table 3. General indices and condition factor in upstream and downstream smallmouth bass.

	Upstream	Downstream
Length (cm)	32.3 ± 6.6	34.2 ± 5.1
Weight (g)	514 ± 287	493 ± 292
Gender		
Males	7	8
Females	8	7
Age (years)	3.9 ± 1.9	4.3 ± 1.5
Spleen organosomatic index[a] (%)[3]	0.08 ± 0.03	0.07 ± 0.03
Testis organosomatic index[a] (%)	0.51 ± 0.23	0.43 ± 0.16
Ovary organosomatic index[a] (%)	1.08 ± 0.66	1.15 ± 0.26
Liver organosomatic index[a] (%)	0.96 ± 0.15	0.79 ± 0.20*
Condition factor[b]	0.013 ± 0.001	0.011 ± 0.001*

Values represent mean ± SD, expect for gender, where they represent absolute numbers of fish collected.

[a] Organosomatic index = (organ weight/body weight) × 100.

[b] Condition factor = body weight/length^3.

* Mean liver organosomatic index and condition factor in downstream fish were significantly ($p < 0.05$) less than for upstream fish.

both sampling locations was negatively correlated with wet weight ($p = 0.001$, rho = -0.77) and lipid-normalized liver PCB sum congener concentrations ($p < 0.01$, rho = -0.64). The same relationship was observed for wet weight ($p < 0.02$, rho = -0.46) and lipid-normalized ($p < 0.001$, rho = -0.70) fillet total PCB concentrations.

Biochemical indices

Hepatic EROD activity was not different between sampling locations when both genders were combined (Table 4). However, males from the downstream site showed a significant ($p = 0.003$) reduction in EROD activity compared with upstream males. Comparison of female and male activity revealed a significant ($p = 0.021$) reduction in EROD activity in upstream females compared with upstream males; however, the same difference was not observed in the downstream fish. A trend for increased activity in downstream females versus upstream females was seen (Table 4).

Hepatic EROD activity in males combined from both sampling locations was negatively correlated with liver sum PCB congener concentrations both on a wet weight ($p = 0.022$, rho = -0.76, $n = 10$) and lipid-normalized ($p = 0.035$, rho = -0.70, $n = 10$) basis (Figure 2). Similarly, EROD activity in male livers was negatively correlated with male fillet total PCB concentrations on a wet weight ($p = 0.032$, rho = -0.57, $n = 15$) and lipid-normalized ($p = 0.043$, rho = -0.54, $n = 15$) basis. This relationship was even more pronounced in males when individual liver PCB congeners were evaluated on a lipid-normalized basis ($p \leq 0.029$, rho = -0.72). However, for females combined from both sampling locations, there was no significant correlation between EROD activity and liver sum congener PCB concentrations or fillet total PCB concentrations (Figure 2).

No significant difference in CYP1A protein expression was seen between the sites when data from both genders were combined. Significantly less CYP1A protein expression was seen in females compared with males at the upstream site

Table 4. EROD activity and relative CYP1A protein concentration in livers from male and female upstream and downstream smallmouth bass.

	Upstream						Downstream					
Gender	EROD activity (pmol min^{-1} mg^{-1} protein)			CYP1A concentration (relative absorbance)			EROD activity (pmol min^{-1} mg^{-1} protein)			CYP1A concentration (relative absorbance)		
	n	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range
Male	7	5.07 ± 0.80⋆§	4.00–6.26	7	0.50 ± 0.13§	0.40–0.77	8	2.06 ± 1.342	1.17–4.56	8	0.48 ± 0.30	0.32–1.21
Female	8	2.32 ± 0.94	1.08–3.39	8	0.31 ± 0.05	0.24–0.37	7	7.80 ± 11.62	0.24–30.77	7	0.35 ± 0.11	0.24–0.52

Relative absorbance is expressed in optical density units (see Materials and methods).
⋆ EROD activity significantly ($p < 0.05$) greater in male upstream fish than in male downstream fish.
§ EROD activity and CYP1A protein (relative absorbance) significantly ($p < 0.05$) greater in male upstream fish than in female upstream fish.

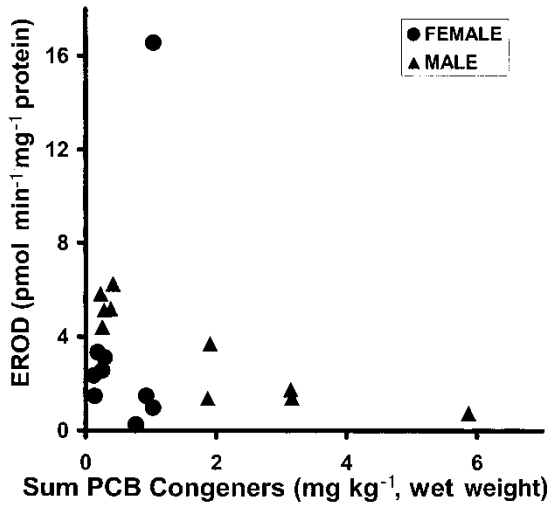

Figure 2. Correlation between male and female EROD activity and total PCBs in liver tissue. Each data point represents the results obtained from a single fish. EROD activity was assessed in a microsomal preparation of liver tissue and was compared with liver sum PCB congener concentrations in the same fish. Liver total PCBs were estimated using the sum of congeners method (see Materials and methods).

($p < 0.01$). No significant difference in CYP1A protein expression between the genders was seen at the downstream site. CYP1A protein expression in males, females or both genders combined was not significantly correlated with liver sum PCB congener concentrations or fillet total PCB concentrations. CYP1A protein expression was positively correlated ($p = 0.003$, rho $= 0.79$, $n = 15$) with EROD activity in upstream fish (both genders combined); however, this relationship was not significant in downstream fish (rho $= 0.13$).

Mean $\pm$SOD activity in spleen (1.87 ± 1.22ng ml^{-1}) and in liver (2.45 ± 1.04ng ml^{-1}) of downstream fish was significantly depressed ($p = 0.001$ and $p = 0.015$, respectively) relative to the same parameters in upstream fish (3.12 ± 1.41ng ml^{-1} and 3.79 ± 0.64ng ml^{-1}, respectively). There were no significant differences in liver or spleen SOD activity between the genders within each sampling location. Spleen SOD activity in fish combined from both sampling locations was negatively correlated with liver sum PCB congener concentrations on a wet weight ($p = 0.016$, rho $= -0.56$) and lipid-normalized ($p = 0.029$, rho $= -0.51$) basis. Spleen SOD activity was negatively correlated with fillet total PCB concentrations only on a lipid-normalized ($p = 0.03$, rho $= -0.40$, $n = 30$) basis. Liver SOD activity in fish combined from both sampling locations was negatively correlated with wet weight ($p = 0.026$, rho $= -0.52$) and lipid-normalized ($p = 0.005$, rho $= -0.66$) liver sum PCB congener concentrations (Figure 3), and both wet weight ($p = 0.002$, rho $= -0.58$) and lipid-normalized ($p = 0.001$, rho $= -0.60$) fillet total PCB concentrations. Similar to EROD activity, relationships between spleen and liver SOD activities and lipid-normalized liver PCB concentrations were more pronounced when individual congeners were evaluated.

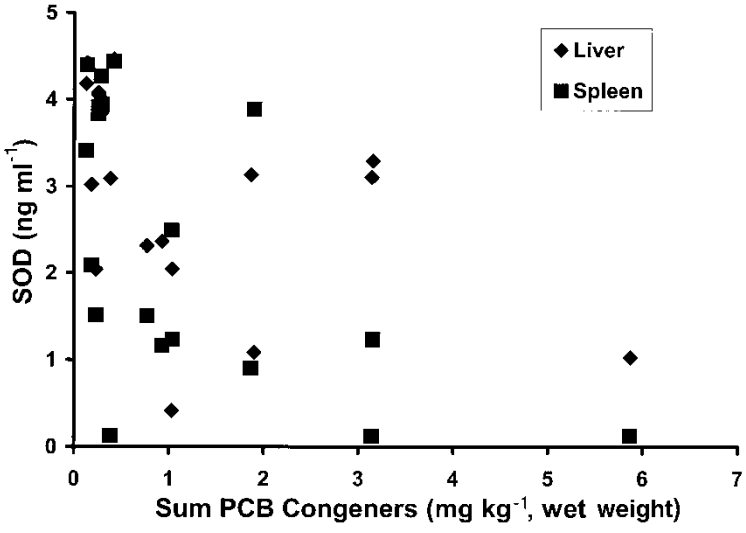

Figure 3. Correlation between SOD activity in liver or spleen (both males and females) and total PCBs in liver tissue. Each data point represents the results obtained from a single fish. SOD activity was assessed in a cytosolic preparation of liver or spleen tissue and was compared with liver sum PCB congener concentrations in the same fish. Liver total PCBs were estimated using the sum of congeners method (see Materials and methods).

Plasma vitellogenin was not detected in males from either sampling location. The mean plasma vitellogenin in upstream females ($454 \pm 435 \mu g\ ml^{-1}$, $n = 7$) was significantly greater ($p < 0.05$) than that of downstream females ($259 \pm 281 \mu g\ ml^{-1}$, $n = 7$). Using plasma vitellogenin levels, gonadosomatic indices and histopathological scoring data for the ovary, it was determined that three of the upstream females that showed no detectable plasma vitellogenin possessed gonadosomatic indices of $< 0.6\%$ and were gonadally regressed (i.e. they contained predominantly immature oocytes). When these three fish were eliminated from the data set, mean plasma vitellogenin concentrations in females combined from both locations were significantly negatively correlated with liver PCB concentration on a lipid basis ($p = 0.035$, $rho = -0.79$, $n = 11$).

Histopathological indices

Histopathological lesions were present in liver, stomach, spleen, kidney, gill, thyroid, ovary and testis of fish collected from both sampling locations. The scoring criteria and lesion descriptions are summarized in Tables 5 and 6. While foreign body granulomas were observed in liver, spleen, kidney and gonads of fish collected from both sampling locations, the distribution of lesion scores indicated a higher frequency ($p < 0.05$) of moderate to severe foreign body granulomas in the organs of upstream fish (Table 7).

Toxicopathic lesions were generally quantified as more severe in the downstream fish (Table 7). Significant differences ($p < 0.05$) in the distribution of lesion scores for liver glycogen depletion and macrophage aggregates were found. FCAs

Table 5. Criteria for scoring histopathological lesions or parameters in liver, stomach, thyroid, spleen, head kidney and trunk kidney.

Organ	Lesion or parameter assessed[a]	Scoring value[b] 1	2	3
Liver	Glycogen depletion	Glycogen vacuoles small, but larger than hepatocyte nuclei	Glycogen vacuoles smaller than hepatocyte nuclei	Glycogen vacuoles absent from the major of hepatocytes
	MAs	1 MA within a × 10 field	2–3 MAs within a × 10 field	> 3 MAs within a × 10 field
	FBGs	< 1 FBG within a × 4 field	2–3 FBGs within a × 4 field	> 3 FBGs within a × 4 field
Stomach	Single cell necrosis	< 5 necrotic cells within a × 40 field	6–10 necrotic cells within a × 40 field	> 10 necrotic cells within a × 40 field
Thyroid	EGLs	< 20 EGLs within a × 40 field	> 20 EGLs within a × 40 field	—
Spleen	Single cell necrosis	< 10 necrotic cells within a × 40 field	11–20 necrotic cells within a × 40 field	> 20 necrotic cells within a × 40 field
	MAs	< 2 MAs within a × 20 field	3–5 MAs within a × 20 field	> 5 MAs within a × 20 field
	FBGs	1 FBG within a × 4 field	2–3 FBGs within a × 4 field	> 3 FBGs within a × 4 field
Head kidney	MAs	< 3 MAs within a × 10 field	4–5 MAs within a × 10 field	> 5 MAs within a × 10 field
	FBGs	1 FBG within a × 4 field	2–4 FBGs within a × 4 field	> 4 FBGs within a × 4 field
Trunk kidney	MAs	< 1 MA within a × 10 field	2–3 MAs within a × 10 field	—
	FBGs	1 FBG within a × 4 field	2–4 FBGs within a × 4 field	—
	PDs	< 1 tubules with PDs within a × 10 field	1–3 tubules with PDs within a × 10 field	> 3 tubules with PDs within a × 10 field

[a] Glycogen depletion characterized by decreased size of hepatocytes, loss of the 'lacy' cytoplasmic vacuolation typical of glycogen, and increased cytoplasmic basophilia; MA, macrophage aggregate, characterized as a cluster of large macrophages packed with coarsely granular yellow-brown pigment; FBG, foreign body granuloma, characterized as a focal accumulation of macrophages, lymphocytes and occasional multinucleated giant cells, clustered around a foreign body (e.g. a parasite); single cell necrosis characterized by cells having eosinophilic cytoplasm with nuclear pyknosis and karyorrhexis; EGLs, eosinophilic granular leukocytes, inflammatory cells characterized by refractile, eosinophilic and cytoplasmic granules; PDs, protein droplets – refractile, brightly eosinophilic, cytoplasmic proteins found in the lining of some renal tubules.

[b] A score value of zero indicates none or infrequently observed. Note: this applies to every lesion or parameter assessed.

Table 6. Criteria for scoring histopathological lesions or parameters in gonads.

Organ	Lesion or parameter assessed[a]	Scoring value[b] 1	2	3
Ovary	MAs	< 2 MAs within a × 4 field	2–4 MAs within a × 4 field	> 4 MAs within a × 4 field
	IMs	< 25% IMs within a × 4 field	25–75% IMs within a × 4 field	> 75% IMs within a × 4 field
Testis	Intersex	< 10 IMs within a × 20 hilar field	10–20 IMs within a × 20 hilar field	> 20 IMs within a × 20 hilar field
	MAs	< 1 MA within a × 10 field	1–3 MAs within a × 10 field	> 3 MAs within a × 10 field
	Sperm production	Small amounts in seminiferous tubules	Seminiferous tubules filled, some accumulation in hilar ducts	Seminiferous tubules and hilar ducts filled
	DM	< 1 DM foci observed within × 40 field	1–3 DM foci observedwithin × 40 field	> 3 DM foci observed within × 40 field

[a] MA, macrophage aggregate, characterized as a clusters of large macrophages packed with coarsely granular yellow-brown pigment; IM, immature oocyte, characterized as a small basophilic oocyte devoid of yolk, lipid or chorion; intersex characterized as the presence of immature oocytes within the seminiferous tubules near the hilus (in some cases immature oocytes were scattered throughout the testicular parenchyma); sperm production was semi-quantitatively estimated by the amount of mature sperm within the seminiferous tubules and hilar ducts within a × 4 field; DM, dysplastic meiosis, an unusual lesion characterized by a variety of different alterations, including degenerative and necrotic spermatogonia, with some or all of the following changes: eosinophilic cytoplasm, granular basophilic cytoplasm, hyperchromatic nuclei, pyknotic nuclei and karyorrhexis.

[b] A score value of zero indicates none or infrequently observed. Note: this applies to every lesion or parameter assessed.

Table 7. Frequency of histopathological lesions or parameter scores at upstream and downstream sampling locations ($n = 14–15$ fish per site).

Organ	Lesion or parameter assessed	Frequency of score value[a]								Fisher p value
		Upstream (reference)				Downstream (assessment)				
		0	1	2	3	0	1	2	3	
Liver	Glycogen depletion	3	4	6	2	3	0	3	9	0.019
	Macrophage aggregates	1	10	4	0	0	4	6	5	0.017
	Foreign body granulomas	NA	3	11	1	NA	10	5	0	0.025
	Neoplasms[b]		0			1				NA
	Foci of cellular alteration[c]		0			3				NA
Stomach	Foci of cellular alteration		0			1				NA
	Single cell necrosis	NA	5	10	0	NA	11	3	1	0.025
Thyroid	Eosinophilic granular leukocytes	0	8	6	1	2	13	0	0	0.005
Spleen	Single cell necrosis	NA	11	3	1	NA	7	4	4	0.280
	Macrophage aggregates	NA	7	4	4	NA	3	8	4	0.257
	Foreign body granulomas	NA	11	2	2	NA	13	2	0	0.533
Head kidney	Macrophage aggregates	1	8	2	3	0	1	9	5	0.006
	Foreign body granulomas	4	3	1	6	12	1	2	0	0.002
Trunk kidney	Macrophage aggregates	NA	2	10	2	NA	0	9	6	0.124
	Foreign body granulomas	NA	5	5	4	NA	13	2	0	0.010
	Protein droplets	9	2	1	2	15	0	0	0	< 0.001
Caudal ovary	Macrophage aggregates	1	5	1	1	0	1	6	0	0.028
	Immature oocytes	NA	3	1	4	NA	1	6	0	0.010
Cranial ovary	Macrophage aggregates	1	5	2	0	0	4	1	2	0.569
	Immature oocytes	NA	3	3	2	NA	2	5	0	0.500
Caudal testis	Intersex	1	2	3	0	0	5	1	2	0.212
	Macrophage aggregates	2	3	1	0	1	4	2	1	1.000
	Sperm production	NA	0	1	5	NA	3	2	3	0.231
	Dysplastic meiosis	2	1	3	0	1	0	3	4	0.180
Cranial testis	Intersex	NA	4	2	1	NA	4	3	1	1.000
	Macrophage aggregates	NA	3	4	0	NA	2	3	3	0.331
	Sperm production	NA	1	1	5	NA	3	3	2	0.212
	Dysplastic meiosis	1	5	0	1	1	1	3	3	0.079

NA, not applicable.

[a] See Tables 5 and 6 for scoring criteria.

[b] Because of the significance of neoplastic lesions, the score is expressed as the number of fish positive for the lesion out of the fish sampled. One assessment fish had a hepatocellular adenoma composed of a mildly pleomorphic population of eosinophilic hepatocytes with large round to oval nuclei. Nuclear pseudoinclusions were common.

[c] Because of the significance of preneoplastic (foci of cellular alteration) lesions, the score is expressed as the number of fish positive for the lesion out of the fish sampled. Foci were characterized as small clusters of hepatocytes distinguished from the adjacent parenchyma by altered staining. Three types of foci were observed: eosinophilic clear cell, amphophilic, and basophilic.

and HTs were observed only in downstream fish. Three fish (two females and one male) possessed liver FCAs, and one male possessed a large hepatocellular adenoma in the liver and multiple foci of glandular hyperplasia in the stomach (Table 7). The distributions of lesion scores for hepatic vascular lesions (i.e. vasculitis and vascular fibrosis) were not significantly different between the sampling locations (data not shown).

Head kidney (i.e. containing haematopoietic and endocrine tissue) and trunk kidney (i.e. primarily excretory tissue) were examined separately. The distribution of lesion scores for head and trunk kidney macrophage aggregates indicated more lesions in downstream fish, but the lesion score distributions were only significantly different ($p < 0.05$) for head kidney. Protein droplets were detected only in trunk kidneys collected from upstream fish (Table 7). The distributions of lesion scores for nephron regeneration, single cell necrosis, glomerulonephritis and intimal thickening of arteries were not significantly different between the sampling locations (data not shown).

Downstream fish possessed slightly higher lesion scores for single cell necrosis and macrophage aggregates (Table 7), but the differences were not significantly different.

Lesion scores for gill showed little or no difference between the sampling locations; these included eosinophilic granular leukocytes, foreign body granulomas, epithelial hyperplasia, mucous cell hyperplasia, cartilage dysplasia and filament fusion. Downstream site fish had slightly higher lesion scores for single cell necrosis in the gill arch and filament epithelia, but these differences were not statistically significant (data not shown).

Lesion scores for eosinophilic granular leukocytes present in thyroid were significantly ($p < 0.05$) higher in upstream fish compared with downstream fish (Table 7). Lesion scores for atrophy and interstitial fibrosis were also higher in upstream fish than in downstream fish, but these differences were not statistically significant (data not shown). Lesion scores for ovary showed that downstream fish had a higher prevalence and severity of macrophage aggregates and a lower severity score for immature oocytes. However, these differences were only statistically significant ($p < 0.05$) in the caudal ovary (Table 7). The higher severity scores for immature oocytes in upstream fish were the result of three fish that were sexually immature (gonadosomatic index $< 0.6\%$). When these fish were removed from the data set, there was no significant difference between the sampling locations for the proportion of immature oocytes estimated in the ovaries. Other lesions, including oocyte atresia and inflammatory foci, were similar between the sampling locations (data not shown).

Macrophage aggregate lesions in the cranial and caudal portions of testes were more prevalent among the downstream fish than the upstream fish, but these differences were not statistically significant. The frequency of intersex (i.e. the appearance of immature oocytes in the testes of male fish) was similar at both sites. Occurrences of testicular lesions characterized by degenerative or necrotic spermatogonia (dysplastic meiosis) tended to be more severe in downstream fish (Table 7), but the differences were not statistically significant.

Discussion

Fish collected from the downstream assessment site showed measurable alterations in biochemical, histopathological, organ and body condition status compared with upstream reference fish. Of the biological indicators assessed in this study, those most affected by the location of fish collection included EROD and SOD activity, plasma vitellogenin, toxicopathic lesions (i.e. macrophage aggregates, FCAs, neoplasms and testicular abnormalities), liver somatic index and body condition factor. A number of the bioindicators were significantly correlated with tissue PCB concentrations. Fish collected from both sampling locations were similar in gender, length, weight and age, suggesting that site differences in biomarker expression were related to other factors, including habitat characteristics (types and availability of food items, water quality parameters, physical disturbance), parasitism, genetics or chemical stressors (PCBs).

Concentrations of total PCBs ranging up to 160mg kg^{-1} dry weight have been measured recently in surface sediments of the Kalamazoo River near the city of Kalamazoo (Blasland, Bouck & Lee 2000). Other contaminants have also been measured in sediments, including volatile organic compounds (methylene chloride and trichloroethene), semivolatile organic compounds (benzo[*a*]anthracene, benzo[*b*]fluoranthene, chrysene, bis(2-ethylhexyl)phthalate, fluoranthene, 4-methylphenol, phenanthrene and pyrene), organochlorine pesticides (dichlorodiphenyldichloroethane [DDD], dichlorodiphenyldichloroethylene [DDE] and dichlorodiphenyltrichloroethane [DDT]) and elevated metals (lead and mercury). However, PCBs are much more widely distributed and occur at much higher concentrations (relative to background) than these other contaminants; because of this, PCBs are the only constituent of concern listed at the site (Blasland, Bouck & Lee 2000).

Of the general organ and body condition indices evaluated, the liver somatic index and body condition factor were significantly lower in downstream fish compared with upstream fish. Both indices were negatively correlated with liver and fillet PCB concentrations. In laboratory studies, PCBs have been shown to cause reductions in fish growth; however, a reduction in growth has been demonstrated in laboratory fish only with tissue burdens of PCBs in excess of 50mg kg^{-1} wet weight (Niimi 1996). Similarly, relatively high exposures to PCBs ($>$ 300mg kg^{-1} daily in the diet for 365days) are required to increase liver size in exposed rainbow trout (*Oncorhynchus mykiss*) (Cleland *et al*. 1988). Differences in food availability or food type between the two sampling sites may have affected these indices and their association with PCBs. For example, Jørgensen *et al*. (1999) showed that PCB accumulation in livers of Arctic charr (*Salvelinus alpinus*) was markedly enhanced in food-deprived fish versus fed fish administered an equivalent dose of PCBs. The authors postulated that these effects were associated with lipid accumulation and mobilization in various tissues. In addition, differences in nutritional status, as assessed by the liver organosomatic index or by body condition factor, may have affected the mobilization of lipids and subsequent accumulation of PCBs in the liver and fillet of all the fish sampled in this study.

Biochemical endpoints evaluated in this study and previously shown to be associated with or modulated by PCBs include liver EROD activity, liver CYP1A

relative protein concentrations, liver and spleen SOD, and plasma vitellogenin concentrations (Anderson *et al*. 1997). Liver EROD activity and CYP1A protein in fish are generally elevated in response to planar halogenated (PHH) and polycyclic aromatic hydrocarbon (PAH) exposure (Stegeman and Hahn 1994). PHHs, including PCBs, have been shown in the laboratory to induce CYP1A protein expression or catalytic function (i.e. EROD activity) in fish (Lech *et al*. 1982, Stegeman and Hahn 1994, Whyte *et al*. 2000) and to inhibit EROD activity (Besselink *et al*. 1998, Whyte *et al*. 2000, Schlezinger and Stegeman 2001). Despite the elevated concentrations of PCBs in fish tissues (Tables 1 and 2), the mean CYP1A protein expression and EROD activity in fish of both genders were not significantly different between the sampling locations. In fact, mean liver EROD activity assessed in upstream and downstream fish was comparable to that of a related fish species, largemouth bass, exposed to clean (non-contaminated) sediments (1.1–3.1pmol min^{-1} mg^{-1} protein; Haasch *et al*. 1993). Reduced induction or non-induction of CYP1A messenger RNA (Yuan *et al*. 2001) and EROD activity (Otto *et al*. 1996, Barron *et al*. 2000, Bello *et al*. 2001) also has been observed in fish collected from other PCB-contaminated waterways.

CYP1 protein expression and catalytic function may be affected by a multitude of non-chemical contaminant-related factors, including fish age, size, gender, reproductive and nutritional status, sampling season, water temperature and sample preparation (Krüner *et al*. 1999, Whyte *et al*. 2000). Apart from nutritional status, the majority of these confounding factors were either controlled for or considered in this study. We found that significant site-related differences in CYP1A protein expression and EROD activity were gender-specific. For example, in the upstream reference fish, relative CYP1A protein levels and EROD activity were higher in male liver than in female liver. Expression of EROD activity is known to be greater in males than in females, particularly sexually mature females (Whyte *et al*. 2000).

The fact that individual liver PCB congeners in male livers and fillets were highly correlated with the reduction in catalytic activity and not the relative CYP1A protein concentration in downstream fish suggests that PCBs may be acting as competitive inhibitors of CYP1A. Similarly, laboratory studies have suggested that PCBs competitively inhibit EROD activity in exposed fish (Boon *et al*. 1992, Besselink *et al*. 1998, Whyte *et al*. 2000, Schlezinger and Stegeman 2001) in an inverted U-shaped concentration-dependent manner (Hahn *et al*. 1993). Unlike in male fish from the downstream site, neither EROD reduction nor inhibition was observed in females. In fact, two of the females from the downstream site showed a marked (10-fold) induction of EROD activity relative to all the other fish sampled. The observed induction of EROD activity in downstream females may be related to the fact that they accumulated significantly less ($p = 0.014$, wet weight basis) EROD-inhibiting liver PCBs than their male counterparts.

SOD is a critical antioxidant enzyme involved in the conversion of superoxide anion to hydrogen peroxide and water. SOD can be elevated in fish exposed to oxidant stress, particularly following exposure to PAHs (Roberts *et al*. 1987, Di Giulio *et al*. 1993, Otto and Moon 1996, McFarland *et al*. 1999). In this study, SOD activity was significantly depressed in liver and spleen of downstream fish

relative to those collected from the upstream site; unlike the change in EROD activity, this depression was significant in both males and females. Tissue concentrations of PCBs were inversely correlated with liver and spleen SOD activity, suggesting that PCBs may impair detoxification (EROD) and antioxidant (SOD) responses in fish, as reported by Otto *et al*. (1996). These effects may be expected to have negative consequences for fish, including the accumulation of PCBs in tissue and the lessened ability to detoxify contaminant stressors such as PAHs that may be metabolized to reactive oxygen species. These may cause DNA damage, tissue necrosis and possibly cancer (Stegeman and Hahn 1994). Nevertheless, additional studies are necessary to elucidate a cause–effect relationship between PCBs and the impairment of detoxification or antioxidant enzymes. It is also possible that other contaminants that covary with sediment PCB contamination, such as heavy metals, PAHs and organochlorine pesticides, and were not directly evaluated in this study may be at least in part responsible for the enzyme modulatory effects observed (Whyte *et al*. 2000).

Induction of vitellogenin, the egg-yolk precursor protein, in male or juvenile fish, has been used as an indicator of oestrogenic effects in fish (Sumpter and Jobling 1995). Some PCB congeners have potentially oestrogenic (Ramamoorthy *et al*. 1997) and anti-oestrogenic (Anderson *et al*. 1996) properties. In this study we found no induction of vitellogenin in the plasma of male fish collected from the upstream or downstream sites, suggesting that potential xenoestrogen-containing substances, including PCBs, alkylphenols (Sumpter and Jobling 1995) and sewage wastewater (Folmar *et al*. 1996), were not of sufficient concentrations in the Kalamazoo River system to elicit vitellogenin production in the males of this species.

In females of equivalent reproductive status from both sampling locations (i.e. the majority of the ovary contains a similar mixture of vitellogenic and pre-vitellogenic oocytes and the ovaries are of a similar size relative to body weight), plasma vitellogenin concentrations were significantly lower in downstream fish and were negatively correlated with PCB tissue concentrations. These findings suggest potential anti-oestrogenic influences on fish from the downstream site. Chen *et al*. (1986) and Anderson *et al*. (1996) showed that PCB mixtures or individual congeners can have anti-oestrogenic activity in fish by reducing the ability of the liver to synthesize vitellogenin in response to 17β-oestradiol. Similarly, Garcia *et al*. (1997) reported that largemouth bass (*Micropterus salmoides*) environmentally exposed to PCBs in a contaminated reservoir showed lower oestrogen receptor binding capacity than fish from a relatively uncontaminated upstream location.

Toxicopathic lesions associated with contaminant exposure were generally more severe in downstream fish, and were consistent with lesions reported in freshwater fish environmentally exposed to PCBs (Teh *et al*. 1997). Pre-neoplastic (FCA) and neoplastic stomach and liver lesions were observed only in downstream fish. Largemouth bass collected from a PCB-contaminated reservoir showed severe liver lipidosis and vacuolated and basophilic FCAs in contrast to upstream fish, which did not contain any of these lesions (Teh *et al*. 1997). Similarly, walleye collected from the PCB-contaminated Lower Fox River and Green Bay, Lake Michigan, Wisconsin, USA (Barron *et al*. 2000) and white sucker (*Catostomus commersoni*)

collected from PCB-contaminated reaches of the Sheboygan River, Wisconsin, USA (Schrank *et al*. 1997) also showed liver FCAs and in some instances neoplasia. Moreover, Atlantic tomcod (*Microgadus tomcod*) collected from the PCB-contaminated Hudson River, USA, exhibited one of the highest prevalences of liver neoplasia in any feral fish population investigated (Dey *et al*. 1993). Although none of the aforementioned studies demonstrate a cause–effect relationship between PCB exposure and hepatic FCAs or neoplasms, there is laboratory evidence that PCBs can act as tumour promoters in fish (Bailey *et al*. 1987, Hendricks *et al*. 1990, Fabacher *et al*. 1991) and in mammals (Safe 1994, IARC 1999), which indicates that PCBs could be causative agents promoting the expression of neoplasia in wild fish in contaminated habitats (Moore and Meyers 1994).

Male feminization, testicular atrophy and intersex gonads have been documented in other fish species, reptiles, birds and mammals from other contaminated environments (Fry 1981, Colborn and Clement 1992). Laboratory studies in fish have shown that PCBs cause testicular lesions, including damage to the spermatozoa (Hacking *et al*. 1977), spermatogenic elements (Sangalang *et al*. 1981) and inhibition of testicular development (Freeman and Idler 1975). Our study identified histopathological lesions in the testes of the downstream and upstream fish. For example, immature, pre-vitellogenic oocytes were present in every testicular sample examined. Although intersex scores for downstream fish were slightly higher than upstream fish, there did not appear to be substantial 'male feminization' (i.e. lesion scores were reported as mild) and vitellogenin was not detected in the plasma of male fish. Interestingly, a potentially more significant lesion, dysplastic meiosis, characterized by a combination of degeneration, necrosis and syncytial cell formation of developing spermatogonia, appeared to be more severe in downstream fish. These lesions may possibly be associated with reduced numbers and viability of sperm produced by the testes. Similar lesions have been identified in domestic animals following exposure to a wide variety of agents, including ionizing radiation, metals, rare earth salts and other chemical compounds (Ladd 1993).

Glycogen depletion present in liver and macrophage aggregates present in liver, kidney, spleen or gonad are non-specific indicators of contaminant exposure, environmental stress, nutritional status or age (Wolke *et al*. 1985). Loss of glycogen is a common, non-specific lesion that can be observed in fish under a variety of stressful conditions, including infection, parasitism and exposure to chemical contaminants (Myers and Hendricks 1985). Hepatic glycogen depletion has also been demonstrated in fish exposed to PCBs in the laboratory (Hacking *et al*. 1977). In our study, the incidence of hepatic macrophage aggregates and glycogen depletion was greater in downstream compared with upstream fish. Hepatic macrophage aggregates develop following parenchymal degeneration and necrosis and have been positively associated with contaminant exposure in fish (Haensly *et al*. 1982, Wolke *et al*. 1985, Wolke 1992). Since age-matched fish from each sampling location were evaluated in this study, the enhanced aggregates found in the downstream fish are most likely reflective of a response to downstream stressors.

It is notable that some toxicopathic lesions that are associated with or caused by PCB, PAH, or heavy metal exposure, including biliary hyperplasia, hepatic necrosis, hepatic fatty changes (Nimmo *et al*. 1975, Myers *et al*. 1991, 1998), nephron regeneration (Cormier *et al*. 1995, Schrank *et al*. 1997), ovarian atresia (Johnson *et al*. 1988) and deformities of the gill (Teh *et al*. 1997), were not observed in this study. Similarly, laboratory exposures of fish to PCBs have shown potentially adverse pathological effects on kidney and spleen (Nestel and Budd 1975) that were not observed in this study. Since no previous biomarker-related laboratory or field studies have involved smallmouth bass, the lack of these lesions suggests there may be differences in susceptibility to PCBs or other chemicals, or differences in the response to environmental stressors (water quality, food availability, disease) between smallmouth bass and the various other fish species that have been evaluated. These differences also may be attributable to the unique sediment contaminant profile found in the Kalamazoo River compared with other aquatic habitats.

Some histopathological lesions were more prevalent or severe in upstream fish. For example, foreign body granulomas were observed more frequently in the liver, spleen, kidney and gonads of the upstream fish. These lesions were usually collocated with an adult metazoan parasite (species not identified). The increased prevalence and severity of metazoan parasites in upstream fish may be associated with the presence of an intermediate host that occurs in the upstream site, but not in the downstream site. For example, during fish collections, freshwater mussels were observed only in the upstream site. Other lesions, including stomach single cell necrosis, thyroid eosinophilic granular leukocytes and kidney protein droplets, were more prevalent or severe in upstream fish. While the aetiology of these lesions is unknown, they are potentially related to infection, parasitism or chemical contaminants unique to the upstream site.

Conclusions

This study has shown that a suite of biochemical, histopathological and organosomatic indices are spatially and statistically associated with PCB exposure in smallmouth bass. Fish from a chemically impacted reach of the Kalamazoo River showed significant alterations in EROD activity, SOD activity and plasma vitellogenin when compared with an upstream site. Similarly, toxicopathic lesions, including neoplasia, FCAs and macrophage aggregates, were more prevalent or severe in fish from the downstream site. Although cause and effect were not conclusively established by this study, the alterations observed suggest that fish within the impacted reaches of the Kalamazoo River are adversely affected by chemical stressors, including PCBs.

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