Supporting Information

Snapping turtles as bioindicators in Canadian Areas of Concern in the Great Lakes Basin. II. Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls and Organochlorine Pesticides in Eggs of Snapping Turtle (*Chelydra serpentina*)

de Solla SR[1], Fernie KJ[1], Letcher RJ[2,3], Chu SG[3] , Drouillard KG[3] and Shahmiri S[2]

[1] Canadian Wildlife Service, Environment Canada, 867 Lakeshore Road, Box 5050, Burlington, ON, L7R 4A6, Canada

[2] National Wildlife Research Centre, Wildlife and Landscape Science Directorate, Wildlife Toxicology and Disease Division, Science and Technology Branch, Environment Canada, Carleton University, Raven Road, Ottawa, ON, K1A 0H3, Canada

[3] Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON, N9B 3P4, Canada

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Detailed Site Description

Algonquin Provincial Park and Tiny Marsh were included as two inland reference sites. Tiny Marsh is an open water cattail marsh, where the surrounding area is farmed for hay production, but the intensity of agricultural use is less than that near AOCs. Eggs were collected from a 40 km long area within Algonquin Provincial Park, but all sites were similar in that eggs were collected from embankments adjacent to dystrophic lakes or ponds.

Turkey Creek (Detroit River AOC) drains both the city of LaSalle and a large industrial zone in Windsor, ON. The St. Clair National Wildlife Area (NWA) is approximately 13 km south of the St. Clair River AOC southernmost boundary. Wheatley Provincial Park is 2.3 km NE of the Wheatley Harbour AOC boundary. The section of Lyons Creek (Niagara River AOC) where eggs were obtained partially drains the Welland Canal. PCB contamination was expected at Lyons Creek (*1*). Cootes Paradise and Grindstone Creek (Hamilton Harbour AOC) are near an industrial zone, commercial harbour, and a large urban area. The Humber River (Toronto Harbour AOC) drains the city of Toronto, and is near an active harbour and industrial zone. Dead Creek, Murray Canal, and Belleville are all within the Bay of Quinte AOC, although the contaminant sources may vary among the three sites; Murray Canal was once used for commercial shipping. A third site, Upper Canada Bird Sanctuary (UCBS), was used as an upstream site from the St. Lawrence River (Canadian side) AOC, whereas Raisin River was considered downstream of the AOC. Although Raisin River drains an industrial area in

Cornwall, the industry is far upstream from where the turtle eggs were collected. Snye Marsh, Akwesasne, is within the St. Lawrence River AOC (US side), and is downstream of localized heavy industry (*2*).

Turtle sampling sites are shown in Fig. S1, where the relative size of the nearby urban centre is given as a function of the natural log transformed population census (Table 1).

Data adjustments and quality control

Quality assurance and quality control included method blanks, duplicate extraction, and injections of standard reference (SRM) material and cleaned-up turtle egg extracts for each block of 5 samples to monitor for quantitative reproducibility and instrument sensitivity. The recoveries based on the added ISs and recovery surrogates were >80% for 1,3,5-tribromobenzene, $^{13}C_{12}$-labeled CB-37, CB-52 and CB153, and BDE71. Therefore, where an external standard method of quantification was used (PCBs and OC pesticides), analyte concentrations were not recovery-corrected. Duplicate compound injections and herring gull SRMs varied on average by <10% and 5%, respectively. The method limit of quantification (MLOQ) was determined as ten times the standard deviation of the average blank signal in the case where a compound was present. For PCBs and OC pesticides the MLOQs ranged between 0.01 to 0.09 ng/g for the eggs samples analyzed at GLIER by GC-ECD, and 0.1 ng/g for the eggs samples analysed at NWRC using GC-MSD and 0.01 ng/g (ww) for PBDEs using GC-MS(ECNI).

Adjusting for differences between GC-ECD and GC-MSD

To account for some differences in the measurement of PCB congeners and OCs by GC-MSD versus GC-ECD, the concentrations in SRM (herring gull egg homogenate) were compared:

$$diff = \frac{[MSD - ECD]}{[ECD]} \quad \text{(EQ 1)}$$

Then, every compound measured in the samples analyzed using ECD was adjusted using the difference between the ECD and MSD analyzed SRM samples;

$$Adj[ECD] = [ECD] + (diff) \times ([ECD]) \quad \text{(EQ 2)}$$

PCB and OC pesticide concentrations in the turtle eggs determined by GC-ECD were adjusted to GC-MSD-based values using the same factors to remove methodological artifacts.

Estimation of Aroclor sources

To estimate the relative contribution of each Aroclor (1242, 1248, 1254, and 1260) to the PCB egg burden, we calculated a theoretical mixture of each Aroclor (*3*) (Table 3) that had the lowest mean squared error (MSE) with the congener pattern in the eggs. Thus, we minimized the MSE:

$$\text{MSE} = \sum_{n} \frac{[(x_1 \times A1242_i + x_2 \times A1248_i + x_3 \times A1254_i + x_4 \times A1260_i) - \text{Sample}_i]^2}{n} \quad \text{(EQ 3)}$$

where *i* is the PCB congener, and *n* is the number of congeners measured, x_1 to x_4 are the coefficients for each Aroclor; starting values were 0.25 each. The MSE was minimized iteratively, using the conjugate gradient method (*4*). We divided each Aroclor coefficient by the sum of x_1 to x_4, which rescaled the coefficients to proportions of the ΣPCBs.

Replacement values for observations below detection limits

Contaminants were expressed as wet weight (ww), and were natural log transformed prior to statistical analysis. Many compounds measured were below MLOQ, ranging from 0% to 91.6%); all compounds in which all 100% were below MLOQ were not included in any analyses. Overall, 29.1% of observations were below MLOQ, and the mean percent of observations below MLOQ varied from 10.1% at Turkey Creek (Detroit River AOC) to 64.9% at Algonquin Provincial Park, one of the reference locations. We calculated replacement values for samples below MLOQ using Maximum Likelihood Estimation (MLE). MLOQ values were used to calculate the initial values of the means and SDs. The Log Likelihood (LL) was maximized:

$$\mathrm{LL} = \sum_{\mathrm{i}} \frac{1}{\sigma\sqrt{2\pi}} \times \int_{-\infty}^{x} e^{\left(-\frac{(MLOQ_i-\mu)^2}{2\sigma^2}\right)} du + \sum_{j} \ln\left(\left(\frac{1}{(2\pi)^{0.5}\times\mu)}\right) \times e^{\left(\frac{1}{2}\times\left(\frac{\ln(x_j)-\mu)}{\sigma}\right)^2\right)}\right), \quad \text{EQ4)}$$

where μ is the initial mean contaminant concentration, σ is the initial SD, *i* is the number of observations below MLOQ, *j* is the number of detectable observations, and x_j is the contaminant concentration. The LL was maximized iteratively, using the conjugate gradient method (*13*), and produces the new estimate for the mean concentration (and SD) for each compound. Two PCB congeners had > 70% observations below MLOQ; those values were given as zero.

Replacement values for MLOQ s should be proportional to the amount of PCBs present; consequently, we used PCB 153 to indicate the relative contaminant. We calculated the mean and SD of the log transformed concentrations of PCB 153, and for each sample, calculated the cumulative probability distribution (CDF), and the CDF of the MLOQ for each PCB congener. For each PCB congener, we rescaled the CDFs of the corresponding PCB 153 value, so that the CDFs ranged from zero to the CDF of the MLOQs:

$$CDF' = \frac{CDF[PCB\,153]}{\max CDF[PCB\,153]} \times CDF\,[MDL] \qquad \text{(EQ 5)}$$

, where: CDF' is the new CDF for the MLOQ value, CDF[PCB 153] CDF[MLOQ] are the CDFs for PCB 153 and MLOQ. The replacement value for observations below MLOQ is then the inverse CDF', using the mean and SD estimated by MLE. For OC pesticides and PBDEs, DDE and PBDE 47 were used, respectively, to indicate the relative contaminant burden for each turtle egg.

References

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Figure S1. Sampling sites where snapping turtle eggs were collected in the lower Canadian Great Lakes 2001-2004. The log transformed population sizes of adjacent urban municipalities is proportional to the size of the symbols. Where there are no symbols, the local population size is essentially zero. Site names that are underlined have substantial industry or known contaminant sources nearby.

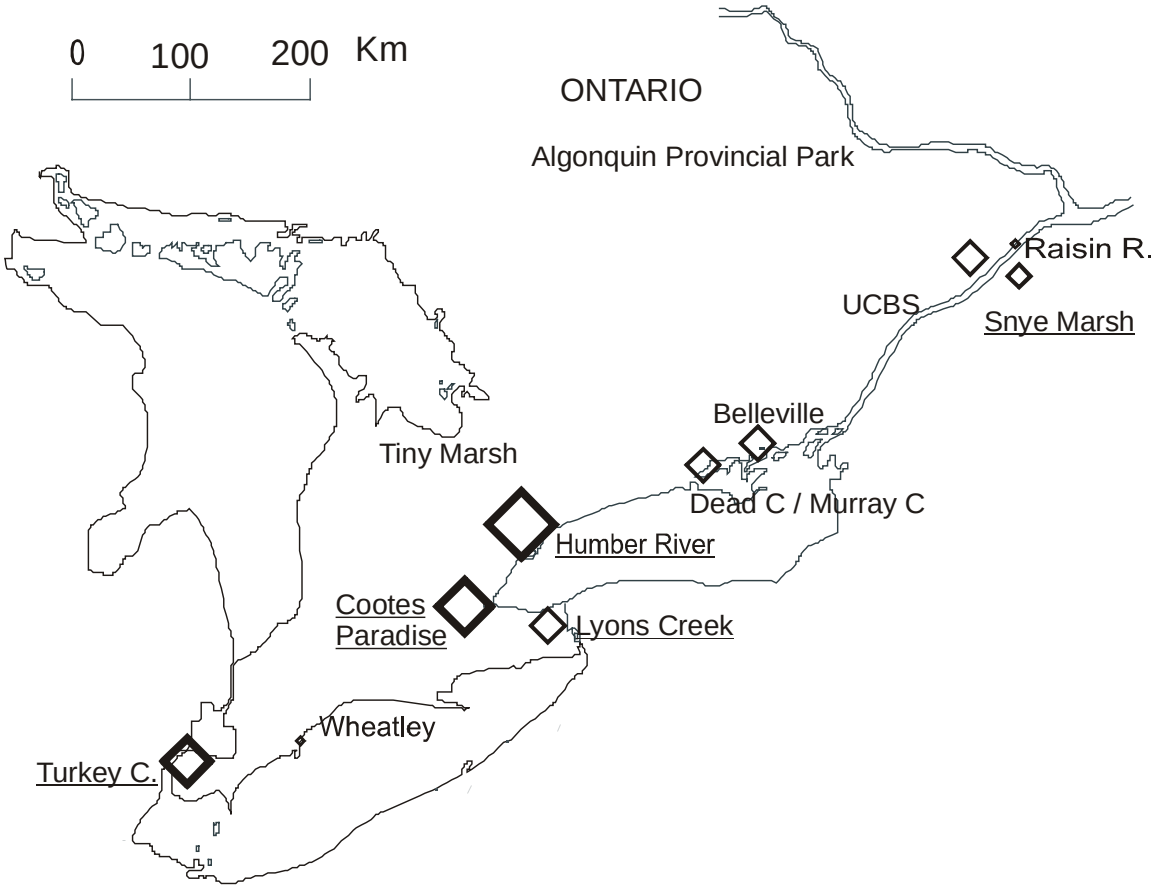

Table S1. Estimated population sizes for urban centres near turtle collection sites, adapted from Statistics Canada, 2006 Population Census (http://www12.statcan.ca/english/census06/data/popdwell/Tables.cfm)

Municipality	Population
Toronto	2503281
Hamilton	504559
Windsor	216473
Belleville	48821
Welland	50331
Quinte West	42697
Cornwall	45965
Wheatley	2248
Elmvale[1]	0
Algonquin[1]	0
Akwesasne[2]	13000
Long Sault	33
Lancaster	825

[1] No urban centre, town or village adjacent or immediately upstream of turtle sampling site.

[2] Not censused by federal government; number given is estimate only.