Contaminant Body Burdens in Wildlife Populations in the St. Clair River Area of Concern

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Executive Summary

Concentrations of organohalogens in frogs from the St Clair River AOC in 2006 and 2007 were generally lower than, or comparable to those in frogs from historically contaminant sites or other AOCs. Otherwise, body burdens were similar to frogs from reference sites. Concentrations of mercury were significantly higher in frogs from parts for the St Clair River AOC relative to frogs from other AOC locations and reference sites. Concentrations of most organochlorines, including sum PCBs, in snapping turtle eggs from St Clair River AOC in 2011 were generally similar to those from reference sites, and were ranked as intermediate relative to eggs from other Great Lakes sites including AOCs. Mercury concentrations in St Clair River AOC eggs were highly variable but among the highest relative to other Great Lakes sites, and were higher than concentrations in eggs from one of two reference sites. No temporal declines in concentrations of organochlorines or mercury were detected in turtle eggs from the 1990s to 2011, which was partially due to limitations in sampling during this time period. Hepatic concentrations of organochlorines in mallards from the St. Clair River area declined between 80.0 and 99.0% from 1985 to 2010. Hepatic PCB and DDT concentrations in liver of mink from the Walpole Delta in 2002 were similar to those from the references sites, and were among the lowest found relative to other Great Lakes sites including other AOCs from 1998-2006. Despite the mink from the St Clair AOC having the highest burdens of mercury relative to other Great Lakes sites, concentrations were similar to one non-AOC reference site and were lower than those from sites elsewhere in Canada where elevated mercury exposure was reported. Concentrations of PCBs and DDT in frogs from the St Clair River AOC in 2006 and 2007 were below those associated with toxic effects in amphibians. Although from 2 areas in the St Clair River AOC had mercury burdens greater than those found in one other study showing toxicity of mercury, there was no evidence of increased incidence of embryonic or young of year deformities frogs at these two AOC locations. Burdens of mercury in turtle eggs were below the only known threshold for mercury. Hepatic PCB, DDT and mercury concentrations in canvasbacks and mallards were below known thresholds for adverse effects, whereas selenium concentrations in one bird exceeded the threshold associated with toxic effects. Hepatic PCB and mercury concentrations in mink were well below maternal concentrations that associated with increased kit or adult mortality. There is some risk to higher trophic-level consumers feeding within the St. Clair River AOC, however, there are a number of factors that influence risk including the type, amount and frequency of tissues consumed and these were not quantified in this study.

Introduction

As an important Great Lakes shipping channel and source of water for local industries, the St. Clair River is approximately 64 kilometres in length and flows southe from Lake Huron and into Lake St. Clair. Prior to entering Lake St. Clair, the River branches into several channels creating an extensive delta known as the Walpole Delta that includes Walpole Island First Nations' Territory. The St. Clair River was designated by the International Joint Commission (IJC) as one of 43 Great Lakes Areas of Concern (AOC) where environmental degradation was thought to be causing harm to the broader Great Lakes ecosystem. The binational AOC includes the main river, its delta channels, and coastal watersheds in Ontario and Michigan. Historical industrial and municipal point sources in the upper reaches of the St. Clair River

have contributed significant loadings of pollutants to the river which have been detected in water, sediment, and biota as well as in regions downstream including Lake St. Clair (Oliver and Bourbonniere 1985; Pugsley *et al.*, 1985; OMOEE and MDNR 1991, 1995). Contaminants of concern associated with these sources include hexachlorobenzene (HCB), octachlorostyrene (OCS), polychlorinated biphenyls (PCBs), and heavy metals such as mercury. Following the designation of the St. Clair River as an Area of Concern (AOC) in 1985, restrictions on discharges, improved industrial and municipal practices as well as sediment remediation projects have reduced contaminant loadings to the river (Mayne, 2008). However, evidence of elevated concentrations of mercury, PCBs and other organochlorines in juvenile spottail shiner (*Notropis hudsonius*) from the St. Clair River suggest that historically-contaminated sediment in the river might be a continued source of chemicals to the food web (Gewurtz *et al.*, 2010).

Degradation of fish and wildlife populations is one benefical use impairment (BUI) used to assess environmental degradation in AOCs. One subcomponent of this BUI relates to assessing body burdens, i.e., the bioaccumulation of contaminants, in fish and wildlife. Contaminants such as PCBs and mercury that accumulate to sufficiently high concentrations in biota can impair reproduction and survivability and, in extreme cases, result in population-level effects. The purpose of this report is to assess whether contaminant burdens in wildlife are sufficiently elevated to adversely impact survivability of wildlife species which feed from the aquatic ecosystem in the AOC. Wildlife species studied include the northern leopard frog (*Rana pipiens*), snapping turtle (*Chelydra serpentina*), canvasbacks (*Aythya valisineria*) and resident mallards (*Anas platyrhynchos*) and mink (*Mustela vison*). All have an aquatic-based diet, accumulate contaminants and are sensitive to the effects of contaminants. Consequently, these wildlife species are frequently used as bio-indicators of contaminants and their effects on wildlife health (Martin *et al.*, 2006, 2011, 2015; de Solla *et al.*, 2007). These species also represent different trophic levels which allows for a more thorough assessment of wildlife body burdens within the St. Clair River AOC food web.

Based on evidence from extensive laboratory and field studies, threshold concentrations for specific compounds and associated with effects on survival have been determined or proposed for some species (e.g., PCBs in mink) and groups of species (e.g., mercury in avian species). For most compounds, however, threshold effect levels resulting in impaired survival in specific species do not exist. Therefore, in order to provide an assessment of body burdens that may impact wildlife at the population-level and also that are specific to localized effects within the AOC, a four-pronged approach is proposed: 1) assess spatial trends in contaminant burdens in the AOC relative to burdens outside of the AOC at suitable Great Lakes reference site(s); 2) assess temporal trends to demonstrate that burdens have declined following remediation activities within the AOC, if historic data are available; 3) assess body burdens against thresholds on survival (if available), and; 4) assess risk to higher trophic-level consumers which feed on these indicator species using fish flesh criteria and Canadian Environmental Quality Guidelines for the protection of wildlife consumers of aquatic biota where available (Newell *et al.*, 1987; CCME, 2001). Table 1 provides a summary of contaminants data available for wildlife species within the AOC for each of the four categories. This multi-faceted and multi-species approach will be used to assess the current status of the body burdens component of the wildlife populations BUI. Contaminants data are

presented on a species-by-species basis followed by an overall summary of risk to higher trophic-level consumers that feed on these species.

Table 1. Contaminant body burden data used to assess survivability of wildlife populations in the St. Clair River AOC. Wildlife data that are available (√) and not available (X) are indicated for assessement purposes. Risk to higher trophic-level consumers are based on concentrations in wildlife tissue that exceed fish flesh criteria and Canadian Environmental Quality Guidelines which are used to assess protection to wildlife that consume these species (Newell *et al.*, 1987; CCME, 2001).

Species	**Spatial Trends**		**Temporal Trends**	**Survival Threshold**	**Risk to Higher Trophic-Level Consumers**
	AOC	**Reference**			
Northern Leopard Frog	√	√	X	X	√
Snapping Turtle	√	√	√	X	√
Canvasback & Resident Mallard	√	X	√ - Mallards	√ - Mercury & Selenium	√
Mink	√	√	X	√ - PCBs	√

With the exception of mink, body burdens for these species have been previously summarized in earlier St. Clair River AOC reports which documented the results of wildlife studies examining reproductive health and development (see Hughes *et al.*, 2014a,b, 2015 for further details of studies and methods). These data have also used to assess the status of the reproduction and deformities in wildlife BUI in the AOC. Contaminants in liver of mink trapped at sites in the lower Great Lakes basin including the St. Clair River AOC have been previously reported in Martin *et al.* (2006, 2011, 2015).

Methods

i) Contaminant Analyses

Detailed methods for chemical analyses can be found in de Solla et al. (2007), Simon and Wakeford (2000) and Martin et al. (2015), and further, including details on ensuring comparability of contaminant data obtained from different laboratories or methods, in Hughes et al., (2014a; 2014b; 2015). A very brief summary of the methods used is outlined here.

The tissues used for chemical analyses and the analytes targeted varied among leopard frog, snapping turtle, mink and canvasback studies, as did the laboratories that did the analyses. However, in general similar methods were used for each study organism, and where applicable efforts were made to ensure the data were comparable for each comparison. PCBs (individual congeners) and organochlorine pesticides (*p,p'*-DDE, sum chlordane, dieldrin and others) were measured at the National Wildlife Research Centre (NWRC) in Ottawa or the Great Lakes Institute of Environmental Research (GLIER) at the University of Windsor in Windsor, Ontario. PCBs and OC pesticides were performed using capillary gas chromatography using a mass selective detector (GC-MSD) or electron capture detection (GD-ECD). Brominated flame retardants (BFRs) including polybrominated diphenyl ethers (PBDEs) were quantified

at the NWRC or GLIER using GC-MSD. Dioxins, furans, and non ortho PCBs were analyzed at NWRC using high resolution GC-MSD.

Chemical analyses for total mercury were conducted at NWRC or the Environmental Analytical Laboratories (EAL) at Laurentian University in Sudbury, Ontario, using an Advanced Mercury Analyzer (AMA-254), or a Direct Mercury Analyzer (DMA-80). Methylmercury was measured using cold vapour atomic fluorescence spectrometry (CVAFS). Chemical analyses for selenium (Se) were conducted at the EAL using hydride generation atomic fluorescence spectrometry, respectively. Other metals were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) or by graphite furnace atomic absorption spectrometry (GFAAS).

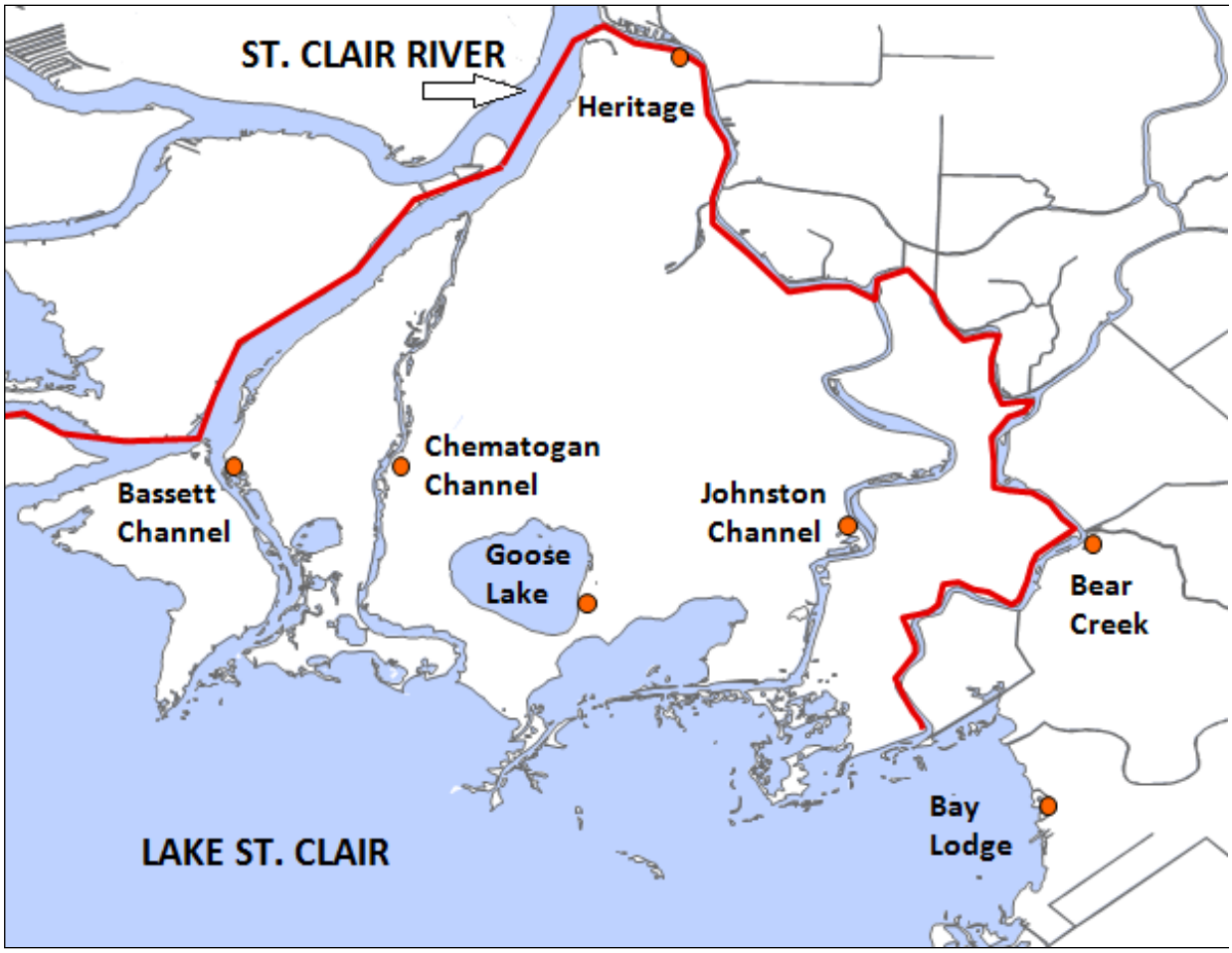
Organohalogen concentrations are reported on a wet weight basis in ng/g and metals are reported on a dry weight basis in µg/g unless indicated otherwise.

ii) Northern Leopard Frogs

Maturing pre-hibernation young-of-year northern leopard frogs were captured by dip net at seven St. Clair River AOC locations (including five locations within the Walpole Island First Nations Territory) and two reference sites in 2006 and 2007 (Figure 1). Frogs were collected at four AOC locations, Bassett Channel, Goose Lake, Johnston Channel, and Heritage, in September of 2006 and an additional three AOC locations, Chematogan Channel, Bear Creek and Bay Lodge, in October of 2007. Frogs were also collected from two reference sites on the south shore of Lake Huron located upstream of the AOC at Port Franks (at the mouth of Mud Creek and the Cut) and at Wood Road which is a series of boating channels adjacent to Kettle Point First Nations. Following collection, frogs were transported to the laboratory where they were euthanized and the kidney/gonad complex removed and preserved for histological assessment. The remainder of the frog was stored at -20^{o}C prior to chemical analysis.

Organochlorine pesticides, PCBs and mercury were analyzed in whole bodies of frogs (minus gonads/kidneys and spleens); frogs were analyzed as pools consisting of two same sex frogs with 5-7 pools of frogs analyzed per site in the two study years, whereas mercury was analyzed on individual frogs in 2006 and 2007 with 9 or 10 individuals analyzed per site.

Figure 1. Study locations within the St. Clair River AOC where northern leopard frogs were assessed for contaminant body burdens in 2006 and 2007. The red line denotes the boundary of the Walpole Island First Nations Territory.



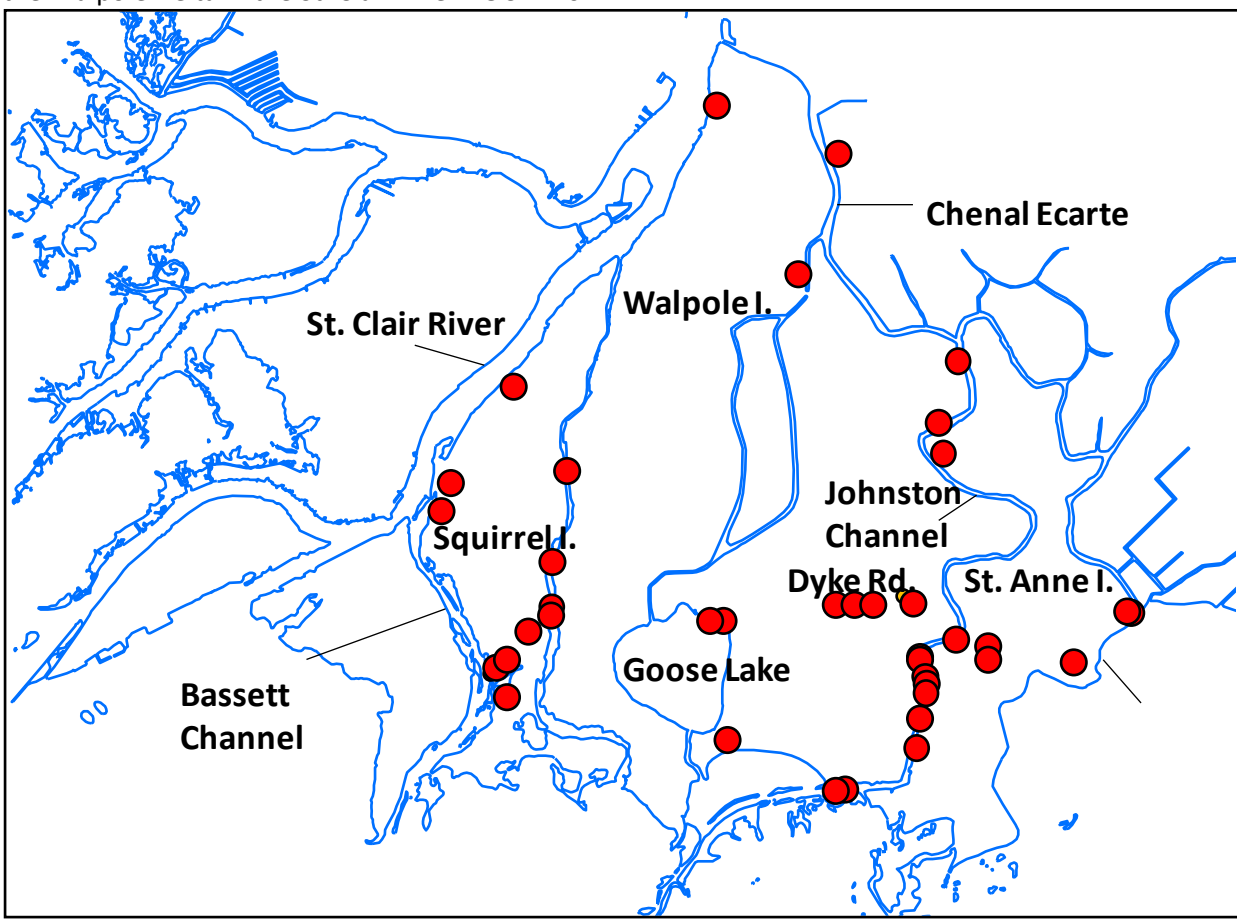
iii) Snapping Turtle Eggs

Snapping turtle eggs were collected from 41 locations in the downstream Canadian portion of the St. Clair River AOC in the Walpole Delta in 2011 (Figure 2). Clutches were collected from the three major islands of the Delta, Squirrel Island, Walpole Island, and St. Anne Island, which were also proximate to four longest channels of the Delta (Bassett Channel, Chematogan Channel, Johnston Channel, and Chenal Ecarte) as well as the lower St. Clair River. Eggs were also collected from a few inland locations at Goose Lake and along Dyke Road on Walpole Island. Three reference non-AOC sites were selected for comparison purposes: 1) Long Point Beach at Long Point on Lake Erie (42°35' N, 80°27' W) in 2013; 2) the St. Clair NWA site consisting of two areas on Lake St. Clair approximately 13 km south of the AOC boundary at Big Point Hunt Club (42°25' N, 82°24' W) and the St. Clair National Wildlife Area (42°23' N, 82°25' W) where eggs were collected in 2001, and; 3) Tiny Marsh located upstream near Elmvale and inland of Lake Huron (44°36' N, 79°56' W) where eggs were collected in 2001-2003.

Entire clutches of eggs were collected within 48 h of oviposition. A subset of eggs, usually five, was selected for contaminant analysis from 37 clutches, and the remainding of eggs from each clutch were

incubated to assess hatching success and frequencies of hatchling deformities; results were reported in Hughes *et al.* (2015). Eggs were analyzed for PCBs, organochlorine pesticides, mercury and

Figure 2. Collection locations of 37 snapping turtle clutches that were analyzed for contaminants in the Walpole Delta in the St. Clair River AOC in 2011.



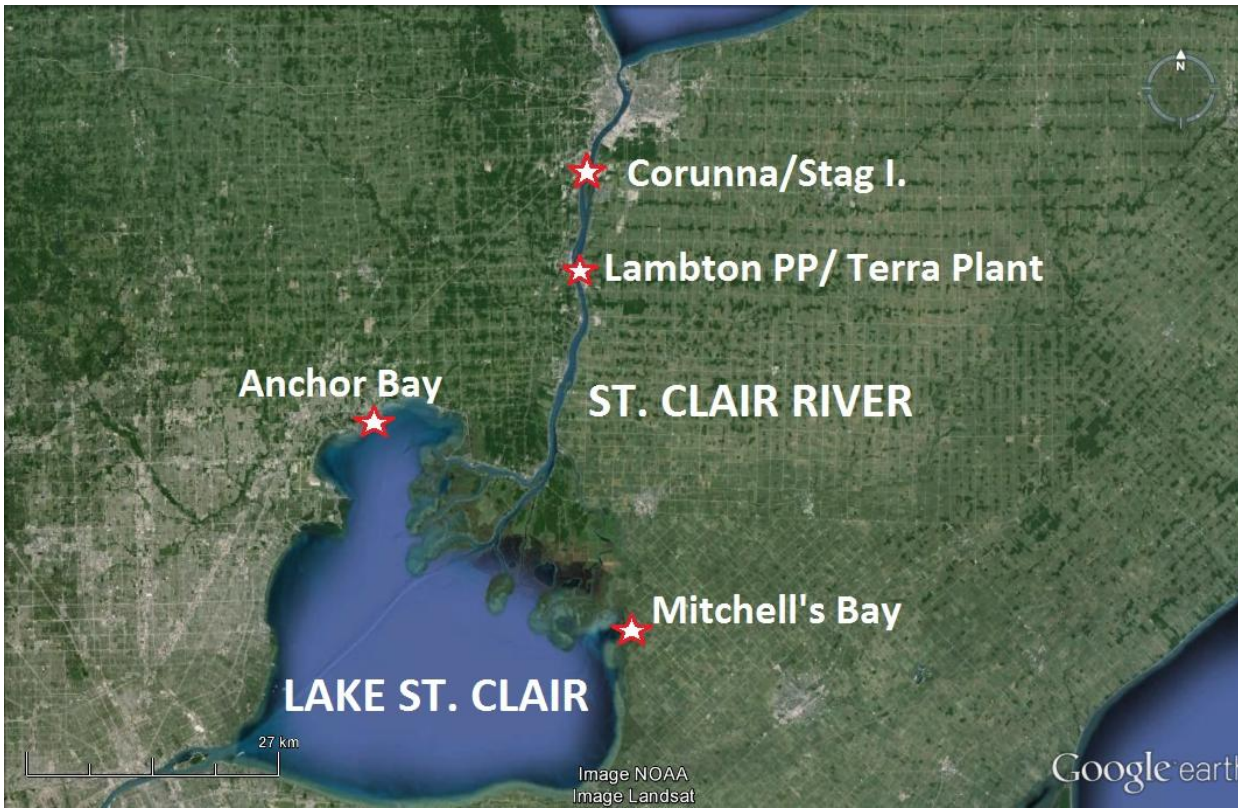
methylmercury. No mercury data are available for eggs collected from the St. Clair NWA reference site in 2001 for comparison purposes.

iv) Canvasbacks and Mallard Liver

Specimen collection for this study was part of a large research project conducted by the Long Point Bird Observatory examining wintering ecology of canvasbacks and redheads (*Aythya americana*) on Lake St. Clair and the Detroit and St. Clair rivers. Canvasbacks (N=148) were shot by firearms over a four month period in 2008/09 from the Lake St. Clair/St. Clair River study area. Birds were collected in November and December from Lake St. Clair where this species typically forages in shallow, open water habitats in the fall and early winter. Following freeze-up of Lake St. Clair, birds move to the open, fast water areas of the St. Clair River where they were collected in January and February. Livers from a subsample of 30 birds consisting of five males and 25 females were selected for contaminant analyses. Collection months and numbers of birds were as follows: November (8), December (8), January (8) and February (6). Three resident mallards were also collected from the study area within the Walpole Delta in the Walpole Island

First Nations Territory in November of 2010. Organohalogens, total mercury (Hg) and selenium (Se) were analyzed in the bird livers.

Figure 3. Main collection locations of canvasbacks collected in November and December of 2008 and January and February of 2009.



v) Mink Liver

Ten wild mink were trapped by licensed trappers at sites on the Walpole Delta within the St. Clair River AOC in 2002 (Figure 4). For comparison purposes, wild mink were also collected from three reference non-AOC sites: 1) Lake St. Clair and within four kilometres of the lake shoreline where 16 mink were trapped in 1999, 2001, 2003 and 2004, 2) Long Point and within one kilometre of the shoreline where 18 mink were trapped at Big Creek Marsh in 1998 and 1999, and; 3) inland Erie where 16 mink were trapped at collection sites ranging between 8 and 38 kilometres inland of the Lake Erie shoreline in 1999.

Livers from mink carcasses were analyzed for organochlorine contaminants in individual mink trapped at Walpole Island, Lake St. Clair, Long Point and inland Erie. Concentrations of dioxin-like PCBs (i.e., non-*ortho* PCBs #77, 126, 169 and 189), dioxins and furans were measured in pooled liver samples of mink collected from Walpole Delta, Long Point and four other Great Lakes sites between 1998-2002. Pooled samples consisted of livers from 4-6 mink per site. Concentrations of 2,3,7,8-TCDD toxic equivalents

(TEQs) were calculated for dioxin-like PCBs, dioxins and furans in livers of mink from all sites using the most recent toxic equivalency factors reported by Van den Berg *et al.* (2006) for mammals.

Total mercury, selenium, arsenic, cadmium and lead were measured in livers of individual mink trapped within the St. Clair River AOC. Metal burdens of three mink trapped in 2003 in the southwestern Ontario region and 10-12 km inland of either Detroit River, western Lake Erie or Lake St. Clair are also included as inland Erie mink for spatial comparisons.

Figure 4. Study area of mink collection sites within the St. Clair River AOC at Walpole Delta in 2002 and Long Point, Lake St. Clair, and at inland Erie sites (i.e., two main locations) from 1998-2004. General locations where mink were trapped are indicated by an arrow or box.

Statistics:

Spatial comparisons were made using a one-way ANOVA and, when significant, means were compared using Tukey's pairwise comparisons. If data failed the assumption of equal variance, the data were either $\log_{10}$ transformed or comparisons were made using a Kruskal Wallis test; post-hoc tests were conducted using non-parametric multiple comparison tests for unequal sample sizes (Zar, 1984). Concentrations of organochalogens were expressed as wet weight and metal data as dry weight. All results were considered significant at $p<0.05$.

Since differences in lipid content can influence contaminant burdens, comparisons of percent lipid content in tissues were conducted prior to spatial comparisons of organochlorine burdens. Significant spatial differences in percent lipid content were found in turtle eggs ($p=0.002$) and mink liver ($p=0.02$).

Following this, organochlorine data for these species were analyzed using an Analysis of Covariance (ANCOVA) using percent lipid as the covariate. When the condition of homogeneity of slopes was met, site effect was examined using an ANCOVA followed by Tukey's pairwise comparisons to examine significant differences in means among sites. Percent lipid content in frogs did not vary significantly among study sites and contaminants data were analyzed using a one-way ANOVA as described above.

In general, contaminant concentrations below detection limits were expressed as one-half of the detection limit. However in turtle eggs, for individual PCB congeners and other organochlorines that were below the minimum detection limit, maximum likelihood estimation was generally used to calculate replacement values. Further details of this method are found in Hughes *et al.* (2015).

Results & Discussion

i) Northern Leopard Frogs - Spatial Trends:

Significant spatial differences in mean concentrations of organochlorine compounds were evident in northern leopard frogs collected from the St. Clair River AOC and reference study sites in 2006 and 2007 (Table 2). PCB concentrations were significantly higher in frogs from Chematogan Channel versus those from Heritage and the two upstream Lake Huron reference sites, Wood Road and Port Franks. Sum PCBs in frogs were low with means generally ranging between 1-2 ng/g at study sites and concentrations below 5.3 ng/g in all samples, with one exception of a pooled sample from Wood Road with a high sum PCB concentration (37.4 ng/g).

Total DDT concentrations, as the sum concentration of *p,p'*-DDE (91.8% of total DDT) , *p,p'*-DDT and *p,p'*-DDD, were largely similar to sum PCB concentrations with means ranging from 1-4 ng/g at most study sites (Table 2). Frogs from Johnston Channel had a significantly higher mean total DDT concentration than frogs from Bassett Channel, Goose Lake, Bear Creek, and Bay Lodge with frogs from the reference sites showing intermediate mean total DDT concentrations. Concentrations of total DDT were highest in two pools of frogs from Johnston Channel (25.3 ng/g and 18.9 ng/g).

Mean concentrations of the remaining organochlorine compounds in frogs from study sites were at most one-third of total DDT concentrations, generally below 1 ng/g and found below the limit of detection for some compounds at some sites (Table 2). With the exception of heptachlor epoxide (HE), significant differences in mean concentrations of these compounds were found among study locations. The mean Sum chlordane was highest (1.25 ng/g) in frogs from Port Franks, but the only significant difference was that concentrations were significantly higher in frogs from Chematogan Channel compared to those from Wood Road and Bassett Channel. Concentrations of deildrin were very low or below MDL in frogs from all sites except for three pooled samples of frogs from Johnston Channel (range=2-8 ng/g). HE and Hexachlorobenzene (HCB) were both very low among all sites, with the only difference in that HCB was significantly higher in frogs from Bassett Channel compared to Goose Lake Goose Lake.

While also considered extremely low, relatively higher concentrations of OCS were found in frogs from Bassett Channel where the mean OCS concentration (0.41 ng/g) was significantly higher relative to frogs from Chematogan Channel, Bear Creek and Bay Lodge. Mirex was not detected in any samples.

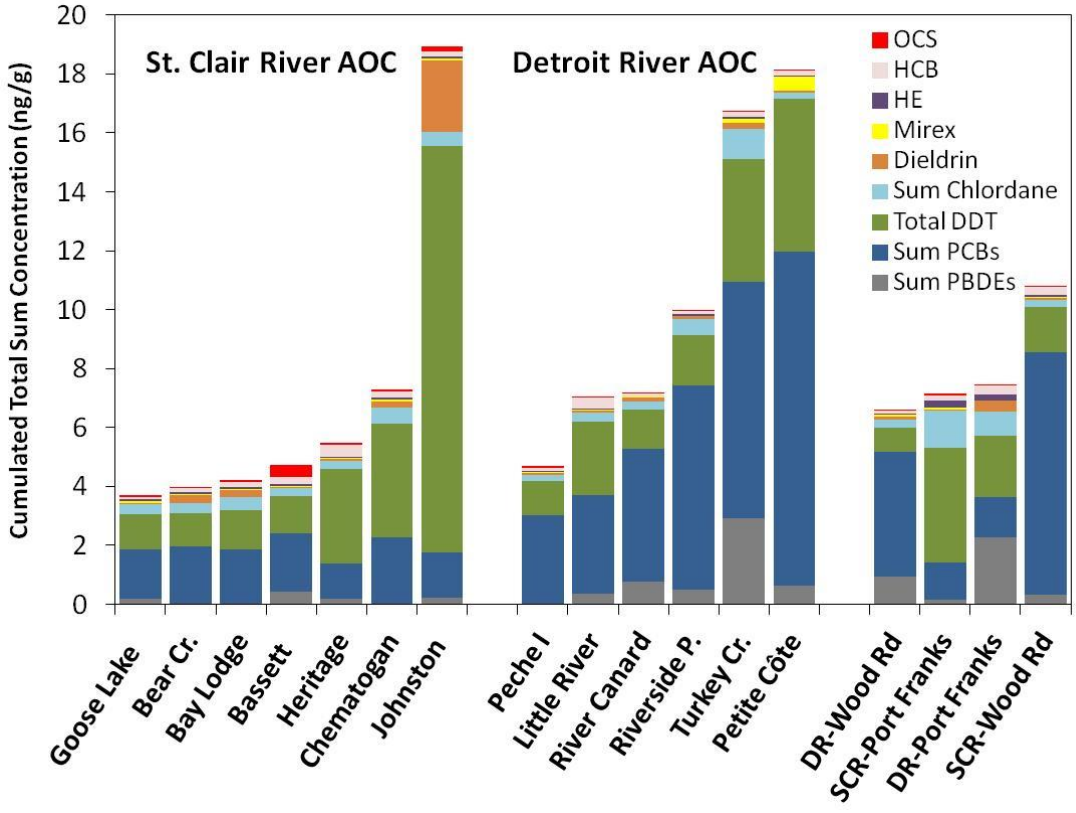
Table 2. Mean (SD) and maximum concentrations (ng/g, wet weight) of organochlorine compounds and sum PBDEs in northern leopard frogs from seven St. Clair River AOC locations and two upstream reference sites in 2006 and 2007. N denotes the number of pooled samples analyzed each consisting of two individuals of the same sex. ND denotes below the level of detection. Means sharing the same uppercase letters are not significantly different; a significant spatial difference was found overall for dieldrin but not between individual site comparisons in the post-hoc analysis.

Site	N	Sum PCBs	Total DDT	*p,p'*-DDE	Sum Chlordane	HCB	Dieldrin	HE	OCS	Sum PBDEs
Bassett Channel	5	1.97 (0.21) 2.13 **AB**	1.28 (0.31) 1.72 **B**	1.18 (0.31) 1.62 **BC**	0.25 (0) 0.25 **B**	0.26 (0.06) 0.31 **A**	ND	ND	0.41 (0.20) 0.63 **A**	0.43 (0.18) 0.73
Goose Lake	5	1.66 (0.22) 1.74 **AB**	1.21 (0.27) 1.61 **B**	1.11 (0.27) 1.51 **BC**	0.31 (0.15) 0.60 **AB**	0.09 (0.04) 0.17 **B**	ND	0.07 (0.05) 0.16	ND **ABC**	0.20 (0.07) 0.30
Heritage	5	1.22 (0.18) 1.47 **B**	3.20 (1.71) 6.17 **AB**	3.10 (1.71) 6.07 **ABC**	0.27 (0.03) 0.32 **AB**	0.40 (0.53) 1.35 **AB**	ND	ND	ND **ABC**	0.18 (0.03) 0.23
Johnston Channel	5	1.54 (0.15) 1.72 **AB**	13.80 (8.31) 25.44 **A**	13.70 (8.31) 25.34 **A**	0.48 (0.31) 0.97 **AB**	0.16 (0.04) 0.18 **AB**	2.44 (3.19) 8.00	0.08 (0.06) 0.18	0.19 (0.10) 0.28 **AB**	0.21 (0.08) 0.34
Chematogan Channel	7	2.28 (0.41) 3.04 **A**	3.84 (4.25) 12.02 **AB**	3.35 (3.83) 10.95 **ABC**	0.54 (0.15) 0.76 **A**	0.20 (0.06) 0.26 **AB**	0.24 (0.09) 0.37	0.07 (0.03) 0.13	0.06 (0.02) 0.11 **C**	-
Bear Creek	6	1.95 (1.63) 5.27 **AB**	1.13 (0.61) 2.26 **B**	0.99 (0.60) 2.16 **C**	0.33 (0.06) 0.43 **AB**	0.14 (0.03) 0.17 **AB**	0.28 (0.32) 0.91	ND	ND **C**	-
Bay Lodge	6	1.85 (0.51) 2.79 **AB**	1.33 (0.35) 1.79 **B**	1.17 (0.27) 1.51 **BC**	0.45 (0.03) 0.51 **AB**	0.19 (0.02) 0.21 **AB**	0.24 (0.10) 0.37	ND	0.06 (0.02) 0.10 **BC**	-
Port Franks	5	1.27 (0.18) 1.48 **B**	3.90 (0.97) 4.81 **AB**	3.80 (0.97) 4.71 **AB**	1.25 (1.22) 3.20 **AB**	0.17 (0.07) 0.27 **AB**	ND	0.26 (0.47) 1.09	ND **ABC**	0.15 (0.03) 0.19
Wood Rd.	5	8.24 (16.28) 37.4 **B**	1.54 (0.37) 1.94 **AB**	1.44 (0.37) 1.84 **ABC**	0.25 (0) 0.25 **B**	0.27 (0.46) 1.10 **AB**	ND	ND	ND **ABC**	0.31 (0.44) 1.10

Mean sum PBDE concentrations ranged from 0.15 ng/g in frogs from Port Franks to 0.43 ng/g in frogs from Bassett Channel. The dominant congeners were BDE-47, BDE-99, BDE-100 and BDE-153 which contributed on average 96.8% to the total sum PBDEs in frogs (range=93.8-100%). One frog from Wood Road had both the highest concentrations of sum PBDEs (1.10 ng/g) and sum PCBs (37.4 ng/g) and was the only frog where hexabromocyclododecane (HBCDD) was detected (0.02 ng/g). BB-101 was below the limit of detection in all samples. While sum PBDE concentrations in frogs were not significantly different among sites sampled in 2006, they were found at higher concentrations relative to some organochlorine compounds. No PBDE data are available for frogs collected from AOC locations in 2007.

A Great Lakes perspective of contaminants in northern leopard frogs represented as a cumulative total sum concentration of mean sum PCBs, seven organochlorines and PBDEs (where data are available) from the St. Clair River and Detroit River locations from 2006-2009 is provided in Figure 5. Total burdens in St. Clair River AOC frogs were generally lower than, or comparable to, total burdens in Detroit River AOC frogs where PCBs burdens were more predominant as well as the upstream Lake Huron reference sites. Frogs from Johnston Channel were the exception where notably high mean DDT and dieldrin concentrations contributed to the highest total burden found in frogs relative to other locations.

Figure 5. Cumulative total sum concentration (ng/g, wet weight) of mean sum PCBs, seven organochlorines and sum PBDEs (where available) in northern leopard frogs from St. Clair River (SCR) AOC locations, Detroit River (DR) AOC locations and two upstream reference sites, Wood Road and Port Franks. Collection years were 2006 and 2007 for SCR locations, 2008 and 2009 for DR locations, and all four years for reference sites. No PBDE data are available for Bear Creek, Bay Lodge, Chematogan, and Peche I. Each location is ranked from the least contaminated to the most contaminated.



Overall concentrations of sum PCBs in frogs from the St. Clair River AOC were generally low (i.e., below 3 ng/g) and were well below concentrations found in leopard frogs from known contaminated ecosystems. These include polluted sites in the Fox River and Green Bay watershed where PCBs in whole bodies of juvenile leopard frogs were notably elevated (10-502 ng/g; Karasov *et al.*, 2005) and an area contaminated by a smoke plume from a PCB warehouse fire (analyzed as composite samples of green frogs (*Rana clamitans*) and leopard frogs, 50-112 ng/g; Phaneuf *et al.*, 1995). Sum PCB concentrations at AOC locations were lower than mean concentrations reported in leopard frogs collected from seven lower Great Lakes sites in the mid-1990s (range=2.8-15.7 ng/g, based on 1.2% lipid content and a factor of 0.8 for conversion of 1:1 PCBs to sum PCBs; Gillan *et al*., 1998). Relatively higher sum PCB concentrations found in frogs from the Detroit River AOC is also consistent with spatial patterns for PCBs in suspended sediments collected from sites on the Detroit River compared to the St. Clair River and Lake St. Clair (Jia *et al.*, 2010).

With respect to concentrations associated with toxic effects in amphibians, no evidence of toxicity was found in northern leopard frogs with a sum PCB body burden of 152 ng/g (Huang *et al.*, 1999). Wood frog tadpoles exposed to PCB-contaminated sediment for 42 days had an increased mortality rate of 10%, increased behavioural abnormalities and a body burden of over 22,000 ng/g while those exposed to control sediment had less than 2% mortality and a body burden of 24 ng/g (Savage *et al*., 2002; Hughs et al). Given that PCB body burdens in this study were far below these concentrations, it is highly unlikely that current PCB body burdens in frogs from the AOC are associated with toxic effects.

Concentrations of *p,p'*-DDE in northern leopard frogs from St. Clair River AOC locations were several orders of magnitude lower than concentrations reported in spring peepers (*Pseudacris crucifer*) from Point Pelee National Park where DDT was used 26 years prior in recreational areas for mosquito control (mean=1,001 ng/g; Russell *et al.*, 1995). DDT concentrations in all St. Clair River frogs were well below the lowest reported toxic effect concentration of 2,400 ng/g in common frog (*R. temporaria*) tadpoles (Cooke, 1972). Largely comparable concentrations of DDT and PCBs found in St. Clair River frogs likely reflect the biphasic life history of this species and the primary difference in exposure through food sources (i.e., aquatic versus terrestrial) between the two compounds.

Mean total mercury (±SD, dry weight) concentrations in frogs from St. Clair River AOC locations and reference sites ranged from 78.98 (±17.47) ng/g in frogs from Bear Creek to 319.01 (±40.34) ng/g in frogs from Bassett Channel (Figure 6; range in mean concentrations on a dry ~~wet~~ weight basis from 18.20 (±4.39) ng/g to 72.01 (±16.14) ng/g)). Overall, mercury concentrations varied significantly among study sites with significantly higher mean concentrations in frogs from Bassett Channel and Goose Lake compared to mean concentrations found in frogs from the other study sites including the two reference sites. The maximum concentration found was in a frog from Bassett Channel at 382 ng/g dry weight (or 106 ng/g wet weight).

Mercury concentrations in frogs from Bassett Channel and Goose Lake were also elevated compared to those from the Hamilton Harbour AOC and reference sites that included Long Point in 2013 (Figure 7). No mercury data are available for frogs collected from the Detroit River AOC in 2008 and 2009 for comparison purposes.

Mercury concentrations in frogs from AOC locations were well below concentrations found in the American toads (*Bufo americanus*) collected from the South River, a mercury-contaminated river in Virginia, where mean concentrations were 2,100 ng/g dw in tadpoles and 600 ng/g dw in adults (Bergeron *et al*., 2010a). In general, mercury concentrations in frogs in this study were on par with concentrations reported in other amphibian species in North America. Similar mercury concentrations were found in one-year-old green frog and bullfrog (*Rana catesbeiana*) tadpoles from Acadia National Park (ANP) in Maine in 2003 (means=25 ng/g and 19 ng/g wet weight, respectively; Bank *et al.*, 2007).

Total mercury concentrations in carcasses (without guts) of southern leopard frog (*Rana sphenocephala*) tadpoles collected from ten wetlands in South Carolina were also within the range found at AOC locations in this study (range in means=90-320 ng/g dw; Unrine *et al.*, 2005).

While the effects of mercury exposure have been documented in numerous piscivorous fish, bird and mammal species (Scheuhammer *et al.*, 2007), relatively fewer studies have examined the toxic effects of mercury in amphibians. As a surrogate threshold for toxic effects of mercury exposure in amphibians, Bergeron *et al.* (2010a) used a whole-body tissue threshold-effect level of 200 ng/g methylmercury (wet weight) proposed to be protective of juvenile and adult fish (Beckvar *et al*., 2005). Methylmercury was not quantified in this study, however, concentrations of total mercury (which is comprised in part of methylmercury) in leopard frogs from the AOC were well below this concentration. In contrast, mean total mercury body burdens of 240 and 400 ng/g dw in southern leopard frog tadpoles were associated with adverse effects including increased mortality, malformations, and increased time to tail resorption

Figure 6. Mean concentrations (SD, ng/g, dry weight) of total mercury in northern leopard frogs collected from seven St. Clair River AOC locations and two upstream reference sites, Port Franks and Wood Road, in 2006 and 2007. Nine or ten individuals were analyzed per site. Means sharing the same uppercase letters are not significantly different

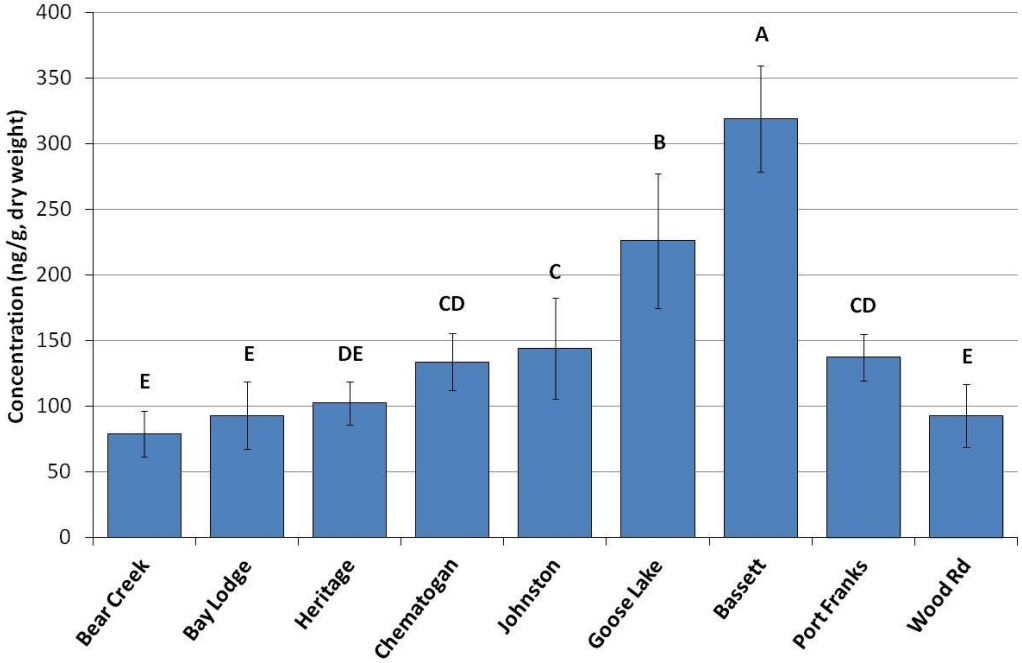
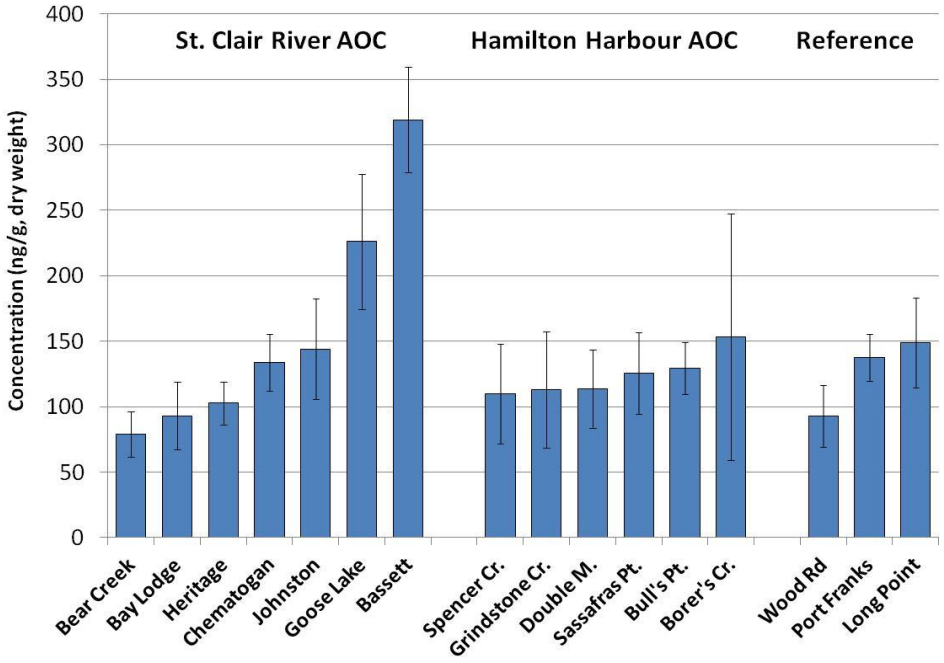


Figure 7. Mean concentrations (SD, ng/g, dry weight) of total mercury in northern leopard frogs collected from St. Clair River (SCR) AOC locations, Hamilton Harbour (HH) AOC locations and reference sites, Wood Road, Port Franks, and Long Point. Collection years were 2006 and 2007 for SCR locations, 2012 and 2013 for HH AOC locations, and 2006, 2007 or 2013 (Long Point) for reference sites. Each location is ranked from the least contaminated to the most contaminated.



when exposed to experimental diets of mercury-enriched a*ufwuchs* (Unrine *et al.*, 2004). Young-of-year northern leopard frogs from Goose Lake and Bassett Channel had comparable mercury concentrations (means=226 ng/g and 320 ng/g dw, respectively) which suggests that they might be potentially exposed to concentrations of mercury associated with such adverse effects. However, there was no evidence of increased incidence of embryonic deformities in lab studies or prevalence of deformities in wild populations of frogs was found at these two AOC locations (Hughes *et al.,* 2014a). Other factors for consideration include the concentration of methylmercury present (as the most toxic form of mercury) which is not known in these frogs and which may differ from concentrations in the Unrine *et al.* (2004) study. As well, the life stages of leopard frogs differ between these two studies (i.e., tadpoles vs young-of-years) so it is difficult to compare potential effects associated with these concentrations. Amphibian species may also vary in their sensitivity to mercury. Wada *et al.* (2011) found no adverse effects of dietary mercury on development or survival of wood frogs (*Rana sylvatica*) with mean body burdens that were much higher than concentrations reported in this study. The presence and bioavailability of selenium may also influence and possibly mitigate the effects of mercury toxicity in amphibians (Bergeron *et al.*, 2010b); this metal was not measured in this study.

ii) Snapping Turtle Eggs - Spatial Trends & Temporal Trends:

Of all organochlorines in snapping turtle eggs collected from the Walpole Delta in 2011, sum PCBs were found at the highest concentrations with a mean of 175.18 ng/g and ranging widely from 7.84 ng/g to 725.73 ng/g in 37 clutches of eggs analyzed (Table 3). Mean concentrations of *p,p'*-DDE and sum

chlordane were found at the next highest concentrations in eggs and were equal to 9.64 ng/g and 9.22 ng/g, respectively. Dieldrin and OCS were found at mean concentrations of approximately 2 ng/g while HCB, HE and mirex were equal to approximately 1 ng/g. Generally, the highest concentrations of organochlorines were found in eggs collected along Bassett Channel, downstream along Chematogan Channel, inland of Johnston Channel along Dyke Road and at the mouth of Goose Lake.

Significant differences in mean concentrations of sum PCBs and the seven other organochlorine compounds were found among eggs collected from the Walpole Delta, Long Point (Lake Erie), St. Clair NWA, and Tiny Marsh (Table 3). For most compounds, including sum PCBs, concentrations in Walpole Delta eggs were not significantly different from eggs from Long Point and the downstream St. Clair NWA site but were significantly higher than eggs from the inland Tiny Marsh reference site. Concentrations of *p,p'*-DDE in Walpole Delta eggs were statistically similar to concentrations at all other sites. Significant spatial differences were found overall for HE and mirex but these differences were not significant between individual site comparisons in the post-hoc analysis. For HCB, there was a significant difference in the relationship between HCB concentrations and percent lipid content in eggs among sites which violated an assumption of the ANCOVA (site x % lipid, p=0.01). Following this, a separate slopes ANCOVA was conducted which resulted in a significant site effect (p=0.0001) as well as a significant site by percent lipid effect (p=0.02). Cognizant of the potential for serious bias (i.e., as a result of the significant site by percent lipid effect), Tukey's HSD post-hoc analysis indicated that mean HCB concentrations in eggs from Walpole Delta, St. Clair NWA and Long Point were similar but all were significantly higher than Tiny Marsh. Mean percent lipid content (±SD) was equal to 4.03 (±1.30)% in Walpole Delta eggs, 5.36 (±0.59)% in Long Point eggs, 4.95 (±0.80)% in St. Clair NWA eggs, and 5.17 (±1.14)% in Tiny Marsh eggs.

A Great Lakes perspective of contaminants in snapping turtle eggs represented as a cumulative total sum concentration of mean sum PCBs, seven organochlorines and PBDEs (where data are available) from Walpole Delta in 2011, two Hamilton Harbour AOC locations and Long Point in 2013 sites and other Great Lakes sites from 2001-2004 is provided in Figure 8. Overall, eggs from Walpole Delta were less contaminated relative to most other Great Lakes sites including other AOCs.

Total mercury concentrations ranged widely in Walpole Delta eggs from 10.55-1,011.80 ng/g with a mean concentration (±SD) of 355.12 (±336.78) ng/g on a dry weight basis (Table 4). The highest mercury concentrations in eggs were from collection locations throughout the Delta that included the northern end of Walpole Island, near Goose Lake, on St. Anne Island, and on Squirrel Island, where concentrations were above 900 ng/g dw (or 210 ng/g ww). Intermediate mercury concentrations ranging from 300-900 ng/g dw were found in clutches collected on Squirrel Island along Bassett Channel and Chematogan Channel. Methylmercury concentrations ranged from 4.41-863.69 ng/g dw with a mean concentration (±SD) of 252.79 (±286.58) ng/g dw (or 54.58 (±63.98), 0.96-193.81 ng/g ww).

Mercury concentrations in Walpole Delta eggs were statistically similar to concentrations in Tiny Marsh eggs but were significantly higher than concentrations in Long Point eggs collected in 2013 (Table 4). Overall, mercury concentrations in eggs from the St. Clair River AOC were among the highest relative to mean concentrations in eggs collected from other Great Lakes sites including other AOCs (Figure 9).

Table 3. Mean concentrations of organochlorines (SD, ng/g, wet weight) in snapping turtle eggs from Walpole Delta in the St. Clair River AOC in 2011, Long Point in 2013 and St. Clair National Wildlife Area (consisting of two sites on Lake St. Clair) and Tiny Marsh in 2001-2003. N represents the number of clutches of eggs collected. Means sharing the same uppercase letters are not significantly different. Significant spatial differences were found overall for HE and mirex but not between individual site comparisons in the post-hoc analysis.

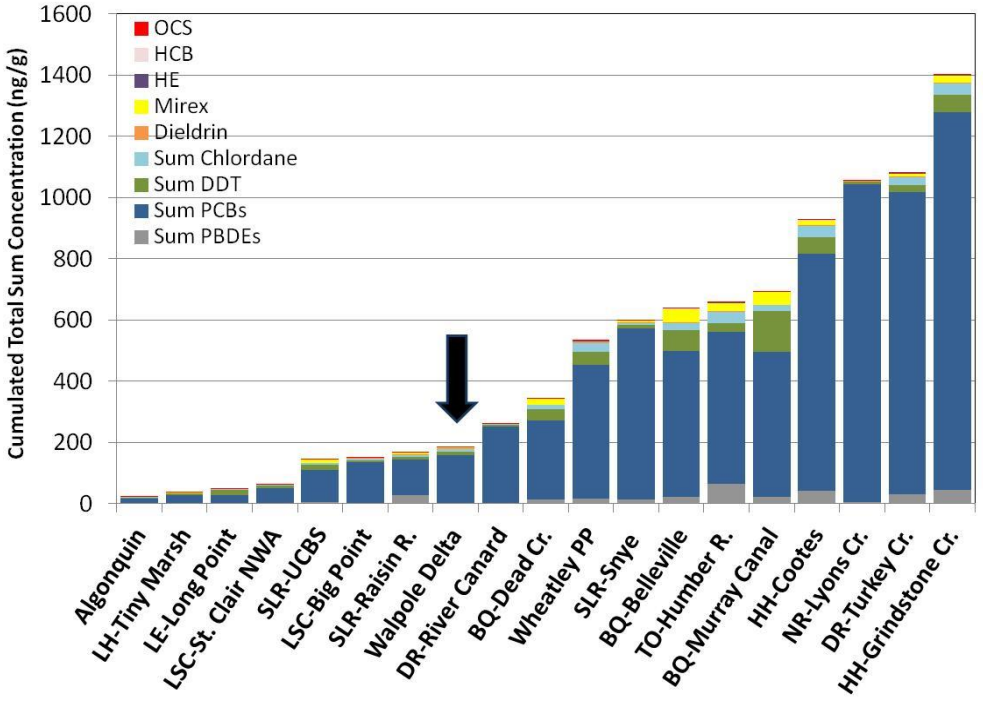
Year (s)	Site	N	Sum PCBs*	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB**	HE***	Mirex
2011	Walpole Delta	37	175.18 (192.43) **A**	9.64 (12.33) **AB**	9.22 (6.32) **A**	2.58 (1.90) **A**	2.24 (2.83) **A**	1.37 (1.63) **A**	0.75 (0.81)	0.67 (0.66)
2013	Long Point	10	30.76 (34.47) **AB**	16.65 (10.60) **A**	3.53 (4.54) **AB**	1.05 (0.77) **AB**	0.05 (0) **AB**	0.25 (0.13) **A**	0.11 (0.14)	0.18 (0.16)
2001	St. Clair NWA	6	101.02 (124.26) **AB**	5.70 (1.52) **AB**	4.43 (3.58) **AB**	1.47 (0.99) **AB**	0.43 (0.40) **AB**	0.60 (0.61) **A**	-	0.24 (0.31)
2001-2003	Tiny Marsh	13	29.28 (34.18) **B**	3.96 (2.21) **B**	1.70 (1.17) **B**	0.42 (0.43) **B**	0.01 (0.01) **B**	0.14 (0.18) **B**	0.14 (0.22)	0.88 (1.97)

* Sum PCBs shown are based on sum concentrations of 34 individual or co-eluting PCB congeners for Walpole Delta eggs and between 36-62 individual or co-eluting PCB congeners for eggs from other sites; however, statistical comparisons are based on the sum of 25 PCB congeners common to all sites.

** See text for further discussion following violation of assumption of ANCOVA.

*** The N value for HE is equal to 9 clutches at Tiny Marsh.

Figure 8. Cumulative total sum concentration (ng/g, wet weight) of mean sum PCBs, seven organochlorines and sum PBDEs (where available) in snapping turtle eggs from Walpole Delta in 2011, two locations in Hamilton Harbour (HH) and Long Point in 2013 and other sites in the Great Lakes basin including AOCs, Tiny Marsh and Algonquin Park in 2001-2004. Two sites on Lake St. Clair (LSC), St. Clair National Wildlife Area and Big Point Hunt Club, where eggs were collected in 2001 are shown separately. No PBDE data are available for the two LSC sites, Tiny Marsh, Walpole Delta, and River Canard.

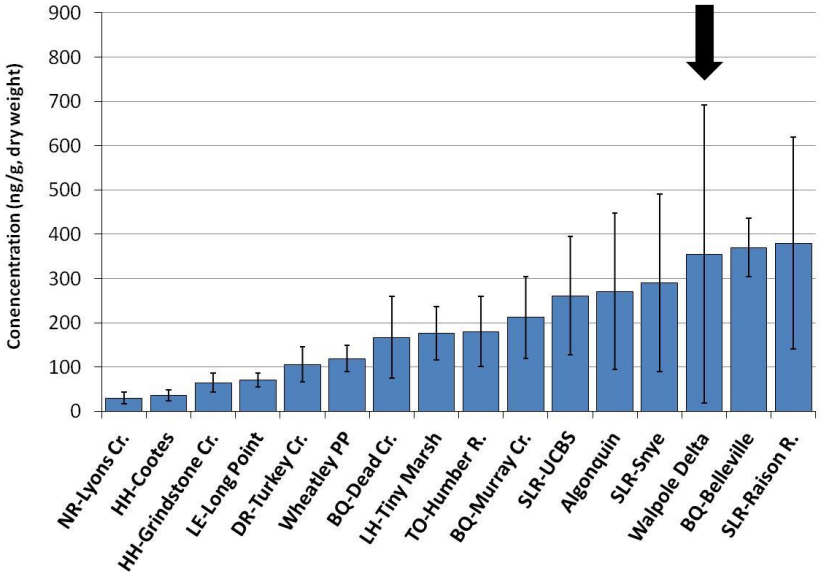


Relative to other biota, few studies have examined the toxic effects of mercury in reptiles. Hopkins *et al.* (2013) demonstrated that total mercury in snapping turtle eggs was negatively correlated with hatching success through increased egg infertility and embryonic mortality. They found that an average mercury concentration of 3,000 ng/g (dry weight) in eggs from a mercury-contaminated study site in Virginia was associated with a 12% reduction in hatching success compared to the reference sites. The mean mercury concentration in eggs from Walpole Delta was approximately one-tenth of this concentration.

Table 4. Mean concentrations (SD, ng/g) of total mercury in snapping turtle eggs from Walpole Delta in the St. Clair River AOC in 2011, Long Point in 2013 and Tiny Marsh in 2002 and 2003. Concentrations are shown as dry weights and wet weights. N represents the number of clutches of eggs collected. Statistical analyses were performed using dry weight concentrations only. Means sharing the same uppercase letters are not significantly different.

Year (s)	Site	N	Total Mercury dry weight	Total Mercury wet weight
2011	Walpole Delta	37	355.12 (336.78) **A**	76.08 (74.70)
2013	Long Point	10	70.53 (15.18) **B**	16.16 (3.83)
2002/03	Tiny Marsh	10	175.80 (60.47) **AB**	31.35 (12.29)

Figure 9. Mean mercury concentrations (SD, ng/g, dry weight) in snapping turtle eggs from Walpole Delta in 2011, two locations in Hamilton Harbour (HH) and Long Point in 2013 and other sites in the Great Lakes basin including AOCs, Tiny Marsh and Algonquin Park in 2001-2004.



Consistent with this, no significant effect on hatching success was found in Walpole eggs compared to the Tiny Marsh reference site when these clutches of eggs were artificially incubated in the laboratory (Hughes *et al.*, 2015). Effects of mercury exposure can be manifested immediately (e.g., at hatch) or delayed long after exposure in natural populations which may be several years in long-lived species such as the snapping turtle. It is difficult to predict long-term effects associated with the highest levels of mercury exposure in individuals although it is unlikely these levels would results in population-level effects. Other factors which are known to influence on population dynamics include harvesting of turtles and road mortality.

High variability in organochlorine and mercury concentrations in eggs among Walpole Delta sites, as a relatively large study area, suggests that exposure is also highly variable. This variability also contributed to the inability to discern significant differences in burdens between eggs from Walpole Delta and non-AOC reference sites. Relatively higher contaminant burdens in Walpole Delta eggs overall may be due to the proximity of collection sites relative to upstream St. Clair River contaminant sources and the pattern of water flow as it moves through the Walpole Delta to the centre of Lake St. Clair and to the Detroit River. This was suggested by Gewurtz *et al.* (2010) who reported significant differences in concentrations of mercury, PCBs, OCS, and HCB in juvenile spottail shiner (*Notropis hudsonius*) between the St. Clair River and nearshore areas of Lake St. Clair where water masses from the St. Clair River rarely mix. High variability in burdens observed among Walpole clutches may be due to differences in turtle foraging areas and prey selected, i.e., inland vs riverine sources, and localized variations in home range contaminant availability. Recent stable isotope analysis of these Walpole Delta clutches of eggs indicated a wide range in stable isotope signatures among collection sites, a pattern that is consistent with turtles feeding on varying types of prey items around the Delta including those from different trophic levels (EC, unpublished). A highly significant positive relationship was also found between total

mercury and stable isotopes of nitrogen in turtle eggs. This suggests that mercury burdens in eggs are related to the trophic level of prey that turtles are feeding on with higher mercury exposure in turtles feeding on higher trophic level prey types. This factor also contributes to variability in mercury concentrations found in turtle eggs in Walpole Delta.

Temporal changes in concentrations of organochlorines in snapping turtle eggs collected from assorted sites in the Walpole Delta in 1995, 1999, 2007, and 2011 are shown in Table 5. Mean concentrations of compounds showed little evidence of declines in concentrations across study years with notable high variability generally found among clutches within each study year. Since the number of congeners used to determined sum PCBs varied across years (ranging from 35-59 congeners), comparisons of sum PCBs are based on the 27 PCB congeners common to all years of analyses. Overall, these 27 congeners represented on average of 91.74%, 85.41%, 93.70%, and 94.31% relative to the total sum of PCBs determined in 1995, 1999, 2007 and 2011, respectively. It is also important to note that collection sites and clutch numbers varied across study years. For instance, five clutches of eggs were collected from the western portion of the Delta on Squirrel Island and Walpole Island in 1995, ten were collected from assorted sites on Walpole Island only in 1999 and 11 were collected from sites on Squirrel Island and on Walpole Island alongside all surrounding channels in 2007. In 2011, 37 clutches were collected from a relatively wider geographic area which also included the eastern side of St. Anne Island along Chenal Ecarte (Figure 2). Such variability in collection sites among years confounds the ability to effectively assess temporal trends. In addition, the largest declines in concentrations of organochlorines have been generally found in Great Lakes biota including sport fish and herring gull eggs since the mid-1970s followed by rates of decline which have slowed or stabilized after the early or mid-1990s (Gewurtz *et al.*, 2010; de Solla *et al.*, 2015). Similar large declines may have occurred in snapping turtle eggs from the Delta in the 1970s followed by a period of slowed decline in the 1990s as in evident in Table 5; however, this is speculative since contaminant data for eggs in the AOC during this early period are lacking.

Mercury concentrations in snapping turtle eggs collected in 2011 were higher than concentrations found in earlier studies of eggs in the AOC. In 1999, the mercury concentration in one pool of eggs collected from Walpole Delta was 110 ng/g dw (Ashpole *et al.*, 2004). In 2007, the mean mercury concentration ($\pm$SD) was 261.16 ($\pm$154.83) ng/g dw in 11 clutches of eggs collected from the area (range=66.51-596.66 ng/g dw). As mentioned above, an assessment of temporal trends is difficult due to variability in collection sites among study years as well as, in this case, few years of egg collections.

Table 5. Mean concentrations of organochlorines (SD, ranges, ng/g, wet weight) in snapping turtle eggs from sites in Walpole Delta in the St. Clair River AOC from 1995-2011. Sum PCBs shown are based on the sum of 27 PCB congeners common to all chemical analyses in the four study years. N represents the number of clutches of eggs collected.

Year	N	Sum PCBs	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	HCB	HE	Mirex
1995	5	194.31 (117.44) 76.35-346.30	17.00 (16.16) 3.30-43.80	13.09 (5.01) 7.30-20.75	2.14 (0.81) 1.10-3.20	1.95 (2.73) 0.15-6.70	1.46 (1.96) 0.20-4.90	1.32 (0.48) 0.80-1.90	1.00 (0.42) 0.70-1.70
1999	10*	153.49 (93.69) 15.00-282.45	8.75 (5.14) 0.50-20.00	47.53 (122.65) 1.60-395.00	14.81 (38.81) 0.05-125.00	1.47 (1.59) 0.05-5.00	1.05 (1.04) 0.50-3.00	1.56 (3.01) 0.05-10.00	0.96 (1.78) 0.05-6.00
2007	11	142.54 (181.22) 15.43-598.43	12.57 (21.56) 0.70-76.28	6.61 (7.34) 1.87-26.89	1.31 (0.49) 0.78-2.48	1.97 (2.50) 0.03-8.04	1.23 (1.43) 0.26-5.01	0.54 (0.35) 0.23-1.48	0.66 (0.67) 0.11-2.11
2011	37	164.14 (179.62) 7.08-670.95	9.64 (12.33) 0.18-63.96	9.22 (6.32) 0.16-28.05	2.58 (1.90) 0.001-8.61	2.24 (2.83) 0.001-10.99	1.37 (1.64) 0.03-7.59	0.75 (0.81) 0.01-2.81	0.67 (0.66) 0.01-2.36

*Denotes the number of pools of clutches in which a pool includes between 1-4 different clutches of eggs collected from a site.

iii) Canvasback & Mallard Liver – Spatial & Temporal Trends:

Of the organochlorines, sum PCBs and *p,p*'-DDE were found most consistently at the highest concentrations in livers of canvasbacks and resident mallards in the Lake St. Clair/St. Clair River study area (Table 6). Mean hepatic sum PCBs and *p,p'*-DDE concentrations in canvasbacks were 18.15 ng/g and 3.10 ng/g, respectively, while relatively lower mean concentrations of sum PCBs (4.51 ng/g) and *p,p*'-DDE (1.38 ng/g) were found in the three mallards collected from the Walpole Delta in 2010. Notably high mean concentrations of HCB (7.96 ng/g) and OCS (39.10 ng/g) were found in livers of canvasbacks from this area. These means, however, were largely influenced by one adult female collected in February with an elevated concentration of both HCB (184.67 ng/g) and OCS (1,046.33 ng/g) which exceeded concentrations of these compounds in other birds by at least 22 and 14 fold, respectively. Removal of this one bird resulted in relatively lower overall mean concentrations (±SD) reported for HCB (1.87 (±2.53) ng/g) and OCS (4.36 (±13.79) ng/g) in canvasbacks. For the remaining organochlorines, concentrations were low and frequently below the limit of detection with means below 0.9 ng/g in canvasbacks and 0.5 ng/g in mallards. Hepatic PCB and DDT concentrations in canvasbacks and mallards were low and were below concentrations that resulted in death of avian species in laboratory feeding studies (Hoffman *et al.*, 1996; Blus, 2011). Hepatic sum PBDE concentrations were also very low in the three canvasback and mallards analyzed.

Mean hepatic concentrations of mercury and selenium in canvasbacks were 0.58 µg/g and 9.07 µg/g dw, respectively (or as means (±SD) of 0.19 (±0.16) µg/g and 2.98 (±2.41) µg/g, respectively, on a wet weight basis; Table 6). Mean concentrations of mercury and selenium in mallards were relatively lower at 0.36 µg/g and 3.08 µg/g, respectively (or as means (±SD) of 0.11 (±0.08) µg/g and 0.98 (±0.06) µg/g, respectively, on a wet weight basis). Mercury and selenium concentrations in all birds were below 1.42 µg/g dw and 18.11 µg/g dw, respectively, with the exception of two hatch year birds that exceeded these concentrations (2.09 µg/g for mercury and 36.60 µg/g for selenium). Mercury and selenium concentrations in the three mallards were low and below 0.66 µg/g and 3.34 µg/g, respectively. Overall, contaminant burdens were highly variable in overwintering canvasbacks as a result of significant temporal increases in burdens observed over the four month period in the study area. These patterns are described further in Hughes *et al.* (2014b).

Table 6. Mean concentrations of organochlorines and sum PBDEs (SD, ng/g, wet weight) and metals (SD, µg/g, dry weight) in livers of canvasbacks and resident mallards from the Lake St. Clair/St. Clair River study area in 2008/2009 and 2010, respectively. N denotes the number of individuals analyzed with the exception of sum PBDEs in canvasbacks where the mean shown is based on analysis of three individuals.

Species	**Canvasbacks**	**Mallards***
N	30	3
Percent lipid	3.6 (1.1)%	5.2 (2.5)%
Sum PCBs	18.15 (31.79)	4.51 (1.81)
p,p'-DDE	3.10 (5.73)	1.38 (1.64)
Sum DDT	3.19 (5.78)	1.43 (1.64)
Sum Chlordane	0.68 (0.75)	0.47 (0.43)
HCB	7.96 (33.47)	0.05 (0.06)

HCB – one high bird removed	1.87 (2.53)	-
Mirex	0.05 (0.08)	0.05 (0.05)
Dieldrin	0.89 (1.93)	0.35 (0.45)
HE	0.74 (1.27)	0.10 (0.07)
OCS	39.10 (190.72)	0.06 (0.04)
OCS – one high bird removed	4.36 (13.79)	-
Sum PBDEs	0.19 (0.01)	0.26 (0.16)
Mercury (µg/g, dw)	0.58 (0.48)	0.36 (0.26)
Selenium (µg/g, dw)	9.07 (6.93)	3.08 (0.27)

* Mallards collected from Walpole Delta

Based on a recent and extensive review of mercury in birds (Shore *et al*., 2011), mercury concentrations in canvasbacks and mallards were well below hepatic concentrations of 2 µg/g and 20 µg/g (wet weights) suggested as thresholds for adverse effects on reproduction and bird survival, respectively. The maximum mercury concentration in this study was 0.70 µg/g ww (or 2.09 µg/g dw). Selenium concentrations were relatively higher in birds and occasionally exceeded threshold levels. Selenium concentrations in the large majority of canvasbacks (67%) were below the threshold concentration of 10 µg/g (dry weight) associated with background exposure; 30% of birds had selenium concentrations in the range of 10-20 µg/g which are considered elevated; and 3% (i.e., one bird) had a selenium concentration of 36.60 µg/g which exceeded the 20 µg/g threshold associated with toxicity in birds (Ohlendorf and Heinz, 2011). All three mallards had selenium concentrations consistent with background exposure.

Hepatic mercury and selenium concentrations were low overall in wintering canvasbacks from the study area in 2008/09 relative to concentrations in other studies of wintering canvasbacks and other diving duck species in the Great Lakes (e.g., Hothem *et al.*, 1998; Custer and Custer, 2000). Hepatic selenium concentrations indicative of increased exposure (i.e., greater than 10 µg/g) or exceeding the concentration associated with toxicity (>20 µg/g) have been frequently reported in overwintering waterfowl from other Great Lakes locations (Custer *et al.*, 2000; Petrie *et al.*, 2007; Ware *et al.*, 2011). Therefore the one canvasback with a hepatic selenium concentration above the toxicity threshold of 20 µg/g is consistent with elevated concentrations observed elsewhere in overwintering waterfowl.

Historical organochlorine data for mallards collected from the St. Clair River area in 1985/86 are also available to examine long-term temporal trends for waterfowl in this area (EC, unpublished). Twelve mallards were collected consisting of adult and immature birds (unknown numbers of each and status as either resident or migratory also unknown). Two pooled sets of liver samples were analyzed consisting of six individuals each. Since levels of contamination varied by a factor of at least two between the two pooled samples, concentrations are reported separately (Table 7). Concentrations of PCBs, which were quantified using an Aroclor 1254:1260 (1:1) mixture in the 1970/80s, were equal to 776.00 ng/g and 147.00 ng/g in the two pooled samples. Based on a conversion factor of 0.56 for Aroclor 1:1 to sum PCBs

Table 7. Concentrations of organochlorines (ng/g, wet weight) in livers of mallards from the St. Clair River area in two time periods. Concentrations in two pooled samples of livers from birds collected in 1985/86 and mean concentrations (SD) for three birds collected within the study area (Walpole Delta) in 2010 (November) and analyzed individually are shown. Percent decline is relative to concentrations in

pooled sample No. 2 and provides a conservative estimate of the relative change in concentrations between 1985/86 and 2010.

Year	1985/86 – Pooled Samples*		2010 – Individuals	Percent Decline 1985/86 & 2010
	No. 1	No. 2	3	
Sum PCBs	434.56**	82.32**	4.51 (1.81)	94.5%
p,p'-DDE	42.00	18.00	1.38 (1.64)	92.3%
Sum DDT	49.00	20.00	1.43 (1.64)	92.9%
Sum Chlordane	28.25	6.75	0.47 (0.43)	93.0%
HCB	311.00	11.00	0.05 (0.06)	99.5%
Mirex	0.25	0.25	0.05 (0.05)	80.0%
Dieldrin	42.00	8.00	0.35 (0.45)	95.6%
Heptachlor Epoxide	13.00	5.00	0.10 (0.07)	98.0%
OCS	493.00	41.00	0.06 (0.04)	99.9%

* Pooled sample No. 1 consists of six birds collected in December/January 1985/86 and No. 2 consists of six birds collected in February 1986.

** Estimated sum PCB concentration based on conversion factor of 0.56 for known Aroclor 1254:1260 PCB concentration.

in muscle tissue of mallards collected from Great Lakes sites (EC, unpublished), estimated sum PCBs concentrations in pooled liver samples in 1985/86 were equal to 434.56 ng/g and 82.32 ng/g. Among the remaining organochlorines, *p,p*-DDE, OCS and HCB were found at the highest concentrations. Mirex was not detected in either sample and is reported as one-half of the detection limit. Overall, large declines in concentrations were found in livers of mallards between 1985/86 and 2010 with percent declines ranging from 80.0% for mirex to 99.9% for OCS. Reported declines are based on comparisons to the pooled sample No. 2 which had relatively lower concentrations and provide a conservative estimate of change in contamination. Since chemical analyses for mercury and selenium were not conducted in 1985/86 pooled liver samples, similar temporal comparisons cannot be performed.

Mink Liver - Spatial Trends:

Overall, concentrations of organochlorines in liver of wild mink from Walpole Delta in the St. Clair River AOC were not elevated relative to mink at the non-AOC reference sites (Table 8). Mean hepatic sum PCB concentrations ranged from 78 ng/g in mink from Lake St. Clair to 263 ng/g in mink from Long Point. The maximum hepatic sum PCB concentration of 1,288 ng/g was found in a mink from Long Point. For sum PCBs, there was a significant difference in the relationship between sum PCB concentrations and percent lipid content among sites which violated an assumption of the ANCOVA (site x % lipid, p=0.02). Following this, a separate slopes ANCOVA was conducted which resulted in a significant site effect (p=0.005) as well as a significant site by percent lipid effect (p=0.04). Cognizant for the potential for bias in the post-hoc analysis, no significant difference in sum PCB concentrations were found in mink from Walpole Delta compared to any of the reference sites. Mean percent lipid content ($\pm$SD) was equal to 3.0 ($\pm$0.9)% in liver of mink from Walpole Delta, 1.9 ($\pm$0.9)% for mink from Long Point, 3.0 ($\pm$1.6)% for mink from Lake St. Clair, and 3.1 ($\pm$1.2)% for mink from inland Erie.

Hepatic concentrations of *p,p'*-DDE were the next highest in mink with means ranging from 7.0 ng/g to 61.7 ng/g at the four study sites followed by sum chlordane where means ranged from 2.7 ng/g to 15.2

ng/g. Mink from Walpole Delta had significantly lower *p,p'*-DDE concentrations relative to mink from Long Point. A significant spatial difference was found for sum chlordane but not between individual site comparisons in the post-hoc analysis. Mean concentrations of dieldrin and OCS ranged from 0.05 ng/g (where it was found below the limit of detection at inland Erie) to 4.9 ng/g. Concentrations of the remaining organochlorines were relatively lower with means below 1.0 ng/g. While considered low overall, mean concentrations of OCS, mirex and HCB in mink from Walpole Delta were significantly higher than concentrations in mink from at least two of three reference sites. No significant differences in mean concentrations of dieldrin and HE were found among sites.

Hepatic burdens of organochlorines represented as a cumulative total mean sum concentration of sum PCBs, *p,p'*-DDE, sum chlordane and other organochlorines in mink trapped at lower Great Lakes basin sites from 1998-2006 are provided in Figure 10. Since percent lipid concentrations in mink liver varied significantly among Great Lakes sites, total cumulative concentrations have been lipid-normalized to allow for spatial comparisons. Overall, total organochlorine burdens in liver of mink from Walpole Delta were among the lowest found at Great Lakes sites during this period. Mink from Long Point had relatively higher cumulative burdens compared to mink from Walpole Delta.

Figure 10. Cumulative mean total sum concentration (SD, µg/g, lipid weight) of sum PCBs and other organochlorine compounds in livers of mink from Walpole Delta and other sites including AOCs in the lower Great Lakes basin from 1998-2006. Sites are ranked from the least contaminated to the most contaminated.

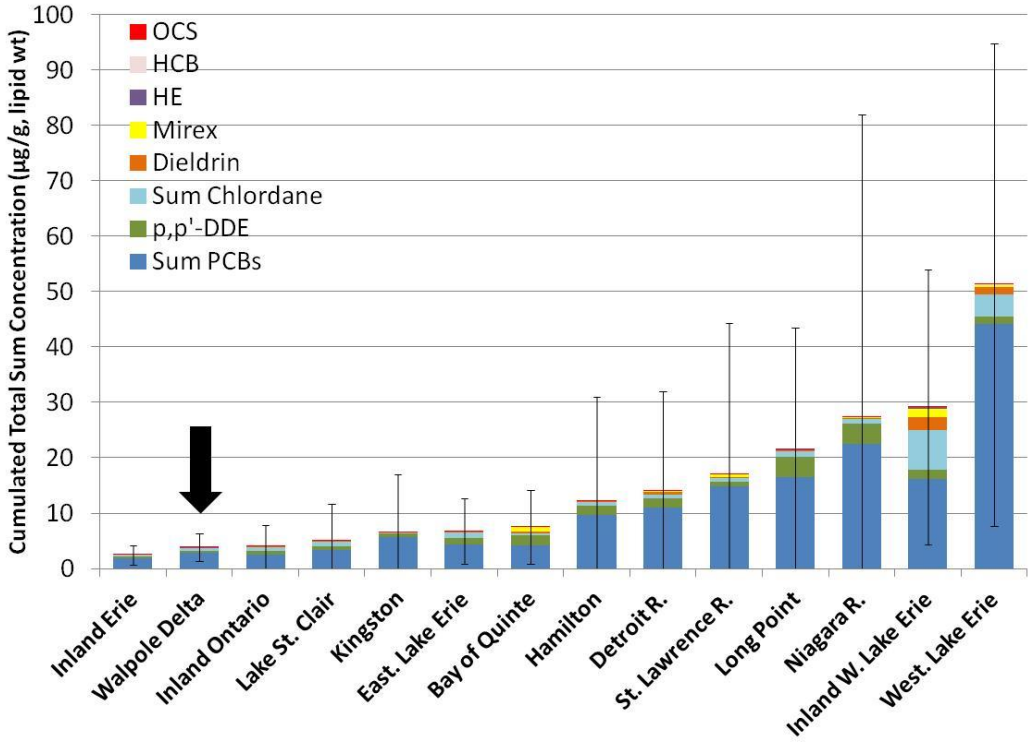


Table 8. Mean (SD) and maximum concentrations of organochlorine compounds (ng/g, weight wet) in livers of wild mink from Walpole Delta, Lake St. Clair and inland Erie (reference site) from 1998-2004. N denotes the number of mink analyzed in which the higher number indicates the number of mink analyzed for sum PCBs only. ND denotes below the level of detection. Means sharing the same uppercase letters are not significantly different; a significant spatial difference was found overall for sum chlordane but not between individual site comparisons in the post-hoc analysis.

Site	Collection Year	N	Sum PCBs	*p,p'*-DDE	Sum Chlordane	Dieldrin	OCS	Mirex	HCB	HE
Walpole	2002	10	82 (63)	12.1 (6.5)	9.6 (9.2)	3.4 (3.2)	2.3 (2.3)	0.44 (0.47)	0.40 (0.31)	0.38 (0.66)
Delta			216 **AB**	26.9 **B**	22.9	8.6	7.4 **A**	1.29 **A**	0.99 **A**	1.89
Long	1998/99	18	263 (341)	61.7 (67.4)	15.2 (22.7)	4.9 (10.1)	0.2 (0.5)	ND	0.23 (0.19)	0.68 (1.04)
Point			1,288 **A**	238.8 **A**	75.2	31.6	1.7 **B**	**B**	0.80 **AB**	3.17
Lake St.	1999-2004	13/16	78 (89)	14.0 (9.5)	13.6 (19.7)	3.5 (7.9)	0.4 (0.8)	0.10 (0.14)	0.17 (0.19)	0.97 (1.26)
Clair			338 **B**	38.3 **B**	72.2	28.0	3.0 **B**	0.50 **B**	0.50 **B**	4.00
Inland	1999	4/15	85 (73)	7.0 (5.8)	2.7 (3.8)	ND	ND	ND	ND	0.16 (0.23)
Erie*			298 **B**	15.0 **B**	8.2		**B**	**B**	**B**	0.50

* three inland mink trapped in the southwestern Ontario region and 10-12 km inland in 2003 were not included (see methods)

Concentrations of the four dioxin-like PCBs, seven dioxins, including 2,3,7,8-TCDD, and 10 furans were generally lowest in the single pooled liver sample of mink from Walpole Delta compared to mink from Long Point and other Great Lakes sites (Table 9). Corresponding TEQ concentrations were consequently lowest in mink from Walpole Delta with a total TEQ concentration of 11.65 ng TEQ/kg. Concentrations of dioxin-like PCBs contributed the greatest proportion to overall TEQ toxicity ranging from 70.4% in mink from Walpole Island to 86.0% in mink from western Lake Erie.

Hepatic PCB concentrations in Walpole Delta mink were well below concentrations associated with impacts on reproduction and survival. Laboratory toxicity testing of ranch mink established a lowest observable adverse effect level (LOAEL) on kit growth corresponding to a maternal liver sum PCB concentration of 998 ng/g wet weight (Restum *et al.,* 1998). Three separate dietary exposure studies found that doses of PCB-contaminated fish fed to mink that resulted in decreased kit survival corresponded to maternal liver PCB concentrations of 2,200 ng/g, 3,100 ng/g and 3,400 ng/g (Heaton *et al.,* 1995; Bursian *et al*., 2006a,b, 2013). Bursian *et al.* (2013) predicted that the concentration lethal to 20% of the population for kit stillbirths was based on a maternal liver sum PCB concentration of 1,700 ng/g and the concentration lethal to 50% of the population for mortality of six-week-old kits was based on a maternal liver sum PCB concentration of 3,000 ng/g. The maximum hepatic concentration found in mink from Walpole Delta was 216 ng/g which was well below concentrations associated with effects in these laboratory studies.

Table 9. Concentrations of dioxin-like PCBs, dioxins and furans with associated TEQs (ng/kg, wet weight) in pooled livers of mink collected from Walpole Delta and four other Great Lakes sites, 1998-2002. TEQs reported are based on the most recent toxic equivalency factors reported by Van den Berg *et al.* (2006) for mammals. N refers to the number of livers included in the single pooled sample.

	Walpole Delta	**West. Lake Erie[a]**	**Long Point**	**East. Lake Erie[b]**	**St. Lawrence R.**
N	**5**	**6**	**4**	**4**	**6**
% lipid	3.59	13.8	8.64	4.08	2.54
PCB-77	5.34	58.8	6.78	5.86	8.30
PCB-126	80	699	156	113	186
PCB-169	6.35	80.3	19.8	14.8	10.3
PCB-189	261	3710	756	944	471
TEQ (d-l PCBs)	**8.20**	**72.4**	**16.2**	**11.8**	**18.9**
% Total TEQs	**70.4**	**86.0**	**79.7**	**80.6**	**82.1**
2378-TCDD	0.52	2.49	1.00	0.62	0.96
12378-PnCDD	0.48*	1.21	0.71	0.44	0.30
123478-HxCDD	0.52	0.72*	0.46*	0.45	0.34
123678-HxCDD	3.8*	10.8	2.54	2.92	4.32
123789-HxCDD	0.63*	1.04*	0.55	0.41	0.45
1234678-HpDD	35.9	33.6	8.17	9.73	65.6
12346789-OCDD	54.2	145	16.2	38.0	368
TEQ (dioxins)	**1.86**	**5.34**	**2.14**	**1.55**	**2.54**
% Total TEQs	**16.0**	**6.34**	**10.5**	**10.6**	**11.0**

	Walpole Delta	West. Lake Erie[a]	Long Point	East. Lake Erie[b]	St. Lawrence R.
2378-TCDF	0.15	0.56	0.16	0.12	0.19
12378-PnCDF	<0.10	0.20*	<0.10	<0.10	<0.10
23478-PeCDF	4.13	18.4	5.79	3.57	4.15
123478-HxCDF	0.54*	1.7	0.46	0.28	0.56
123678-HxCDF	0.98	2.46	0.75	0.59	0.81
123789-HxCDF	<0.10	<0.10	<0.10	<0.10	<0.10
234678-HxCDF	1.58	3.96	1.06	1.03	1.26
1234678-HpCDF	0.83	1.53	0.60	0.56*	3.25
1234789-HpCDF	0.13*	0.47*	0.14*	0.14*	0.56
12346789-OCDF	1.76	2.54	1.04	1.2*	29.3
TEQ (furans)	**1.59**	**6.42**	**2.00**	**1.29**	**1.59**
% Total TEQs	**13.6**	**7.63**	**9.83**	**8.85**	**6.88**
Total TEQs	**11.65**	**84.19**	**20.36**	**14.61**	**23.05**

[a] 4 of 6 mink in pool were domestic farm escapees based on subsequent DNA analysis.

[b] 2 of 4 mink in pool were domestic farm escapees

*These values are outside the 15% quality control limits around iron abundance and should be viewed with caution.

Total mercury concentrations in liver of mink from Walpole Delta in 2002 were the highest of all metals and ranged from 2.42 µg/g to 17.10 µg/g with a mean of concentration of 7.31 µg/g on a dry weight basis (or 2.22 µg/g, range=0.72-5.59 µg/g, on a wet weight basis; Table 10). Mean mercury concentrations were statistically similar between mink from Walpole Delta and Long Point and were significantly higher compared to mink from Lake St. Clair and inland Erie. Concentrations of selenium were not significantly different among study sites with means ranging from 2.43 µg/g dw at Long Point

Table 10. Mean (SD) and maximum concentrations (µg/g, dry weight) of total mercury and other metals in livers of mink from Walpole Delta, Long Point, Lake St. Clair and inland Erie from 1998-2004. N denotes the number of mink analyzed in which the higher number indicates the number of mink analyzed for total mercury only. Means sharing the same uppercase letters are not significantly different.

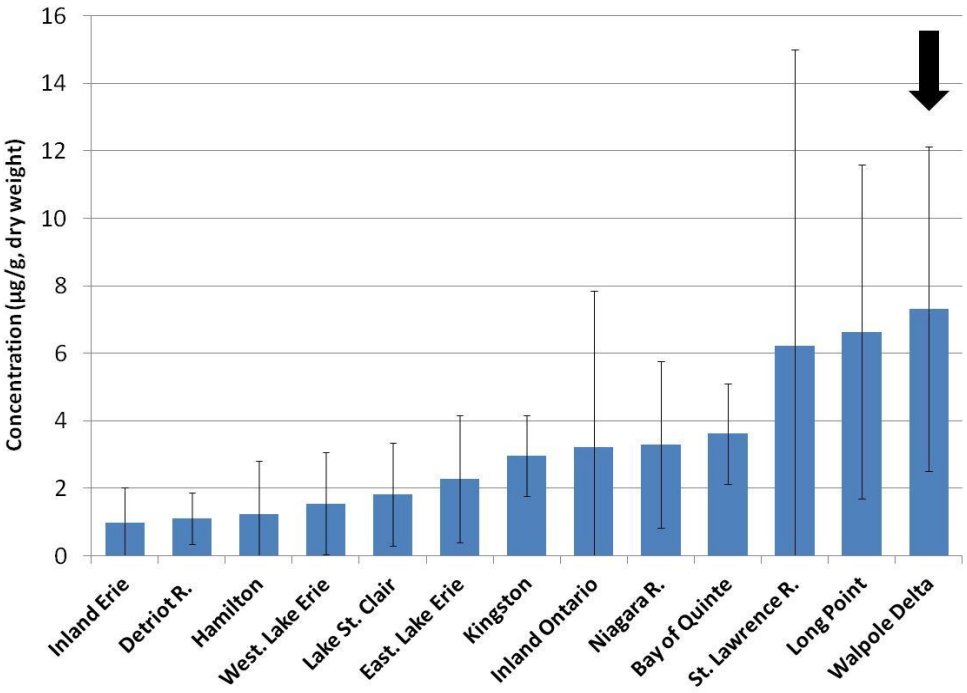
Site	Year	N	Total Mercury	Selenium	Cadmium	Arsenic	Lead
Walpole Delta	2002	10	7.31 (4.81) 17.10 **A**	3.19 (1.08) 5.11	0.21 (0.18) 0.66 **AB**	0.20 (0.06) 0.30 **A**	0.85 (1.55) 5.11
Long Point	1998/99	6/18	6.64 (4.95) 21.33 **A**	2.43 (0.39) 2.99	0.08 (0.04) 0.14 **B**	0.14 (0.04) 0.18 **AB**	0.19 (0.03) 0.24
Lake St. Clair	1999-2004	4/13	1.81 (1.53) 36.82 **B**	3.00 (0.55) 3.50	0.23 (0.19) 0.46 **AB**	0.09 (0.09) 0..17 **B**	0.12 (0.05) 0.20
Inland Erie	1999 & 2003	8	0.99 (1.02) 3.12 **B**	2.58 (0.48) 3.14	0.48 (0.31) 0.86 **A**	0.21 (0.07) 0.33 **A**	0.27 (0.31) 1.01

to 3.19 µg/g dw at Walpole Delta. Significant differences for concentrations of cadmium and arsenic were found in liver of mink among the four study sites. Lead concentrations were not significantly different among sites.

Overall, mean hepatic total mercury concentrations were highest in mink from Walpole Delta relative to mink trapped at other sites in the lower Great Lakes basin sites from 1998-2006 (Figure 11). Mink from Long Point had the second highest mean mercury concentration of all Great Lakes sites including Areas of Concern during this period. Wetland environments, such as at Long Point and Walpole, can act as natural sites of mercury methylation where methylmercury, as the most toxic form of mercury, is produced and subsequently becomes available to bioaccumulate in fish and fish-eating wildlife. There are no historical industrial sources of mercury at Long Point, however, Big Creek Marsh is a managed wetland with impoundment where mercury concentrations may be elevated relative to other wetland habitat types (Eagles-Smith and Ackerman, 2014). Methylation dynamics and bioaccumulation of methylmercury in the food web are complex processes controlled by many local biotic and abiotic factors. Nevertheless, hepatic mercury concentrations in Walpole Delta mink were nearly an order of magnitude lower than concentrations in mustelids which died of methylmercury intoxication in experimental studies or which were found dead or dying in the wild. In these studies, mean hepatic total mercury concentrations typically exceeded 25 µg/g ww (Aulerich *et al.*, 1974; Wobeser *et al*., 1976; Wobeser and Swift, 1976; Wren *et al*., 1987). Mercury concentrations in mink from Walpole Delta were low relative to those found in other wild mink populations where elevated mercury exposure was noted (two sites in Quebec [3.7 µg/g ww, Fortin *et al.*, 2001; 13.0 µg/g dw, Klenavic *et al.*, 2008] and inland Nova Scotia [18 µg/g dw, Klenavic *et al.*, 2008]).

Hepatic cadmium concentrations were several orders of magnitude lower than critical renal concentrations associated with kidney dysfunction in humans and experimental mammals (100-200 µg/g ww, Scheuhammer, 1987). Similarly, lead concentrations were also largely associated with normal background concentrations (i.e., 0.05-0.50 µg/g ww; Mason and Wren, 2001) and were below the 10 µg/g dw threshold associated with diagnosing acute lead poisoning in mammalian wildlife (Ma, 1996). Overall, the ages of 24 trapped mink collected from Walpole Delta in 2001 and 2002 ranged from 0 years to 5 years old and represents a wide range of ages that were trapped in mink from the lower Great Lakes (Martin *et al.*, 2015). The average life span for a wild mink is 7-8 years (Fruth, 1986).

Figure 11. Mean total mercury concentrations (SD, µg/g, dry weight) in livers of mink from Walpole Delta in the St. Clair River AOC and other sites including AOCs in the lower Great Lakes basin from 1998-2006. Sites are ranked from the least contaminated to the most contaminated.



Risk to Higher Trophic-level Consumers:

Risk to higher trophic-level consumers in the St. Clair River AOC food web was assessed by comparing tissue burdens measured in wildlife against guidelines that have been developed to protect consumers against contaminant–induced adverse effects on reproduction and survival. Consumers, for example, would include birds and mammals feeding on frogs or turtle eggs and bald eagles (*Haliaeetus leucocephalus*) feeding on overwintering waterfowl such as canvasback or mallards. In addition, while mink have few natural enemies, they may be occasionally eaten by foxes, coyotes, wolves, and birds of prey. The Canadian Environmental Quality Guidelines are one set of guidelines developed for the protection of wildlife consumers of aquatic biota (CCME, 2001). Another set of guidelines known as fish flesh criteria were developed to protect piscivorous wildlife eating contaminated fish – in this case, contaminated wildlife – from adverse effects such as mortality, reproductive impairment and organ damage (Newell *et al*., 1987).

To assess risk to consumers, tissue concentrations cited for wildlife species in this report were directly compared to these two sets of guidelines for most compounds. For methylmercury however, since this was not directly measured in tissues of frogs, canvasbacks, mallards, and mink, concentrations were estimated based on total mercury concentrations and further details are provided in Martin *et al.* (2011) and Hughes *et al.* (2014a,b). Methylmercury in turtle eggs are based on measured concentrations (Hughes *et al.*, 2015). In addition, estimates of TEQs for sum PCBs in frogs, turtles, and waterfowl were based on concentrations of three mono-*ortho* PCBs (#105, #118 and #156) only and do not include concentrations of the more toxic dioxin-like PCBs such as #77, #126, and #169. As a result, determinations of total TEQs for sum PCBs are underestimated and therefore a complete assessment against this guideline is not possible; nonetheless, exceedences reported would constitute a minimum percentage. Separate CCME

guidelines for TEQ for sum PCBs have been developed for mammals and avian species since modes of toxicity differ in these two groups. Following this, comparisons to these two guidelines are based on toxic equivalency factors specific to birds and mammals (Van den Berg *et al.*, 1998, 2006).

Table 11 provides an overall summary of tissue samples that exceeded the two sets of guidelines associated with protection to wildlife consumers, i.e., an assessment of risk to higher trophic consumers that feed on these wildlife tissues. Percentages of tissue samples for frogs, turtles, canvasbacks and mallards, and mink from the St. Clair River AOC with a corresponding reference site (where available) are shown. For wildlife consumers of frogs, this assessment indicates that there is little to no risk to survival since few exceedences were evident. For wildlife consumers of snapping turtle eggs however, there may some risk since guidelines were exceeded in approximately one-third of eggs for total DDT, TEQ for sum PCBs (mammals), and methylmercury. Furthermore, consumption of canvasback and mallard livers may pose some risk to higher trophic consumers due to high levels of (estimated) methylmercury found in approximately two-thirds of livers. For mink, exceedences of the CCME guidelines were found in liver samples that included a single pooled liver sample analyzed for TEQs for sum PCBs (as shown in Table 9). For many of the other organochlorines, such as sum chlordane and dieldrin, exceedences of fish flesh criteria guidelines were rarely found in tissues of any species. As expected, the frequency of exceedences increased for consumers feeding at a relatively lower trophic level, i.e., frogs, compared to those feeding on turtles, waterfowl and mink.

These results indicate that there is some risk to higher trophic-level consumers feeding within the St. Clair River AOC. Relative to the references sites, risk to consumers of mink liver was not elevated in the AOC compared to Long Point while this was not the case for consumers of turtle eggs in the AOC where risk was notably higher relative to Tiny Marsh. It is important to note that this represents a very limited assessment of consumer health risk since risk is highly dependent on a number of factors that includes the amount and frequency of consumption of these tissues as well as the tissue type consumed. For example, burdens in liver of waterfowl would be much higher relative to muscle tissue for bald eagles feeding on them. Also, as mentioned earlier, there is some uncertainty regarding methylmercury concentrations since these were not directly measured and, for most species, were based on estimated concentrations. Finally, both sets of CCME and fish flesh criteria guidelines were developed to protect aquatic consumers and are based on the most sensitive wildlife species. Different consumers will differ in their sensitivity to these compounds.

Table 11. Percentages of tissue samples in wildlife from the St. Clair River AOC and reference sites that exceeded guidelines for protection of wildlife consumers of aquatic biota and fish flesh criteria (Newell *et al.*, 1987; CCME, 2001). TEQs for sum PCBs are based on concentrations of PCB #105, #118 and #156 for all species except mink which are based on concentrations of other dioxin-like PCBs, dioxins and furans. Methylmercury concentrations are estimated from total mercury concentrations in tissues of frogs, canvasbacks, mallards and mink using methods described in Martin *et al.* (2011) and Hughes *et al.* (2014a,b) and are based on measured concentrations in turtles at Walpole Delta (Hughes *et al*., 2015). Values in brackets represent the number of tissue samples.

Guideline Type	**Contaminant**	**Tissue Residue Guideline**	**Northern Leopard Frogs – Whole Bodies**		**Snapping Turtle – Eggs**		**Canvasback & Mallard – Liver**	**Mink – Liver**	
			SCR AOC (39)	L Huron REF (10)	SCR AOC (37)	Tiny M REF (13)	AOC (33)	SCR AOC (10)	Long P REF (18)
Wildlife Consumers of Aquatic Biota	Total DDT	14.0 ng/g	5% (2)	0%	30% (11)	0%	6% (2)	30% (2)[e]	94% (17)[e]
	TEQ for Sum PCBs	0.79 ng TEQ/kg[a]	0%	0%	35% (13)	0%[c]	0%	100% (1)[f]	100% (1)[f]
		2.4 ng TEQ/kg[b]	0%	0%	8% (3)	0%[c]	0%	100% (1)[f]	100% (1)[f]
	Methylmercury	33.0 ng/g	0%	0%	49% (18)	10% (1)[d]	64% (21)	100% (10)	100% (18)
Fish Flesh Criteria	Total PCBs	110 ng/g	0%	0%	41% (15)	8% (1)	3% (1)	30% (3)	44% (8)
	Total DDT	200 ng/g	0%	0%	0%	0%	0%	0% [e]	11% (2)[e]
	HCB	330 ng/g	0%	0%	0%	0%	0%	0%	0%
	OCS	20 ng/g	0%	0%	0%	0%	6% (2)	0%	0%
	Sum Chlordane	500 ng/g	0%	0%	0%	0%	0%	0%	0%
	Mirex	330 ng/g	0%	0%	0%	0%	0%	0%	0%
	HE	200 ng/g	0%	0%	0%	0%[c]	0%	0%	0%
	Dieldrin	120 ng/g	0%	0%	0%	0%	0%	0%	0%

[a] guideline protective of mammals

[b] guideline protective of avian species

[c] reference site = Long Point (N=10 clutches) since PCB 156 not quantified individually at Tiny Marsh and therefore a comparable estimate could not be made.

[d] based on 10 clutches analyzed for mercury and estimated concentrations for methylmercury

[e] based on *p,p'*-DDE concentrations only since problem with quantification of *p,p'*-DDT in these samples.

[f] based on analysis of a single pooled liver sample (Table 9)

Conclusions

In general, burdens of legacy contaminants in wildlife have declined considerably throughout the Great Lakes including at AOCs since the 1970s (Ashpole *et al.*, 2004; de Solla *et al.,* 2015). Using the herring gull (*Larus argentatus*) as an example, such declines coincided with a period of increased growth in nest numbers at breeding colonies (e.g., Toronto and Region AOC). Unfortunately, no colonial waterbirds nest within the St. Clair River AOC and so similar temporal patterns cannot be used to suggest improved health of aquatic-feeding wildlife populations. However, reproduction and health effects examined in relation to contaminant burdens in frogs and turtles from the St. Clair River AOC revealed no evidence of impairment based on the endpoints measured (Hughes *et al.*, 2014a, 2015). Since reproductive effects are tightly linked to population-level effects, these findings support the hypothesis that current levels of exposure of legacy contaminants are unlikely to adversely impact survivability of wildlife that feed from the aquatic ecosystem in the AOC. This report serves to explore this further by examining historical and current contaminants data using a multi-faceted and multi-species approach to assess whether population-level effects might be expected following lethal exposure to these compounds. Contaminants of concern (i.e., HCB, OCS, PCBs, and mercury) were frequently found at higher concentrations in wildlife foraging in the St. Clair River AOC compared to other Great Lakes locations highlighting the effectiveness of these species as bio-indicators of contaminant exposure in the AOC. Overall, current concentrations of organochlorines (HCB, OCS) were not sufficiently elevated to adversely impact the survival of frogs, turtles, birds and mink feeding with the St. Clair River AOC. Mercury body burdens were elevated in wildlife relative to other Great Lakes locations. However, based on the weight-of-evidence and approach presented in this study, it is unlikely the current levels of mercury would result in population-level effects in wildlife species foraging in the AOC.

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