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Characterization of contaminants in snapping turtles (*Chelydra serpentina*) from Canadian Lake Erie Areas of Concern: St. Clair River, Detroit River, and Wheatley Harbour

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"Capsule": *Organochlorine contaminant levels in some snapping turtle eggs in Canadian Lake Erie Areas of Concern exceed both minimum human consumption and Environmental Quality Guidelines.*

Abstract

PCBs, organochlorine pesticides and dioxins/furans in snapping turtle eggs and plasma (*Chelydra serpentina*) were evaluated at three Areas of Concern (AOCs) on Lake Erie and its connecting channels (St. Clair River, Detroit River, and Wheatley Harbour), as well as two inland reference sites (Algonquin Provincial Park and Tiny Marsh) in 2001–2002. Eggs from the Detroit River and Wheatley Harbour AOCs had the highest levels of *p,p′*-DDE (24.4 and 57.9 ng/g) and sum PCBs (928.6 and 491.0 ng/g) wet weight, respectively. Contaminant levels in eggs from St. Clair River AOC were generally higher than those from Algonquin Park, but similar to those from Tiny Marsh. Dioxins appeared highest from the Detroit River. The PCB congener pattern in eggs suggested that turtles from the Detroit River and Wheatley Harbour AOCs were exposed to Aroclor 1260. TEQs of sum PCBs in eggs from all AOCs and *p,p′*-DDE levels in eggs from the Wheatley Harbour and the Detroit River AOCs exceeded the Canadian Environmental Quality Guidelines. Furthermore, sum PCBs in eggs from Detroit River and Wheatley Harbour exceeded partial restriction guidelines for consumption. Although estimated PCB body burdens in muscle tissue of females were well below consumption guidelines, estimated residues in liver and adipose were above guidelines for most sites.

Keywords: Polychlorinated biphenyls; Pesticides; Eggs; Environmental guidelines; Body burdens

1. Introduction

The 1987 Protocol to the 1978 Great Lakes Water Quality Agreement has committed both Canada and the United States to the "virtual elimination of persistent toxic substances and toward restoring and maintaining the chemical, physical and biological integrity of the Great Lakes Basin Ecosystem" (International Joint Commission United States and Canada, 1988). As a means to this goal, the International Joint Commission designated 42 geographical regions as Areas of Concern (AOCs) within the Great Lakes basin, based upon impairment of beneficial use or ability of the regions to support aquatic life. Impairment of beneficial use is defined as "…a change in the chemical, physical, or biological integrity of the Great Lakes System sufficient to cause [a high incidence of developmental problems, restrictions of human use or consumption of wildlife, loss of habitat, or similar impairment]" (International Joint Commission United States and Canada, 1988). Delisting of AOCs is dependent upon the remediation of the causes of beneficial use impairment (Table 1). The Canadian Wildlife Service (CWS) monitors contaminant burdens and reproductive and developmental abnormalities in wildlife

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Table 1
Causes and sources of impairment of Canadian Areas of Concern in Lake Erie basin

AOC	Impairment of beneficial use[a]	Cause(s) of impairment
Detroit River	• Loss of wildlife and fish habitat • Restriction on wildlife and fish consumption • Fish deformities or tumours • Restrictions on dredging • Restrictions on drinking water • Beach closures • Degradation of benthos • Degradation of aesthetics	• Industrial discharges • Storm relief sewer outlets • Contaminated sediment
Wheatley Harbour	• Bacterial contamination and eutrophication • Restrictions on dredging activities • Loss of wildlife and fish habitat • PCBs in sediment (not assessed)	• Food processing discharges • Septic bed leakage
St. Clair River	• Loss of wildlife and fish habitat • Restriction on wildlife and fish consumption • Wildlife deformities or reproduction problems • Restrictions on dredging • Restrictions on drinking water • Beach closures • Degradation of aesthetics	• Discharges from petroleum and chemical industries • Sewer overflows/treatment plants • Spills

[a] Based upon Great Lakes Water Quality Agreement, Annex 2, Section 1 (c).

within these AOCs and other areas, through long term monitoring projects involving herring gulls (*Larus argentatus*; Hebert et al., 1994), double-crested cormorants (*Phalacrocorax auritus*; Ryckman et al., 1998), snapping turtles (*Chelydra serpentina*; Bishop et al., 1998), and mink (*Mustela vison*; Martin et al., unpublished manuscript).

Nineteen areas in the Canadian or binational Great Lakes basin have been declared Areas of Concern (AOCs) based on the IJC-GLWQA criteria. In the Canadian Lake Erie basin, the Detroit River and Wheatley Harbour have been declared as AOCs. Although the St. Clair River AOC is classified within the Lake Huron region, it is within the Lake Erie watershed, as it drains into Lake St. Clair and subsequently into Lake Erie through the Detroit River. The health of wildlife and fish species is currently being assessed through a program recently established by Environment Canada. The program seeks to evaluate the health of snapping turtles, mink, herring gulls, and fish, as well as contaminant burdens in these animals and the sediment in selected Canadian AOCs in the lower Great Lakes. As part of this program, this study reports the initial findings relating to contaminant burdens and patterns in snapping turtles.

PCBs, non-*ortho* PCBs, dioxins and furans, and organochlorine pesticides and related compounds are reported for snapping turtle eggs sampled from the Lake Erie AOCs. Our objectives were to characterize and contrast the contaminant loads among the AOCs and reference sites, but also to determine the degree of (dis)similarity among AOCs. We determined if the levels of organochlorine contamination in snapping turtle eggs exceed Environmental Quality Guidelines, both to protect human and wildlife consumers (CCME, 1998; OMOE, 2001). Finally, we estimated the body burdens of PCBs and *p,p′*-DDE, based upon published relationships between eggs and maternal tissues of snapping turtles (Pagano et al., 1999; Russell et al., 1999).

2. Materials and methods

2.1. Study areas

Five sites at three AOCs within the Lake Erie basin were investigated (Fig. 1). Canard River and Turkey Creek (42° 14′ N, 83° 6′ W) are both within the Detroit River AOC. Turkey Creek drains both the city of LaSalle, and an industrial zone in Windsor, Ontario, and has previously been dredged because of contaminated sediments. Canard River drains into the Detroit River south of Windsor, partially draining through a large privately owned cattail marsh. The St. Clair National Wildlife Area (NWA) (42° 23′ N, 82° 25′ W) and Big Point Hunt Club (42° 25′ N, 82° 24′ W) are approximately 13 km south of the St. Clair River AOC southernmost boundary. The NWA and the Big Point Hunt Club are approximately 4 km apart, and thus were treated as one site. Muddy Creek (42° 4′ N, 82° 28′ W) is within the Wheatley Harbour AOC, while Wheatley Provincial Park (42° 5′ N, 82° 26′ W) and Hillman Marsh Conservation Area (42° 2′ N, 82° 30′ W) are 2.3 km NE and 3.5 km SW of the Wheatley Harbour AOC boundaries, respectively. Although turtle eggs were obtained from Wheatley Provincial Park and Hillman Marsh, adult snapping turtles but not eggs were collected at Muddy Creek in the Wheatley Harbour AOC. At all three AOCs, contaminant burdens in fish, wildlife and/or sediment, are important causes for impairment (Table 1). Both the Detroit River AOC and St. Clair River AOC have high levels of organic contamination due to industrial discharge, while Wheatley Harbour has had PCB discharges from fish processing plants and the unconfined disposal of dredged harbour sediment on land immediately adjacent to Muddy Creek (Table 1; Bedard, 1995). Furthermore, the areas surrounding all AOCs involve

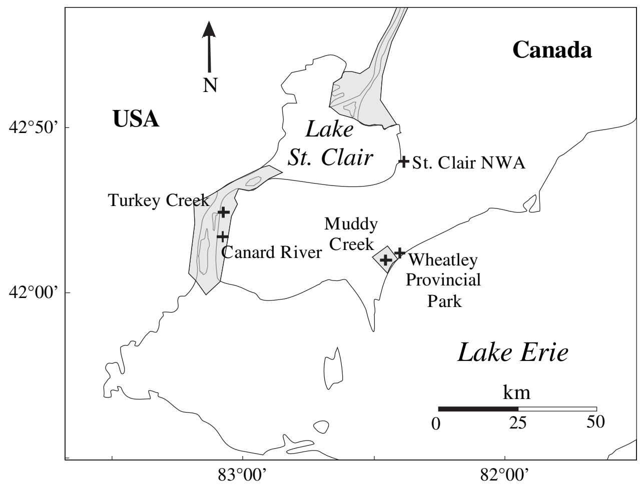

Fig. 1. Study site locations in Lake St. Clair, Detroit River, and Lake Erie relative to the Areas of Concern (AOCs; shaded areas). The Big Creek Hunt Club is included within the St. Clair National Wildlife Area. The AOC boundaries are approximate.

intensive agriculture and pesticide use from agricultural and/or urban sources.

Two sites outside of the Lake Erie basin, Tiny Marsh (44° 36′ N, 79° 56′ W) and Algonquin Provincial Park (45° 36′ N, 78° 41′ W), were used as reference sites. Tiny Marsh is a cattail marsh with open water, and was drained in the early to mid 1900s, but then subsequently reflooded and dyked in the late 1900s. Although the surrounding area is farmed, largely for hay production, the intensity of agricultural use is less than that near or in the three AOCs. Tiny Marsh is both a provincially significant (Class 1) wetland and a Provincial Wildlife Area. Although Algonquin Provincial Park is currently logged, there has been no industry otherwise, and it is often used as a reference site (Bishop et al., 1998). Eggs were collected from a variety of sites within Algonquin Park, but all sites were similar in that eggs were collected from embankments adjacent to dystrophic lakes or ponds.

2.2. Sampling of eggs and plasma for contaminant analysis

Snapping turtle eggs were collected from each site in southern Ontario for contaminant analysis and artificial incubation in June 2001 and 2002, except for Canard River and the St. Clair AOC, which were sampled in 2001 only. Five eggs were selected from each clutch, unless the total number of eggs in the clutch was less than 25, in which case fewer eggs were selected to maintain a final clutch size of 20 each. If a clutch had 21 or fewer eggs, only one egg was selected for contaminant analysis. Eggs were selected in a pseudo-random but stratified manner; eggs were ordered from first egg laid to the last, and each clutch was divided into five groups of approximately equal size. Within each group, an egg was selected haphazardly. Subsequently, the egg contents for each clutch were pooled and weighed, and frozen in hexane-cleaned amber glass jars at −20 °C.

In both years, snapping turtles were caught using hoop traps baited with fish from May to July. The traps were left overnight and checked the following day. Whenever possible, the sex of males was positively identified by eliciting eversion of the penis (de Solla et al., 2001); otherwise the sex of adults was identified by nesting behaviour and relative size of the precloacal area (Mosimann and Bider, 1960). Blood samples were generally taken in the early to late afternoon. Depending on the body mass of the turtle, approximately 5–8 ml of blood was taken from the caudal vein using 10 ml sodium heparin coated vacutainers and 22 gauge double sided needles. The blood samples were stored in a cooler on ice until centrifuged for 5 min in a clinical centrifuge. The blood plasma was then transferred to cryovials and stored in liquid or gaseous phase nitrogen in Dewar cryogenic containers. Once brought back to the laboratory, the samples were stored in a −80 °C freezer until analyzed.

2.3. Chlorinated hydrocarbon analysis

In 2001, eggs from the Wheatley AOC were analyzed at the National Wildlife Research Centre (NWRC, Ottawa, ON), while the remaining samples from both years were analyzed at the Great Lakes Institute of Environmental Research (GLIER, University of Windsor, Windsor, ON). The egg samples were thawed to room temperature and extracted with dichloromethane (DCM):hexane (1:1 v/v) after the samples were dehydrated with anhydrous Na_2SO_4. The lipids and biogenic material were removed using gel permeation chromatography and cleaned by florisil column chromatography. All the 2001 samples were analyzed using capillary gas chromatography, coupled with a mass selective detector (GC/MSD), and samples from 2002 were analyzed with an electron capture detector (GC/ECD). Although up to 59 congeners were detected, the different GC detectors identified a different number of PCB congeners, so only 36 individual or coeluting congeners common to all analyses were included (IUPAC numbers 31/28, 42, 44, 56/60, 52, 64/41/71, 66/95, 70/76, 74, 87, 97, 99, 101, 105, 110, 118, 138, 141, 146, 149, 151, 153, 158, 170/190, 171, 172, 174, 180, 183, 187/182, 194, 195, 196/203, 200, 201, 206). Sum PCBs were the sum of all 36 congeners. The organochlorine pesticides that were analyzed included dichlorodiphenyltrichloroethane (*p,p′*-DDE) and *p,p′*-DDD, hexachlorobenzene (HCB), octachlorostyrene (OCS), mirex, dieldrin, *cis*- and *oxy*-chlordane, *cis*- and *trans*-nonachlor, heptachlor epoxide (H.E.), and α- and γ-hexachlorocyclohexane. *trans*-Chlordane and *p,p′*-DDT were not included because of interference for the GC/ECD analysis. Each cleaned sample was injected to determine organochlorine compounds by using 21 organochlorine standards. The method quantification limits (10× the detection limits) ranged between 0.01–0.09 ng/g for the eggs samples analyzed at GLIER and 0.1 ng/g for the egg and plasma samples analyzed at NWRC using GC/MSD. Non-detectable concentrations were treated as one-half the detection limits.

There were some differences in the measurement of residues in the reference material (herring gull, [NWRC, GLIER] and carp homogenate [GLIER]) using GC/ESD and GC/MSD (results not shown). To account for these differences, the percent difference between methods was calculated for each PCB congener and pesticide in the reference material. Then, for each sample, the contaminant concentration was adjusted using the %difference between the samples measured using GC/ESD and GC/MSD. This adjustment reduces the differences between the GC/ESD and GC/MSD methods to 0 for each compound in the reference material, and adjusts the turtle egg residues by the same difference.

Concentrations of PCDDs and PCDFs and six non-*ortho* PCBs were measured using GC/MS by Axys Analytical Services Ltd. (Victoria, BC, Canada), in eggs from Turkey Creek, Wheatley Park, Tiny Marsh, and Algonquin Park. Subsamples of the homogenate from each clutch were pooled for each site and analyzed as a single sample. Detailed methodology can be found in Simon and Wakeford (2000).

2.4. Statistical analyses

The %lipid in eggs did not vary among sites and there was no relationship between percent lipid levels and the concentrations of the organochlorine compounds (results not shown). There was a positive, though nonsignificant, linear relationship between %lipids and PCBs and *p,p′*-DDE in blood plasma. Since the sample size ($n = 14$) and thus power was low, we used %lipid as a covariate for analyses of contaminants in plasma despite the lack of significance. Contaminants were expressed on a wet weight basis for comparisons. Patterns of PCBs and OCs in eggs were examined using ANOVA and Principal Components Analysis (PCA) using varimax normalized rotation on untransformed contaminant concentrations. Individual OCs and PCB congeners were expressed as a percentage of the sum OCs and PCBs, respectively, for the PCA. Sum-chlordane, *p,p′*-DDE, mirex, dieldrin, and 10 PCB congeners (74, 99, 105, 118, 138, 170/190, 180, 183, 187, 201) were included in the PCA analysis. Contaminants in eggs were log transformed prior to analysis using ANOVA to reduce heterogeneity of variance and to reduce skewness. Squared Mahalanobis distances were calculated to determine the relative distances among sites in terms of organochlorine contaminants. Essentially, Mahalanobis distances are the distances between each observation and the group (site) mean representing the average contaminant concentrations in multidimensional space; the greater the distance between the group means, the greater the difference between the sites.

Toxic equivalency concentrations (TEQs) were calculated for total PCBs, PCDD/Fs, and non-*ortho* PCBs in the eggs, using congener-specific toxic equivalency factors (TEFs). TEFs have not been estimated for turtles, but we used TEFs developed for the World Health Organization (van den Berg et al., 1998), which were also used by the Canadian Council of Ministers of the Environment (CCME, 1998) to develop tissue residue guidelines for the protection of wildlife that consume aquatic biota. We estimated body burdens (leg muscle, liver, and adipose) of female snapping turtles for both sum PCBs and *p,p′*-DDE. We converted the concentrations reported in snapping turtle egg, maternal liver and adipose tissues (Pagano et al., 1999) into wet weight concentrations, and estimated the mean ratios for PCBs and *p,p′*-DDE between eggs with liver and adipose. Similarly, Russell et al. (1999) calculated the ratio of contaminants between egg and leg muscle in snapping turtles. As they used individual congeners for their analysis, we used the mean ratio (8.4:1, egg:muscle) for sum PCBs, and the ratio 6.1:1 (egg:muscle) for *p,p′*-DDE (Russell et al., 1999).

Statistica 6.1 (StatSoft, Inc., 2003) was used for all statistical analyses.

3. Results

3.1. Summary of contaminants

Total PCBs, sum-chlordane, HCB, OCS, *p,p′*-DDE, mirex, H.E., and dieldrin in eggs varied among sites ($F_{5,35} = 32.6$, $P < 0.0001$; $F_{5,35} = 29.6$, $P < 0.0001$, $F_{5,35} = 22.0$, $P < 0.0001$; $F_{4,32,} = 6.0$, $P = 0.0011$; $F_{5,35} = 29.8$, $P < 0.0001$; $F_{5,35} = 3.7$, $P = 0.0081$; $F_{5,35} = 6.3$, $P = 0.0003$, respectively), and were highest in eggs from the Detroit River AOC and near the Wheatley Harbour AOC (Table 2). Mirex was highest in eggs from Turkey Creek, but not different among the other sites ($F_{4,16} = 3.63$, $P = 0.0274$). Octachlorostyrene was detectable, but low, in eggs from near the St. Clair River and Wheatley Harbour AOCs, and from Turkey Creek, but was not detected in eggs from Canard River and the two reference sites (Algonquin Park or Tiny Marsh; Table 2).

Plasma samples from adult turtles captured in Muddy Creek (Wheatley Harbour, $n = 9$), St. Clair NWA ($n = 2$), and Tiny Marsh ($n = 3$) were also analyzed for organochlorines. Most pesticides and some PCB congeners that were present in eggs were undetectable in blood plasma. Since we had only two samples from St. Clair NWA, we did not include it in the statistical

Table 2
Mean (SD) organochlorine contaminants and sum PCBs (ng/g wet weight) in snapping turtle eggs from sites among three AOCs and reference areas, 2001 and 2002

AOC	Reference		St. Clair	Detroit River		Wheatley
Site	Algonquin Park	Tiny Marsh	St. Clair NWA	Turkey Creek	Canard River	Wheatley Provincial Park
N	6	9	6	8	4	8
%Lipid	4.43 (1.02)	5.45 (1.28)	4.95 (0.80)	5.16 (0.93)	5.95 (1.28)	5.35 (0.77)
Sum PCBs	15.7 (7.81)	41.1 (27.3)	74.2 (90.1)	928.6 (510)	200.5 (281.3)	491 (220.7)
	A	**AB**	**AB**	**C**	**B**	**C**
Sum-chlordane	1.22 (0.63)	2.78 (1.19)	4.58 (3.7)	24.4 (17.9)	2.20 (2.62)	32.3 (12.0)
	A	**A**	**A**	**B**	**A**	**B**
HCB	0.16 (0.08)	0.25 (0.18)	0.66 (0.67)	2.42 (0.64)	0.38 (0.25)	0.93 (0.33)
	A	**AB**	**BC**	**D**	**ABC**	**C**
OCS	N.D.	N.D.	0.39 (0.36)	1.31 (0.71)	N.D.	0.91 (0.28)
			A	**B**		**B**
p,p'-DDE	1.33 (0.28)	4.92 (1.98)	5.93 (1.58)	24.4 (8.63)	4.73 (3.21)	57.9 (89.0)
	A	**B**	**B**	**C**	**AB**	**C**
Mirex	0.21 (0.27)	2.08 (2.91)	0.24 (0.34)	6.65 (4.40)	0.18 (0.27)	1.24 (1.22)
	A	**AB**	**AB**	**B**	**AB**	**AB**
H.E.[a]	0.29 (0.11)	0.44 (0.14)	0.53 (0.43)	1.39 (1.29)	0.06 (0.12)	1.95 (1.04)
	A	**A**	**A**	**A**	**B**	**A**
Dieldrin	0.45 (0.37)	0.59 (0.28)	1.16 (0.83)	4.11 (1.74)	0.11 (0.21)	5.86 (2.40)
	AB	**BC**	**BC**	**BC**	**A**	**C**

Similar letters in rows indicate no significant difference among sites (Tukey Honest Significant Test, $\alpha = 0.05$). Data were $\log_{10}$ transformed prior to analysis.
[a] Heptachlor epoxide.

analyses. No chlordane isomers were detected in the plasma of adult turtles from Tiny Marsh, but both *cis*- and *trans*-nonachlor were detected in plasma from the Wheatley and St. Clair River AOCs (Table 3). Wheatley Harbour turtles had significantly higher concentrations of *p,p'*-DDE than Tiny Marsh turtles ($F_{1,9} = 7.92$, $P = 0.0202$), but there were no other differences between sites.

Although no statistical analysis was possible because the samples were pooled, levels of PCDD/Fs and non-*ortho* PCBs appeared to be highest at Turkey Creek, and lowest at the two reference sites (Table 4).

3.2. Principal component analysis

PCA was used to contrast differences in the pattern of pesticides in eggs among sites. Five principal components were extracted, accounting for 81.1% of the total variance, and the first component explained 27.4% of the variance. The first three factor scores varied among sites (PC1, $F_{4,32} = 6.0$, $P = 0.0010$; PC2, $F_{4,32} = 8.2$, $P = 0.0001$; PC3, $F_{4,32} = 6.5$, $P = 0.0006$). PC1 was highly positively correlated (>0.7) with PCBs 170/190, 180, 183 (Table 5), which are characteristic of Aroclors 1260 (Frame, 1997), and negatively correlated (<0.7) with PCB 74. The mean factor score for PC1 was lowest at Algonquin, and highest at Turkey Creek and Wheatley Provincial Park, whereas Tiny Marsh was not different from Turkey Creek. St. Clair eggs did not differ from any site. Thus, Turkey Creek and Wheatley Provincial Park eggs were associated with Aroclor 1260. PC2 was positively correlated with PCB 201 (Table 5), which is found in three Aroclor mixtures, and negatively correlated with PCBs 105 and 118 (Table 5), which are associated with Aroclors 1254 and 1248/1254, respectively (Frame, 1997). The mean factor score for PC2 was higher at Algonquin Park and Wheatley Provincial Park compared to other sites, thus the other sites were more associated with Aroclor 1254. PC3 was negatively correlated with mirex and positively correlated with PCB 187 (Table 5), which is characteristic of Aroclor 1260 (Frame, 1997). The mean factor score for PC3 was higher for Turkey Creek, Wheatley Provincial Park and St. Clair NWA compared to Tiny Marsh, whereas Algonquin Park did not differ with any site. Although absolute levels of mirex were highest at Turkey Creek (Table 2), mirex constituted a higher proportion of the total pesticide residues in eggs from Tiny Marsh.

Table 3
Mean (SD) HCB, *p,p'*-DDE, and sum PCBs (ng/g wet weight) in snapping turtle plasma from Lake Erie AOCs and Tiny Marsh, 2001

	Wheatley Harbour AOC	St. Clair River AOC	Reference (Tiny Marsh)
N	9	2	3
%Lipid	0.60 (0.24)	0.70 (0.15)	0.38 (0.17)
HCB	0.13 (0.38)	0.72 (0.53)	0.18 (0.3)
trans-Nonachlor	1.12 (1.11)	0.82 (1.17)	N.D.
cis-Nonachlor	0.45 (0.38)	0.23 (0.32)	N.D.
p,p'-DDE	4.63 (1.98) **A**	3.61 (1.47)	0.58 (0.74) **B**
Sum PCBs	87.9 (55.1)	96.3 (85.5)	15.2 (11.1)

Similar letters in rows indicate no significant difference among sites. Data were $\log_{10}$ transformed prior to analysis. Only Wheatley and Tiny Marsh samples were included in ANCOVA, using %lipid as a covariate.

Table 4
Non-*ortho* PCBs, dioxins and furans with associated TEQs (pg/g wet weight) in pooled snapping turtle eggs from four sites, 2002

Compound	Turkey Creek	Wheatley Provincial Park	Tiny Marsh	Algonquin Park
PCB-37	10.1	1.79	1.24	0.982
PCB-77	125	37.1	6.45	2.74
PCB-126	402	198	30.6	17.9
PCB-169	34.6	24.1	5.05	3.48
PCB-189	1420	884	79.2	52.6
TEQ (non-*ortho* PCBs)	40.56	20.04	3.11	1.83
2378-TCDD	4.8	2.3	0.385	0.373
12378-TCDD	4.21	1.95	0.644	0.501
123478-HCDD	0.583	0.641	0.201*	0.148*
123678-HCDD	7.19	2.37	0.513	0.401
123789-HCDD	1.16	0.422	<0.100	0.146*
1234678-HCDD	2.11	1.53	0.192	1.09
12346789-OD	2.91	8.19	0.231*	0.506*
2378-TCDF	0.1*	0.272	<0.100	0.106
12378-PCDF	0.104	0.178	<0.100	<0.100
23478-PCDF	2.42	2.65	0.762	0.638*
123478-PCDF	0.101*	<0.100	<0.100	<0.100
123678-PCDF	0.353	0.226*	<0.100	<0.100
234678-PCDF	0.193	0.127	<0.100	<0.100
1234678-PCDF	0.314	<0.100	<0.100	<0.100
TEQ (PCDD/Fs)	11.22	6.01	1.48	1.28

Note: the TEQs are based upon toxic equivalency factors developed by van den Berg et al. (1998). Asterisk (*) indicates that compound was outside the 15% quality control limit around the theoretical ion abundance ratio and thus should be viewed with caution.

We tested if the squared Mahalanobis distances were significantly different from 0 for each pair of sites. The pairs of sites that were most similar, based upon squared Mahalanobis distances calculated from contaminant levels in eggs, were St. Clair NWA and Turkey Creek with Canard River (7.27 and 10.51, respectively). These were the only pairs of sites in which the Mahalanobis distances were not significantly different (Table 6).

Table 5
Factor loadings of organochlorine pesticides and PCBs in turtle eggs with the first three principal components

Compound	PC1	PC2	PC3
PCB 105	−0.15	**−0.70**	0.09
PCB 118	−0.16	**−0.87**	−0.27
PCB 170/190	**0.89**	0.02	−0.21
PCB 180	**0.81**	0.34	−0.34
PCB 183	**0.82**	0.09	0.26
PCB 187/182	0.21	0.26	**0.77**
PCB 201	0.11	**0.76**	−0.11
PCB 74	**−0.72**	−0.08	0.13
Mirex	0.23	0.02	**−0.78**
Explained variance	3.18	2.57	2.27
Proportion of total variance	0.23	0.18	0.16

Only compounds in which the loadings were larger than ± 0.7 were included.

Table 6
Squared Mahalanobis distances between sites based on organochlorine pesticides and related compounds in snapping turtle eggs, 2001 and 2002 (the larger the distance, the more dissimilar the pair of sites)

	Tiny Marsh	St. Clair NWA	Algonquin Park	Wheatley Provincial Park	Canard River
Tiny Marsh	0	–	–	–	–
St. Clair NWA	19.56*	0	–	–	–
Algonquin Park	22.11*	23.71*	0	–	–
Wheatley	39.70*	34.66*	39.09*	0	–
Canard River	23.32*	7.27	30.80*	36.29*	0
Turkey Creek	32.60*	18.86*	34.25*	16.98*	10.51

*Significant at $\alpha = 0.05$.

Wheatley Harbour and Tiny Marsh, and Wheatley Harbour and Algonquin Park were the most dissimilar pairs of sites (Table 6). Although clearly Canard River and Turkey Creek differ in terms of some absolute and relative contaminant levels (Table 2, Fig. 2a,b), the relative similarity probably reflects their close geographic proximity. Nevertheless, the differences indicate that contaminant exposure can vary considerably within the Detroit River AOC. There appeared to be differences in contaminants even among the four egg samples from Canard River (results not shown).

The mean TEQs for sum PCBs was highest at Turkey Creek, followed by Wheatley Harbour and Canard River (Fig. 3). Only eggs from Tiny Marsh and Algonquin Park did not exceed the Canadian Environmental Quality Guidelines of 0.79 ng/kg for PCBs. The Environmental Quality Guidelines for *p,p′*-DDE is 14.0 ng/g wet weight; some clutches at Turkey Creek exceeded this level, and all clutches at Wheatley Provincial Park exceeded this guideline. Generally, the non-*ortho* PCBs contributed most to total TEQs, followed by PCBs, and lastly by PCDD/Fs (Table 4, Fig. 3).

The mean estimated body burdens of both PCBs and *p,p′*-DDE were low in muscle tissue (Fig. 4a,b), and did not approach the partial consumption guidelines for PCBs (Fig. 4a). However, estimated levels in fat exceeded either partial or complete consumption limits at all sites except Algonquin Park (Fig. 4a), whereas sum PCBs in liver exceeded the partial consumption guidelines at Wheatley Provincial Park and Turkey Creek. Assuming the same guidelines for *p,p′*-DDE as PCBs, adipose levels exceeded the *p,p′*-DDE partial consumption guidelines for Wheatley Provincial Park, and were just under the limit at Turkey Creek (Fig. 4b).

4. Discussion

Contaminant burdens in snapping turtle eggs varied among AOCs both in absolute levels and relative proportions of the total contaminant burdens. Some of

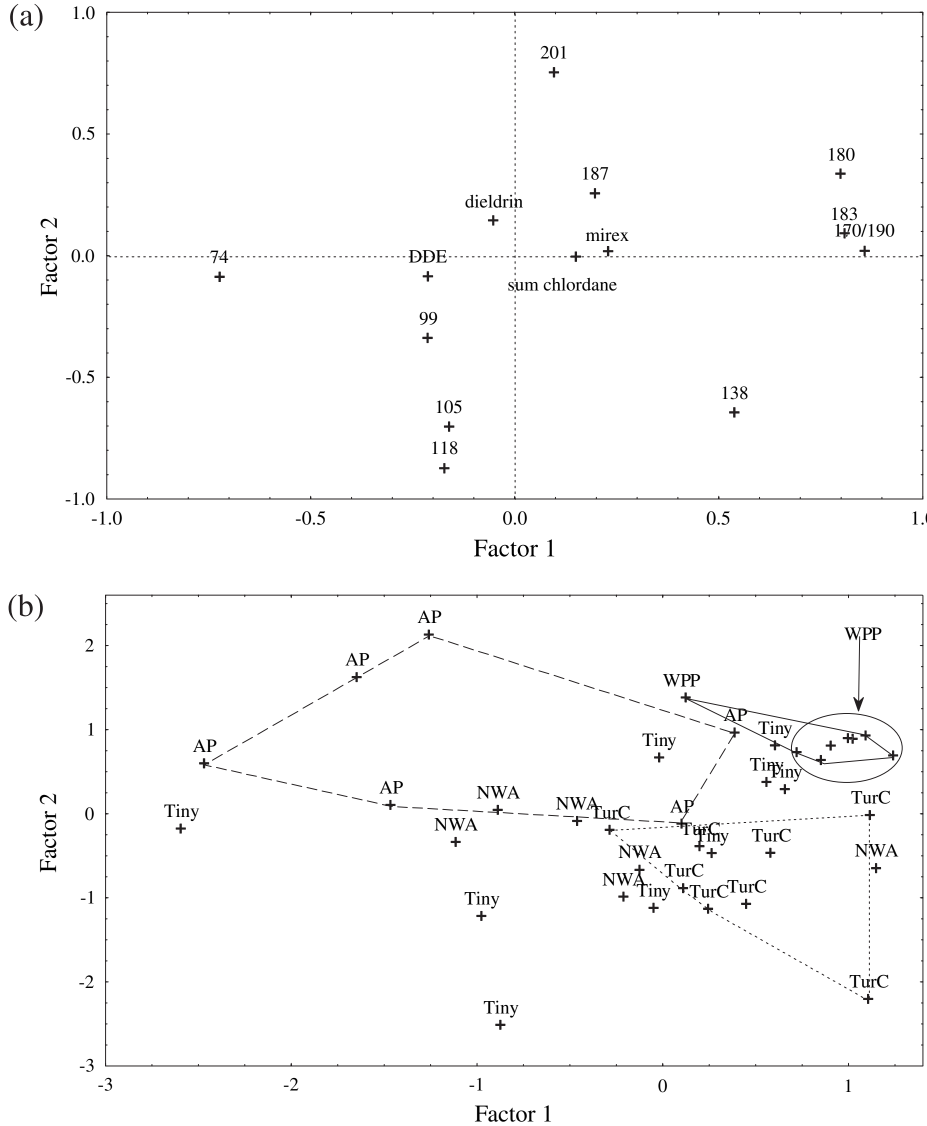

Fig. 2. (a) Principal component loadings of PCB congeners and organochlorine contaminants in snapping turtle eggs from all sites. PC1 is dominated by higher chlorinated biphenyls associated with Aroclor 1260; (b) Factor scores from egg samples for each location, showing separation of Turkey Creek (TurC, Detroit AOC), Wheatley Harbour AOC (WPP), and Algonquin Provincial Park (AP).

the study sites could be differentiated from others based upon the profile of the organochlorine compounds in the turtle eggs. The organochlorine profile of the eggs from near the Wheatley Harbour AOC was, in particular, different from the profile of the other sites. As well, the snapping turtle eggs from Canard River were somewhat similar in their organochlorine profile to the profiles of the eggs from both Turkey Creek and the St. Clair River AOC; this similarity can be partially explained by the close proximity of the various sites to each other.

PCB concentrations were highest in the snapping turtle eggs from Turkey Creek, followed by Wheatley Provincial Park and Canard River. PCB contamination in Detroit River has been well documented (Hamdy and Post, 1985; Kaiser et al., 1985), although the Canadian side of the Detroit River AOC contributes only approximately 10% of the contaminant load (Hamdy and Post, 1985). On the Canadian side of the river, Turkey Creek, Canard River, Little River, and the West Windsor sewage treatment plant were local sources of PCBs in the 1980s (Hamdy and Post, 1985). In our study, the PCB levels in the turtle eggs were moderate at the St. Clair River AOC and the Canard River in the Detroit River AOC. In the early 1980s, PCB contamination in surficial sediments from the Detroit River was consistent with a large presence of Aroclor 1260, while Lake St. Clair was associated with Aroclor 1242 (Oliver and Bourbonniere, 1985). The PCB congener profile seen here in the turtle eggs from Turkey Creek reflect the historical Aroclor 1260 sources. The main source of PCB contamination in Wheatley Harbour is ultimately Lake Erie fish, with refuse from the fish processing

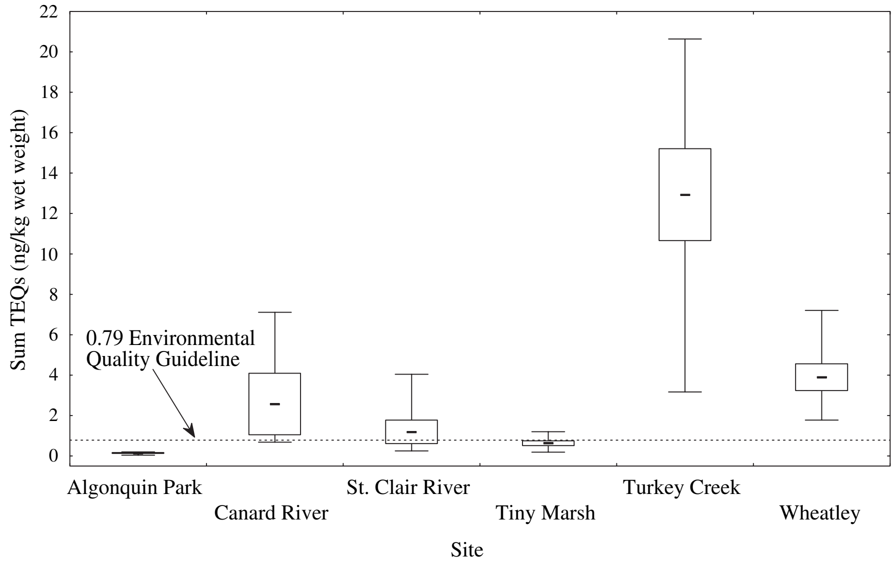

Fig. 3. Mean, SD, and range of toxic equivalencies (TEQs) of sum PCBs (ng/kg wet weight) in turtle eggs from Lake Erie relative to Environmental Quality Guidelines for safe consumption of wildlife. TEQs are based upon toxic equivalency factors (TEFs) calculated by van den Berg et al. (1998).

plants being discharged into the harbour, and so depositing PCBs into the sediments (Bedard, 1995). Although the congener profile of the PCB source is unknown at Wheatley Harbour, the PCB profile in the turtle eggs suggests an Aroclor 1260 source. MacDonald et al. (1992) suggested that the larger proportion of higher chlorinated PCB congeners in herring gull eggs from Middle Island (southwest of Wheatley Harbour) relative to other Great Lake sites, were from continued contamination or resuspension of contaminated sediments from the Detroit River. Again, the PCB congener profile of the turtle eggs from near the Wheatley Harbour AOC is consistent with MacDonald et al. (1992). Since Algonquin Park has no known local PCB sources, the PCB concentrations reported here in the turtle eggs likely represents background airborne exposure to PCBs. Turtle eggs from Tiny Marsh appeared to have intermediate levels of PCBs compared to eggs from Algonquin Park and near the St. Clair River AOC, although there were no significant differences. It appears likely that non-*ortho* PCBs contribute most to TEQs, followed by total PCBs, and lastly by PCDD/Fs in turtle eggs in the Lake Erie basin.

There are multiple sources of dioxin throughout the Detroit River (Kannan et al., 2001), but the major sources of dioxins and furans appeared to be the Conners Creek sewer overflow (Kannan et al., 2001) and the outflow of the Monguagon Creek in the Trenton Channel (Marvin et al., 2002). Homologue profiles of dioxins and furans in the lower Detroit River suggested a point source, possibly from historical chlor-alkali production (Marvin et al., 2002). Dioxin levels at both Tiny Marsh and Alqonguin Park probably represent ambient or near ambient background levels. Although we do not know the source of dioxins at Wheatley Harbour, it is likely that the source was suspended sediments from the Detroit River.

The *p,p′*-DDE concentrations in the turtle eggs were highest in the areas near the Wheatley Harbour AOC and at Turkey Creek, similar in the eggs from Tiny Marsh, St. Clair NWA AOC and Canard River, and lowest in the eggs from Algonquin Park. A similar pattern was found with sum-chlordane and dieldrin concentrations, except that Algonquin Park was not different from any site except Turkey Creek and Wheatley Harbour AOC. Detroit River and particularly Wheatley Harbour AOC, have intensive pesticide use, which is reflected by the organochlorine pesticide levels in the eggs. The eggs collected from near the St. Clair River AOC, Tiny Marsh, and Canard River had similar levels of DDE, sum-chlordane, and dieldrin. Eggs from Algonquin Park generally had the lowest levels for each pesticide compound, and again likely reflect background exposure.

Bryan et al. (1987) suggested that the relative concentration of PCBs in various snapping turtle tissues (excluding blood) remained approximately the same regardless of the absolute concentrations. The ratio of chemical concentrations in adipose tissue and blood in humans and rats is equal to the ratio of lipid in adipose tissues and blood (Haddad et al., 2000), and is independent of octanol:water partition coefficients. This implies that the lipophilic compounds in the blood plasma should reflect that of the body burden. However, Russell et al. (1999) found that ratio of contaminants between eggs and muscle did not agree with the equilibrium partitioning model, and concentrations were approximately 2.4 times higher in muscle than eggs than expected based upon the ratio of lipids between tissues, even though partitioning was independent of octanol: water partition coefficients. Nevertheless, Martin et al.

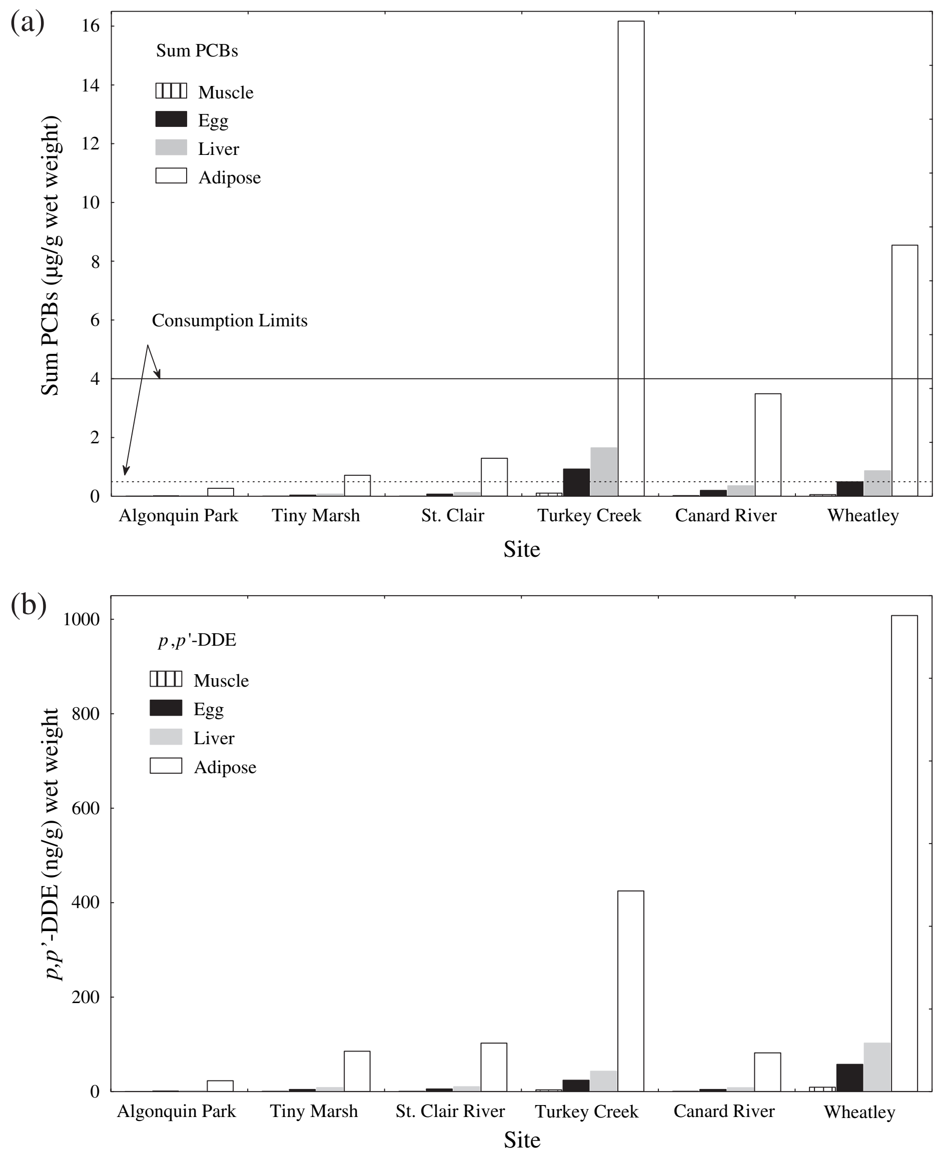

Fig. 4. (a) Mean sum PCBs measured in eggs, and estimated concentrations of sum PCBs in muscle, liver, and adipose tissue in the maternal snapping turtles (μg/g wet weight). Horizontal lines depict the partial (0.5 ppm) and total (4.0 ppm) consumption limit guidelines; (b) mean *p,p'*-DDE measured in eggs, and estimated concentrations of *p,p'*-DDE in muscle, liver, and adipose tissue in the maternal snapping turtles (ng/g wet weight). Muscle residues were estimated using egg:muscle ratios derived from Russell et al. (1999), whereas liver and adipose residues were estimated using egg:tissue ratios derived from Pagano et al. (1999).

(2003) found that contaminants measured in the blood of osprey chicks (*Pandion haliaetus*) better reflected local contamination than those measured in the eggs. However, migration may explain some of these species differences; migratory animals and their young may be exposed to different contaminant sources, whereas all life stages of turtles would be exposed to the same sources. In any case, Bishop et al. (1994) argued that the majority of the contaminants deposited in snapping turtle eggs were from the recent diet instead of body adipose stores.

The levels and patterns of mirex found in the turtle eggs were somewhat surprising. The largest source of mirex in the Great Lakes basin was Hooker Chemicals and Plastics Corporation on the Niagara River at the eastern end of Lake Erie, where mirex was manufactured (Sergeant et al., 1993). However, mirex was found in dreissenid mussels in western Lake Erie and Lake. St. Clair, but not eastern Lake Erie (Robertson and Lauenstein, 1998). Mirex concentrations in herring gull eggs progressively declined in an increasingly westerly direction across western Lake Erie (Weseloh et al., 1990). However, mirex concentrations did not show this geographical pattern here in the snapping turtle eggs: the highest and lowest mean concentrations of mirex occurred within the Detroit River AOC, with the more easterly Wheatley area eggs having the third highest mean concentrations (Table 2). Although the ground application of mirex has been banned since 1978 in the United States, unregistered use in fireworks was permitted until 1998 (Binational Toxics Strategy, 1998). Approximately 20 000 kg of mirex were sold to six companies within the Lake Erie watershed, most of which went to a company in Adrian, MI, near Detroit (Environment Canada, 1977). No mirex was manufactured in Canada. Although concentrations of mirex in dreissenid mussels are lower in Lake Erie than Lake Ontario, mirex may have been released in the area between western Lake Erie and Lake St. Clair (Robertson and Lauenstein, 1998), which is consistent with our data.

Octachlorostyrene was found at very low concentrations in the eggs from near the St. Clair River AOC, Wheatley Harbour AOC, and Turkey Creek, and was virtually undetectable in the eggs from the other sites. The only known important source of octachlorostyrene in the Great Lakes is from "taffy tar" generated by the use of graphite anodes during chlorine production (Kaminsky and Hites, 1984). The largest likely source of octachlorostyrene in the St. Clair and Detroit River AOCs was Dow Chemicals in Sarnia, through treated leachate from landfills and sewer runoff (Battelle Memorial Institute, 1998); up to 38, 160 ng/L of octachlorostyrene was measured in the sewer outflow (King and Sherbin, 1986). Currently, octachlorostyrene is likely no longer being produced in the Great Lakes, as the chlorine producing process was replaced in the early 1970s (Pugsley et al., 1985).

4.1. Implications for AOC remediation goals

Recent evaluation of contaminant inputs into Lake Erie have shown that the Detroit River is the largest single source of the majority of organics, including PCBs and organochlorine pesticides (Kelly et al., 1991). Although on a smaller scale, contaminant inputs into the Wheatley Harbour AOC are from local sources, largely through the processing of fish. These sources of contaminants in both the Detroit River and Wheatley Harbour AOCs are reflected in the relative contaminant burdens in turtle eggs collected from or near these sites. Although pesticide residues were generally low in the snapping turtle eggs, some of the PCB levels were high enough to be of concern based on the Ontario provincial guidelines. Ontario guidelines for sport fish consumption recommend that consumption be partially restricted when PCB levels exceed 0.5 ppm, and completely restricted at 4 ppm (OMOE, 2001). Snapping turtle eggs in some clutches from Turkey Creek, Canard River, and Wheatley Harbour exceeded the 0.5 ppm partial restriction guideline, and thus exceeds the minimum restriction level for consumption. No clutches exceeded the maximum restriction level of 4 ppm. The muscle tissue residues we estimated using previously published relationships between eggs and maternal tissues (Pagano et al., 1999; Russell et al., 1999) were below consumption restriction levels, but the concentrations estimated for livers and especially adipose tissues exceeded consumption restriction levels for all sites except Algonquin Park. Consumption of turtle meat may be problematic, as the inclusion of even small amounts of fat may dramatically elevate contaminant levels (see Bryan et al., 1987). Normal cooking methods for snapping turtle would likely include much more fat than what was present in the muscle tissue taken by Russell et al. (1999), thus our estimated muscle residues would underestimate actual exposure through consumption of turtle meat. Following these guidelines, human consumption of turtle eggs, and thus presumably adult turtles, would be partially restricted at both the Detroit River and Wheatley Harbour AOCs.

The main route of contaminant exposure for wildlife in aquatic ecosystems is the consumption of contaminated aquatic prey species such as fish. The Canadian Environmental Quality Guidelines have developed tissue residue guidelines to protect wildlife that consume aquatic biota in freshwater ecosystems (CCME, 1998). The guidelines are based upon the levels of contaminants in aquatic biota. The Environmental Quality Guideline for p,p'-DDE is 14.0 ng/g wet weight; some clutches at Turkey Creek exceeded this level, and all clutches at Wheatley Provincial Park exceeded this guideline. Although there are no guidelines for PCB concentrations, the CCME has guidelines for TEQs for PCBs; for mammalian species the maximum safe TEQ for PCBs is 0.79 ng/kg. Mirex was also measured in turtle eggs, but the concentrations were considerably lower than consumption guidelines.

Overall, we found that contaminant levels were sufficiently high in turtle eggs to be of concern in the Canadian AOCs, particularly near the Wheatley Harbour and Detroit River AOCs. At both AOCs, PCBs exceeded the concentrations set for both human consumption, and p,p'-DDE and sum PCB concentrations exceeded those set by the Environmental Quality Guidelines. Furthermore, the concentrations of these various contaminants may be associated with changes in the reproduction and development of snapping turtles as indicated by preliminary findings (Fernie et al., unpublished manuscript).

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